

# BOOK OF ABSTRATCS

6TH MEETING  
OF YOUNG RESEARCHERS OF UNIVERSITY OF PORTO



ijmp '13



*ijur* '13

6TH MEETING OF YOUNG RESEARCHERS OF  
UNIVERSITY OF PORTO

# CREDITS

Livro de Resumos IJUP'13

6º Encontro  
de Investigação  
Jovem da U.Porto

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WEDNESDAY, 13<sup>th</sup>

THURSDAY, 14<sup>th</sup>

FRIDAY, 15<sup>th</sup>

08:40	09:00	REGISTRATION	REGISTRATION	REGISTRATION
09:00				
09:30	09:30	<b>PARALLEL ORAL SESSIONS I:</b> A1 - Biomaterials II A2 - Biomaterials I A3 - Biomaterials A4 - Biomaterials	<b>PARALLEL ORAL SESSIONS II:</b> A5 - Biomaterials III A6 - Biomaterials IV A7 - Biomaterials V A8 - Biomaterials VI	<b>PARALLEL ORAL SESSIONS III:</b> A9 - Biomaterials VII A10 - Biomaterials VIII A11 - Biomaterials IX A12 - Biomaterials X
10:00	10:00	<b>AL - LUNCH BREAK</b>	<b>AL - LUNCH BREAK</b>	<b>AL - LUNCH BREAK</b>
10:30	10:30	<b>AL - OPENING SESSION</b> AL1 - Opening Session I AL2 - Opening Session II AL3 - Opening Session III AL4 - Opening Session IV AL5 - Opening Session V	<b>AL - OPENING SESSION</b> AL6 - Opening Session VI AL7 - Opening Session VII AL8 - Opening Session VIII AL9 - Opening Session IX AL10 - Opening Session X	<b>AL - OPENING SESSION</b> AL11 - Opening Session XI AL12 - Opening Session XII AL13 - Opening Session XIII AL14 - Opening Session XIV AL15 - Opening Session XV
11:00	11:00	<b>AL - OPENING SESSION</b> AL16 - Opening Session XVI AL17 - Opening Session XVII AL18 - Opening Session XVIII AL19 - Opening Session XIX AL20 - Opening Session XX	<b>AL - OPENING SESSION</b> AL21 - Opening Session XXI AL22 - Opening Session XXII AL23 - Opening Session XXIII AL24 - Opening Session XXIV AL25 - Opening Session XXV	<b>AL - OPENING SESSION</b> AL26 - Opening Session XXVI AL27 - Opening Session XXVII AL28 - Opening Session XXVIII AL29 - Opening Session XXIX AL30 - Opening Session XXX
11:30	11:30	<b>AL - OPENING SESSION</b> AL31 - Opening Session XXXI AL32 - Opening Session XXXII AL33 - Opening Session XXXIII AL34 - Opening Session XXXIV AL35 - Opening Session XXXV	<b>AL - OPENING SESSION</b> AL36 - Opening Session XXXVI AL37 - Opening Session XXXVII AL38 - Opening Session XXXVIII AL39 - Opening Session XXXIX AL40 - Opening Session XL	<b>AL - OPENING SESSION</b> AL41 - Opening Session XL I AL42 - Opening Session XL II AL43 - Opening Session XL III AL44 - Opening Session XL IV AL45 - Opening Session XL V
12:00	12:00	<b>AL - OPENING SESSION</b> AL46 - Opening Session XL VI AL47 - Opening Session XL VII AL48 - Opening Session XL VIII AL49 - Opening Session XL IX AL50 - Opening Session XL X	<b>AL - OPENING SESSION</b> AL51 - Opening Session XL XI AL52 - Opening Session XL XII AL53 - Opening Session XL XIII AL54 - Opening Session XL XIV AL55 - Opening Session XL XV	<b>AL - OPENING SESSION</b> AL56 - Opening Session XL XVI AL57 - Opening Session XL XVII AL58 - Opening Session XL XVIII AL59 - Opening Session XL XIX AL60 - Opening Session XL XX
12:30	12:30	<b>AL - OPENING SESSION</b> AL61 - Opening Session XL XXI AL62 - Opening Session XL XXII AL63 - Opening Session XL XXIII AL64 - Opening Session XL XXIV AL65 - Opening Session XL XXV	<b>AL - OPENING SESSION</b> AL66 - Opening Session XL XXVI AL67 - Opening Session XL XXVII AL68 - Opening Session XL XXVIII AL69 - Opening Session XL XXIX AL70 - Opening Session XL XXX	<b>AL - OPENING SESSION</b> AL71 - Opening Session XL XXX I AL72 - Opening Session XL XXX II AL73 - Opening Session XL XXX III AL74 - Opening Session XL XXX IV AL75 - Opening Session XL XXX V
13:00	13:00	<b>LUNCH BREAK</b>	<b>LUNCH BREAK</b>	<b>LUNCH BREAK</b>
13:30	13:30			

# PROGRAM

14:00	
14:00	
14:30	<b>REKAMATI, 2014, SESIUNING VI</b> A1. Etnografi (1007) A2. Psikologi A3. Sastra (1001) I A4. Sastra (1001) II
15:00	
15:30	
16:00	
16:30	
17:00	
17:30	<b>REKAMATI, 2014, SESIUNING VII</b> A1. Etnografi (1007) A2. Psikologi (1001) I A3. Sastra (1001) II
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## FOREWORD

O programa de iniciação à I&D da U.Porto (IJUP) tem no seu encontro anual o momento de maior visibilidade.

O encontro deste ano - já a 6ª edição - reúne mais de 1000 participantes, maioritariamente estudantes de primeiro ciclo e de mestrado integrado. Os resultados dos trabalhos em que participam vão ser apresentados nas cerca de 500 comunicações, orais e na forma de poster. Um acontecimento único num local simbólico - o edifício da Reitoria, representando bem o espírito de cooperação que pretendemos criar dentro da nossa universidade.

Mas o IJUP é mais do que este encontro anual. Ele revela o dinamismo e a abertura da nossa comunidade científica para acolher estes estudantes, permitindo-lhes uma experiência de aprendizagem única. Nele se revelam novas vocações e paixões para a investigação científica e para a prática do método científico, num processo que aproxima a criação do conhecimento dos seus destinatários. E isso é ainda mais relevante na medida em que criará canais facilitadores da ligação do sistema científico e tecnológico às entidades onde, no futuro, esses estudantes irão exercer a sua atividade profissional.

O IJUP deve ser motivo de orgulho para toda a comunidade U.Porto. Sem incentivos de políticas públicas, tem sido possível colocar de pé um programa único no ensino superior Português e até mesmo a nível internacional. Com este programa, encontraram-se pretextos para maior cooperação entre centros de investigação e unidades orgânicas. Com este programa a U.Porto mostra-se e passa a conhecer-se melhor... E este maior conhecimento do nosso potencial só nos pode dar razões para enfrentar o futuro com confiança!

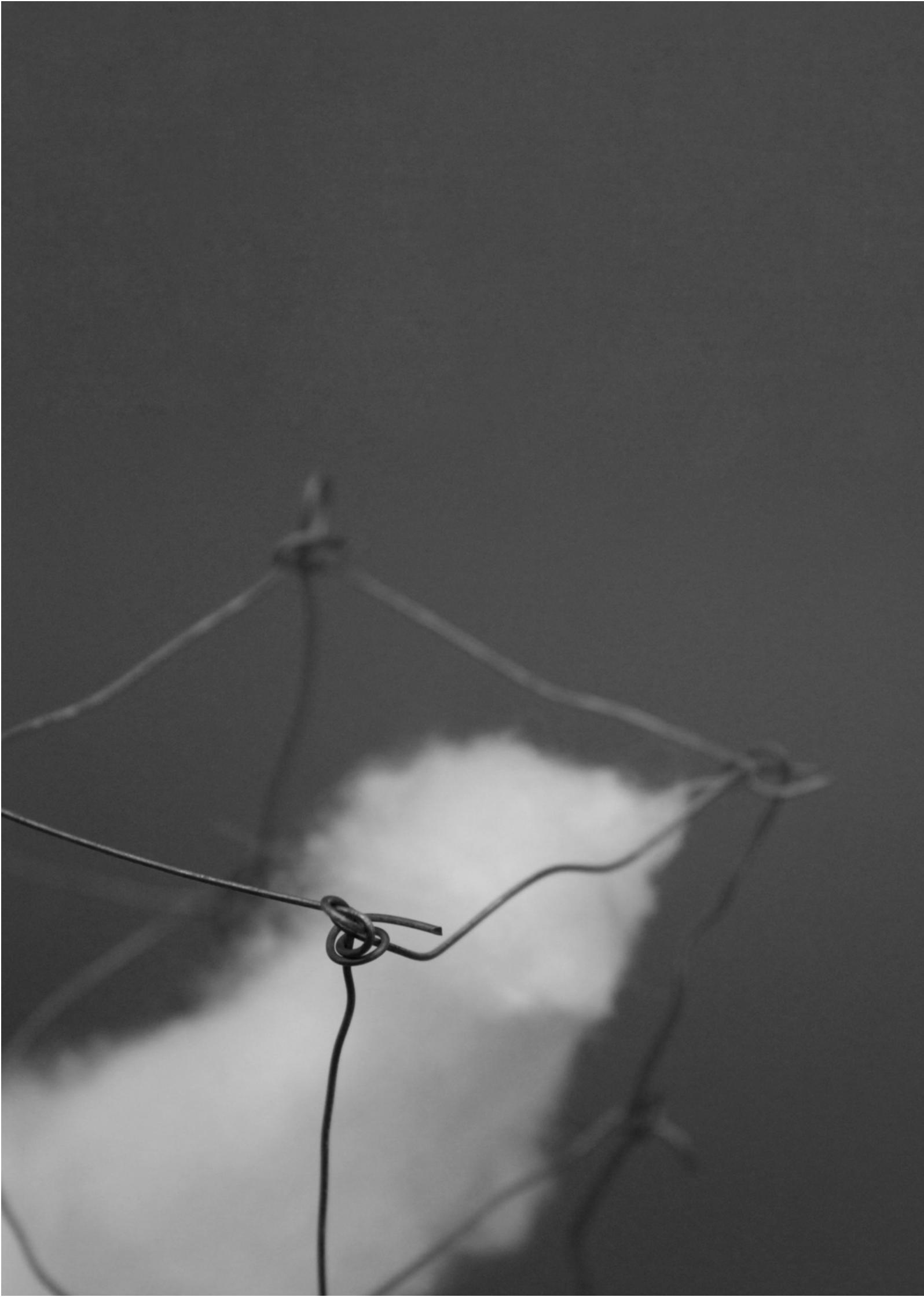
O nosso obrigado a todos os que tornam este programa possível e que deste modo também materializam a nossa ambição de nos diferenciarmos enquanto universidade e afirmarmos a U.Porto como uma universidade de investigação.

Bem hajam!

J. C. Marques dos Santos  
Reitor da Universidade do Porto







**ORAL SESSIONS**

**I**



Oral Sessions ▶ A1

# Biomedicine I



# **EFFECT OF THE PREBIOTICS NUTRIOSE® FB 06 AND RAFTILOSE® P95 UPON THE UPTAKE OF BUTYRATE AND UPON ITS EFFECT ON CELL PROLIFERATION, VIABILITY AND DIFFERENTIATION IN INTESTINAL EPITHELIAL CELLS**

**C. Costa, P. Gonçalves, A. Correia-Branco and F. Martel**

Department of Biochemistry, Faculty of Medicine of Porto, University of Porto, Portugal.

Colorectal cancer (CRC) is one of the most common cancers worldwide. Many epidemiological studies show an inverse relationship between dietary fiber intake and the incidence of CRC. The short-chain fatty acid butyrate (BT), produced in the intestinal lumen by bacterial fermentation of dietary fiber, plays a key role in colonic epithelium homeostasis, having multiple regulatory roles at that level. One of the proposed beneficial effects of BT on human colonic health is the prevention/inhibition of colon carcinogenesis. BT is transported into colonic epithelial cells by two specific carrier-mediated transport systems: the electroneutral H<sup>+</sup>-coupled monocarboxylate transporter 1 (MCT1) and the Na<sup>+</sup>-coupled monocarboxylate cotransporter 1 (SMCT1). MCT1 and SMCT1 were recently proposed to function as tumor suppressors.

The aim of this study was to evaluate the effect of the prebiotics Nutriose® FB 06 and Raftilose® P95 upon (a) uptake of <sup>14</sup>C-BT, and (b) the effects of BT on cell proliferation, viability and differentiation, in a rat normal intestinal epithelial cell line (IEC-6 cells). Uptake of <sup>14</sup>C-BT was measured by liquid scintillation counting. MCT1 and SMCT1 mRNA expression was quantified by qRT-PCR. Cell viability, proliferation and differentiation were quantified with the lactate dehydrogenase (LDH), sulforhodamine B and alkaline phosphatase activity assays, respectively.

This study shows that acutely (1h, 3h, 6h), the prebiotics (up to 100 mg/ml) did not affect neither <sup>14</sup>C-BT uptake nor cellular viability and proliferation. However, chronic exposure (48h) to Nutriose® FB 06 (50 mg/ml) or Raftilose® P95 (20 mg/ml) increased <sup>14</sup>C-BT uptake, independently of changes in cell viability or proliferation. This increase appeared to involve SMCT1 but not MCT1. Prebiotics had no effect on SMCT1 and MCT1 mRNA expression levels. BT (5 mM; 48h) markedly decreased cellular viability and proliferation and increased IEC-6 cell differentiation. In general, combination of prebiotics with BT did not significantly modify these parameters, with the exception of Nutriose® FB 06 50 mg/ml, which decreased the effect of BT upon cell viability.

In conclusion, the results show that chronic exposure to Nutriose® FB 06 and Raftilose® P95 increases uptake of <sup>14</sup>C-BT by intestinal epithelial cells. Although Nutriose® FB 06 and Raftilose® P95 did not modify the effects of BT upon cell viability, proliferation and differentiation, we cannot exclude that they can interfere with the effect of BT at other intracellular targets (e.g. apoptosis, inhibition of NF-κB activation), and thus contribute to colonic epithelium homeostasis.

Acknowledgements: This work was supported by Fundação para a Ciência e a Tecnologia (FCT) and COMPETE, QREN and FEDER (PTDC/SAU-OSM/102239/2008). Authors would like to thank Dr. M. A. Vieira-Coelho (Department of Pharmacology and Therapeutics, Faculty of Medicine of Porto) for the generous gift of the prebiotics.

# Activation of P2X<sub>7</sub> and P2Y<sub>1</sub> receptors by adenine nucleotides counteract cardiodepression induced by adenosine in the spontaneously-beating rat atria

**S. Nogueira-Marques<sup>1</sup>, N. Oliveira-Monteiro<sup>1</sup>, B. Bragança<sup>1</sup>, A.C. Pereira<sup>1</sup>, A.P. Fontes-Sousa<sup>1</sup>, P. Correia-de-Sá<sup>1</sup>**

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Purines such as ATP and its metabolite adenosine (ADO) are involved in the pathophysiology of numerous cardiovascular diseases (*e.g.* ischemia-reperfusion, arrhythmias and hypertension). In the mammalian heart, adenine nucleotides and nucleosides regulate chronotropism, dromotropism and inotropism [1]. ATP exerts pleiotropic effects in the cardiovascular system through the activation of both ionotropic P2X and metabotropic P2Y purinoceptors. The actions of ATP may be cut-short by its rapid catabolism via a cascade of membrane-bound ecto-NTPDases leading to ADO formation [2]. The cardiodepressant effect of ADO mediated by A<sub>1</sub> receptors (A<sub>1</sub>AR, P1 purinoceptor) activation is well known. Studies using the isolated paced left atrium, demonstrated that inotropic effects of ATP and its metabolite ADO appear in a different time scale and are often conflicting [3]. This may be due, in part, to a lack in our knowledge concerning the role of ATP (and related nucleotides) on automatism of the spontaneously-beating atria.

The experiments were performed on isolated atria from Wistar rats (*Rattus norvegicus*; 250-300 g) of either sex, continuously superfused with Tyrode's solution (95% O<sub>2</sub> + 5% CO<sub>2</sub>; 37°C). Isometric muscle tension was monitored on a computer screen via a PowerLab data acquisition system (Chart 5, v.4.2 software; AD Instruments, USA).

Both ATP and ADP (100 μM, *n*=20 and *n*=12; controls) transiently decreased muscle tension (negative inotropism) by 12±2% and 6±3%, respectively. Sustained and more robust decreases in the rate of atrial contractions (negative chronotropism) were obtained less than 1 min after application of ATP and ADP (21±3% and 17±4%). Selective blockade of A<sub>1</sub>ARs with DPCPX (100 nM, *n*=6-7) significantly (*P*<0.05) attenuated the negative inotropic and chronotropic responses of ATP and ADP. POM-1 (100 μM), a potent ecto-NTPDase inhibitor, potentiated the negative chronotropic action of ATP (100 μM, 42±6% *n*=7), without affecting inotropism. Selective blockade of P2X<sub>7</sub> receptors with A438079 (3 μM) sensitized atria to the negative inotropic action of ATP (100 μM, 31±4% *n*=6), without affecting the chronotropic effect. Likewise, the negative inotropic effect of ADP was amplified to 26±8% by blocking P2Y<sub>1</sub> receptors with MRS2179 (0.3 μM, *n*=4).

Data indicate that ADO (via A<sub>1</sub>AR) contributes significantly to ATP-induced cardiodepression in the spontaneously-beating rat atria. Rapid inactivation of ATP by ecto-NTPDases may cut-short the negative chronotropic effect of the nucleotide. Regarding the negative inotropic action of ATP, it appears that activation of ionotropic P2X<sub>7</sub> and metabotropic ADP-sensitive P2Y<sub>1</sub> receptors play a role to counteract the depressant effect of ATP due to ADO formation in the spontaneously-beating rat atria. Understanding of the interplay between P1 and P2 purinoceptors and the enzymatic cascade regulating the purinergic signals in the heart will certainly give the opportunity to design new pharmacological tools for therapeutic intervention in cardiovascular diseases.

Work supported by FCT (FEDER funding, PEst-OE/SAU/UI0215/2011).

[1] Vassort, G. (2001), *Physiol Reviews*, 81, 767-806.

[2] Burnstock, G. (2006), *Trends in Pharmacological Sciences*, 27, 166-176.

[3] Frolidi, G. et al. (1994), *Naunyn-Schmiedeberg's Arch Pharmacol*, 349, 381-386.

# Binding mechanism of new potential antitumor molecules to albumin: a fluorescence and computational study applied to xanthone derivatives

**R. Vieira<sup>1</sup>, D. Marques<sup>1</sup>, J. Soares<sup>1</sup>, C. Azevedo<sup>1</sup>, A. Palmeira<sup>1</sup>, Carlos M. M. Afonso<sup>1</sup>, M. S. Reis<sup>2</sup>, M. M. Pinto<sup>1</sup>**

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In the pipeline that drives “hit” compounds to drug candidates, special attention must be given to both pharmacodynamic and pharmacokinetic behaviors. One of the most important studies that are required is the binding of molecules to plasma proteins, since it affects dramatically absorption, distribution, metabolism and excretion of drugs. As human serum albumin (HSA) is the most abundant and important plasma protein, its potential interactions with a series of *in-house* small molecules have been analysed using *in silico* and fluorescence techniques <sup>[1]</sup>.

Xanthenes are a group of *O*-heterocyclic compounds, which are well known for their wide variety of biological activities. Our group has been focusing on the synthesis of xanthone derivatives with antitumor activity. A compound from our *in-house* library, XC10, showed a promising antitumoral activity and thus several analogues were prepared <sup>[2]</sup>. The binding of XC10 and its newly synthesized analogues to HSA was investigated by Förster resonance energy transfer technique <sup>[3]</sup>. In this technique, the distance between the bound compound and the albumin tryptophan residue located in the subdomain IIA is measured <sup>[3]</sup>. This residue is located in a hydrophobic pocket which is one of the main binding sites of several drugs to albumin.

In order to have more detailed information about the binding of XC10 and analogues to HAS, an *in silico* study was also performed <sup>[4]</sup>. Using AutoDock Vina program, molecular docking to a HSA crystal structure (pdb code: 2VUE) was performed, and the ligand conformations and docking scores were analysed.

The obtained results allowed us to enhance our knowledge about the binding of XC10 and its analogues to HSA, suggesting and orienting the design of new drug candidates with better pharmacokinetic profile.

## References:

- [1] Kerns, E.H. and Di L. (2003), *Pharmaceutical Profiling in Drug Discovery*, Drug Discovery Today, 8 (7), 316-323.
- [2] Pinto, M.M.M., Sousa, M.E. and Nascimento, M.S.J. (2005), *Xanthone derivatives: New insights in biological activities*, Current Medicinal Chemistry, 12 (21), 2517-2538.
- [3] Kalanur, S., Seetharamappa, J. and Kalalbandi, V.K.A. (2010), *Characterization of interaction and the effect of carbamazepine on the structure of human serum albumin*. Journal of Pharmaceutical and Biomedical Analysis, 53, 660–666.
- [4] Sochacka, J. and Baran, W. (2012), *The Investigation of the Binding of 6 Mercaptopurine to Site I on Human Serum Albumin*. The Protein Journal 31, 689–702.

## Acknowledgments:

This work is funded through national funds from FCT – Fundação para a Ciência e a Tecnologia under the project CEQUIMED – PEst-OE/SAU/UI4040/2011, FEDER funds and COMPETE program under the project FCOMP-01-0124-FEDER-011057 and U.Porto/Banco Santander Totta (PP\_IJUP2011-237).

# Effects of olive leaves supplementation on the constituents of blood, oxidative stability of RBCs and pork meat quality

**F. Monteiro-Silva<sup>1</sup>, T. Ribeirinha<sup>1,2</sup>, R. Gonçalves<sup>1,2</sup>, V. Pinheiro<sup>3</sup>, J. Mourão<sup>3,4</sup>, D. Outor-Monteiro<sup>3,4</sup>, L. Belo<sup>5,6</sup>, A. Santos-Silva<sup>5,6</sup> and F. Paiva-Martins<sup>1,2</sup>**

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<sup>5</sup> IBMC and <sup>6</sup>Department of Biological Sciences, Faculty of Pharmacy, University of Porto.

Olive polyphenols are believed to prevent the development of heart and vascular diseases. Therefore, olive leaf extract supplements have been marketed as a promoter of heart health. However, scientific research supporting these claims is lacking. Limited human trials have been conducted but encouraging results were obtained with leaves extract in the reduction of hypertension in borderline hypertensive patients [1].

The present study was designed as a single-center, randomized, prospective pilot comparison of the effect of different concentrations of olive leaf supplements on a cohort of young, healthy pigs. Emphasis was placed on the impact of supplementation on the hematological and lipid profile of blood and on the oxidative stability of red blood cells. These cells are particularly susceptible to oxidative damage because of their role as oxygen carriers and are closely related to vascular tonus regulation. In a recent study [2], leaf supplementation showed to decrease the weight gain of pigs and to increase the tocopherol content of meat. Pigs have a digestive tract similar to humans and have shown to be an excellent model to the study cardiovascular disease [3].

24 pigs were fed with a conventional pig diet until they weighted 70-90 kg, and afterwards, they were randomly assigned to 3 experimental diets: a control group fed with the conventional diet (OL0) and other two groups fed with the conventional diet supplemented at 5% (OL5) and 10% (OL10) olive leaves during 8 weeks. In the present study, no significant reductions were found for LDL and oxidized-LDL levels after supplementation but a statistically significant reduction in triglycerides concentration was observed in the group OL10 (Fig. 1) whereas red blood cells showed lower stability against oxidative injury. The present study may corroborate the well-known duality of phenol's biological activity, namely the concentration-dependent balance of their cytoprotective vs cytotoxic activity. Moreover, other constituents in leaves besides phenols, may contribute for the negative effects. Further studies are needed in order to support the beneficial activity and safety of olive leaf supplements.

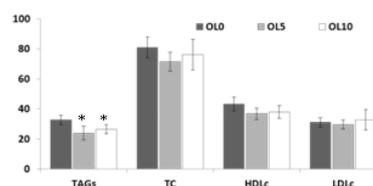


Fig. 1 – Blood analysis values obtained for different feed value groups.

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[2] Paiva-Martins *et al* (2009), *Meat Science*, 82 (4), 438-443.

[3] Dyson *et al* (2006), *Comparative Medicine*, 56 (1), 35-45.

# Experimental therapeutics in a zebrafish model of cardiac insufficiency by mitochondrial dysfunction: insights for Friedreich's ataxia

**A. Fonseca-Silva<sup>1</sup>, B. R. Pinho<sup>1</sup>, J. M. Mendes<sup>1</sup>, R. Maia-Amaral<sup>1</sup>, M. M. Santos<sup>2</sup>, and J. M. A. Oliveira<sup>1</sup>**

<sup>1</sup>*REQUIMTE*, Departamento de Ciências do Medicamento, Laboratório de Farmacologia, Faculdade de Farmácia, Universidade do Porto; (2) *CIIMAR*, Faculdade de Ciências, Universidade do Porto

Mitochondrial respiratory chain dysfunction underlies cardiac impairment in Friedreich's Ataxia (FRDA), a non-coding trinucleotide repeat expansion disorder causing decreased frataxin levels [1]. Ubiquinone (UQ) analogs and lysine deacetylase inhibitors (KDACi) are putative FRDA therapeutics [2]. We aimed to develop an *in vivo* zebrafish (*Danio rerio*) model of cardiac insufficiency by mitochondrial dysfunction and test the rescuing potential of UQ analogs (idebenone-IDB, decylubiquinone-DCB) and pan-KDACi (valproic acid, VPA; and trichostatin A, TSA).

Fertilized zebrafish eggs were randomly distributed in 12-well plates (10 embryos/well). Mitochondrial dysfunction was induced by acute treatment of zebrafish (56 hours post fertilization; hpf) with mitochondrial inhibitors: rotenone (RTN), myxothiazol (MYX), antimycin (ANT), or oligomycin (OLG). Phenotype recovery essays were performed by co-exposure of UQ analogs at 56hpf or pre-incubation of KDACi from 4-56 hpf to allow for gene expression changes prior to RTN exposure. Embryos were scored for presence/absence of circulation and heartbeat at 10min intervals until generalized asystole. For each treatment, 30-60 embryos, from 2-3 independent clutches, were assayed in 2-3 independent trials. Statistics: Kaplan-Meier with Log rank test; Significance level = 0.05.

*Acute exposure essay:* RTN decreased the heart rate after 30min of exposure, arresting circulation and heartbeat in 50% of the embryos after 111 and 173min exposure, respectively. MYX and ANT induced a similar phenotype but with different time rates. IDB and DCB delayed RTN induced cardiovascular insufficiency by 61% and 79%, and asystole by 52% and 104%, respectively, but failed to protect embryos from MYX or ANT. OLG delayed RTN-induced heartbeat arrest by 18%, suggesting ATP synthase reversal by RTN. *Chronic exposure assays.* VPA or TSA delayed RTN induced asystole by, respectively, 27% or 31%, but failed to modify circulation arrest.

In summary, *in vivo* modeling of cardiac impairment is feasible in zebrafish and can be used for testing experimental therapeutics. Our findings in zebrafish suggest that cardioprotection by UQ analogues stems from enhanced ATP synthesis rather than antioxidant properties. The pan-KDACi failed to prevent cardiac insufficiency in our experimental paradigm, albeit delaying asystole and suggesting some degree of cardioprotection as recently reported for other model organisms [3]. While UQ analogues may improve cardiac function in FRDA, the primary advantage of pan-KDACi in this disease may rely on the possibility of enhancing frataxin expression.

ACKNOWLEDGMENTS: Fundação para a Ciência e a Tecnologia (PTDC/NEU-NMC/0237/2012; PEst-C/EQB/LA0006/2011; SFRH/BD/63852/2009); Universidade do Porto & Santander Totta, IJUP2011#118.)

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# Influence of postmenopausal osteoporosis in maxillary and mandibular bone structure: an experimental study in the rat

**R. Araújo Silva<sup>1</sup>, B. Colaço<sup>2</sup>, C. Santos<sup>3</sup>, M.J. Carmezim<sup>3</sup>, M.H. Fernandes<sup>1</sup>, P. Gomes<sup>1</sup>**

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Osteoporosis is a metabolic disease that causes microarchitectural changes in bone and in its biomechanical properties [1]. The effect of osteoporosis in the bone structure has been studied in detail, despite that little is known regarding the affection of the cranio-maxillofacial complex [2].

The aim of this study is to evaluate the influence of the osteoporotic condition in the bone tissue of the oral cavity, using an animal model that reproduces the human postmenopausal osteoporotic condition (the ovariectomized rat).

Twelve female Wistar rats, eight weeks old were used. They were randomly selected for bilateral ovariectomy (n=6, OVX group) or sham surgery (n=6, Sham group). After 3 months, the animals were euthanized and mandibles, maxillas, tibias, femurs, and calvarial bones were harvested. The radiographic optical density and the microtomographic histomorphometrical analysis were conducted in all harvested bones. The thickness of the periodontal ligament and the relative amount of bone support were also determined for mandibles and maxillas. Furthermore, a biomechanical assay was performed, (a three point bending test) in samples of mandibles and femurs.

The mineral bone density of mandible, tibia, femur and calvarial bones, as assessed by x-ray, was significantly decreased in OVX group. Also, in the OVX group, the thickness of periodontal ligament increased 30% and the bone support was reduced about 35%, in mandible samples; while maxillas showed an increased thickness of periodontal ligament of about 40%, and a 10% reduction in the bone support, comparing with Sham group. The histomorphometrical analysis showed that, in OVX group, a decreased number of trabeculae and increase of marrow space were attained, in association with a decreased connective density and bone volume. The results of three point bending test showed that the failure load was decreased in the bones of Ovx group.

Osteoporosis, in the evaluated animal model, seems to cause alterations on bones of the cranio-maxillofacial complex, such as changes in mineral density, bone microarchitecture, biomechanical strength, bone support and thickness of periodontal ligament, which may be of clinical significance for bone-related oral rehabilitations of osteoporosis-affected individuals.

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Oral Sessions ▶ A2

# Arts



# From glass to print: Creation of glass matrixes for printmaking

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The present project aims to explore several glass modulation alternative possibilities for imprinting by introducing experimental methods that may develop its knowledge as plastic support material. The technical revision, innovative at the national context, follows the development of unconventional production methods that will encounter to the artistic specific vocabulary from the printmaking and relief contexts. Investigation procedures assume an original comparative study - under paper support (Fig.1) - to confirm the applicability to contexts of independent edition and design items. Appropriating traditional printing techniques, such as wood engraving and linocut or collography, and potentially similar glass techniques, like kilncasting and sandcasting, hand and sandblasting and pâte-de-verre, it is intended to strength its joint on the contemporary art context.

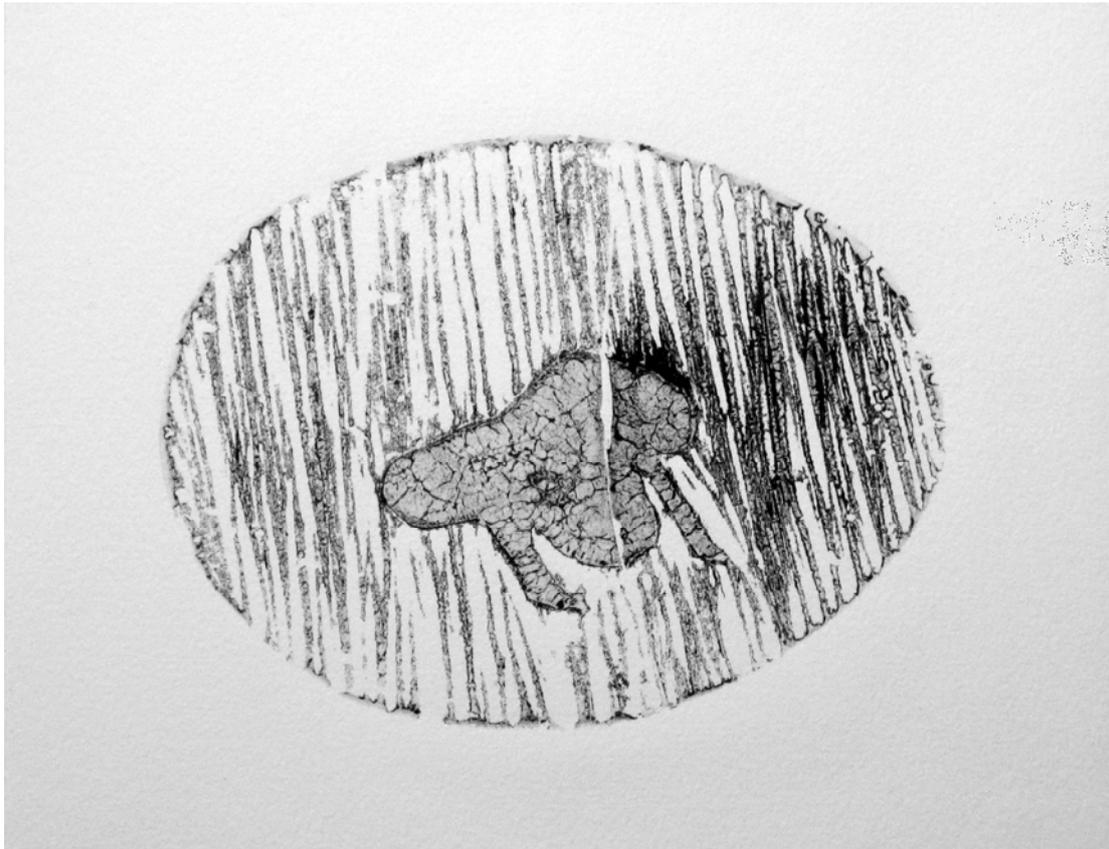


Figure 1

Untitled. Printmaking from glass matrix (kilncasting from a rubber cast), 25 x 25 cm, 2012.

# The Image on Glass: exploration of glass as substrate and plastic material

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The present research project is being developed around an interdisciplinary relationship between the practice of painting, drawing, printmaking techniques and glass technologies, and integrated in “Projectos Pluridisciplinares IJUP 2011” n°262: “*Vidro e Impressão: criação de substratos e matrizes de impressão alternativas*”.

The glass is used as operative and conceptual instrument in an attempt to discover relationships that can be established with the concepts of image and representation. Given the particular characteristics of this material, it is possible to work on both sides, or even overlapping layers and observe multiple realities simultaneously. When we work with glass surface, the image on glass extends beyond itself by shadow and reflection. The transparency of glass nullifies the background image and it appears as if floating in the space, without limits and without spatial barriers. Hence I am interested in seeking to establish a dialogue between the vitreous support, the image, the chromaticism, the light and the exhibition space.

In the context of laboratory studies and technology, I am developing comparative testing of different materials and techniques. In the work of handling the glass surface I am testing etching techniques with diamond points and acid [1]; manual and mechanical abrasion (sandblasting and handblasting, respectively); kilncasting [2]; slumping; fusing; lamination [3]; comparative testes on direct and photomechanical image transfer [4]; ink and vehicles compatibility between printmaking and painting oil inks and enamels for glass.

The strategies for building an artwork depart from the combination of processes and procedures of various origins. Based on painting I intend to explore the artistic expressiveness of materials, focusing on issues of transparency, opacity, density and tonal variation. The printing techniques arise here as a source of creation procedures, transformation and image transfer. From drawing, I am interested in performativity of actions (reserve, scrape, erase, hide, reveal, add and subtract), the unpredictability, the sudden, the accident. The process of building the image defines the existence of various states of embodiment. It is therefore crucial to this work, the importance of the process for the production of images, which strengthens the artistic practice.

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## Light and Shadow

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Junichiro Tanizaki, in his book “In Praise of Shadows”, distinguishes the differences between oriental and occidental culture, making explicit that oriental people are fascinated by shadow (present in architecture, literature...) and occidental seduced by light. Inspired by the book, I propose a comparison between the two distinct cultures, using light and shadow to describe them. This research is seeking to extend the search for light and shadow phenomena, accompanied by interests on turning the sacredness of art produced until XVI century contemporary, offering to the present day the enchanted view that once characterized artistic production. To execute it, painting was readapted in a distinguished way from the traditional one, using printing techniques to reproduce images. Serving the purpose, it has been undertaken several image making methods that allow the reproduction of a set of images on a specific period of art history, as much as the reprint of images. Research has been carried out to identify alternatives to the usual printing plates, using instead new matrices (glass) and unusual substrate of printing (textile). This presentation will outline the unique plastic qualities achieved by such glass techniques, showing in detail the qualities of each process as much as the different procedures applied to create a specific shadow and light game and depth effects..



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# SILENCE

Mafalda Portocarrero

Department, of Sculpture, Faculty of Fine Arts, University of Porto, Portugal

*...All art intuitively apprehends coming changes in the collective unconscious.*  
[1]

Silence started as a search for that something that could not be perceived, as a way of proving the ineffectiveness of our senses.

Bearing in mind Jung's concept of *Collective unconscious* and considering the new developments in science, especially the ones evolving from the Quantum physics, I'm lead to dream of a new *Collective unconscious* only this time a cosmic one accessed trough frequency.

I see myself merging with another, the others, the whole. I let myself go on an idealistic vision: I picture the actual world's synchronicity but feel it on a very wrong frequency: The one of great fear from recent economic/political developments.

It is this "Now" I'm interested in, this moment of sharing; how to rescue the individual from this desperate collectiveness.

There's no conclusion to such question, it evolves from a constant research and my practice follows my thoughts. Working with transparent glass it is the relation matter / process that interests me: I alter usual procedures stressing the effect. I repeat this obstinately producing endless series of identical objects hoping to be rewarded – each and every time - with their particular differences.



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## The false *ready-made*: relevance of support in painting

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The painting and the support where it takes place are a relationship that requires a conscious and committed reflection with the practice of painting itself. Thinking about the relevance of support over this practice is to think about the relationship of the produced object with reality. The result is something that intends to provoke and stimulate the thinking about the work of art and its way of intervention.

The illusion between representation and reality is an important point in this Painting research. And on the other hand, so is the delusion, that is, the understanding of pictorial object. Although conflicting these are characteristics that make their way to many interpretations of the work (Fig.1).



Figure 1

Carlos Mensil, Auto-retrato (Self-portrait). oil on plexiglass, 60 x 60 cm, 2011.

## **Gesture as Subject and Object in the Drawing**

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Analyzing various drawings with different as a result of actions and intentions in that the gesture is subject and object of the proceedings.

Based on the analysis of the drawings of Trisha Brown, *It's a Draw- Live Feed*.2003 and Tom Marioni, *Drawing a Line as Far as I Can Reach*, 1972; the use of gesture is common to both artists, taking advantage of what the body has to think about new ways of representing the movement of the body. Here, the drawing and performance are at the same level.

While Trisha Brown as an example of the translation of dance and performance to other media, we understand the body as a territory and drawing as a process. Here, the drawing is understood as an extension of dance, allows the archeology register movements, the dance movements represent a different way of thinking about the body moving through the space. The drawing is the result of investigations tracks the movements and the body is a body that draws.

Tom Marioni performs an action that draws a line to the fullest extent of his arm, ceiling arm sets the ceiling drawing.

These drawings are the result of a particular time and space that contribute to different results.

The gesture is in practice subject and object of the action when the drawings become autonomous.

Oral Sessions ▶ A3

# Environment



# Efficiency of a new solar photo-Fenton homogeneous process using iron (III) complexes in the degradation of oxytetracycline

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Conventional wastewater treatment plants do not treat toxic or/and non-biodegradable organic pollutants, such as antibiotics like oxytetracycline (OTC) [1]. Advanced Oxidation Processes (AOPs), as photo-Fenton, can be a solution. This study aims to assess the photo-Fenton process efficiency in the degradation/removal of OTC, using different Fe (III) complexes, like Fe (III)-oxalate, Fe (III)-citrate and EDDS. This iron complex presents a higher solubility which allows working at higher pH values and broader UV spectrum than the normally used Fe (II) species [2]. Experiments were done with 20 mg/L OTC in distilled water and different Fe (III)-oxalate concentrations (1, 2, 2.5 and 5 ppm), at 25 °C and different initial pH values. These solutions were recirculated during 45 minutes through a CPC (compound parabolic collector) under simulated solar radiation (Suntest XLS+). HPLC-DAD (High Performance Liquid Chromatography-Diode Array Detector) and TOC analysis were performed to evaluate degradation efficiency and contaminant mineralization, respectively. Results with Fe (III)-oxalate show similar mineralization and H<sub>2</sub>O<sub>2</sub> consumption rates for all the iron concentrations tested. Higher OTC degradation rates occurred for an iron concentration of 5 ppm. However, for all the iron concentrations tested, after 10 minutes of reaction the OTC concentration was below the detection limit of the equipment. As 2 ppm is the total iron concentration that can be discharged into water bodies according to the Portuguese legislation [3], this concentration represents the best option for photo-Fenton treatment with Fe (III)-oxalate at tested pH values. The conditions in terms of iron complex type and concentration, pH, temperature and irradiation were optimized. The process was also applied to the treatment of a real domestic wastewater after biological oxidation spiked with OTC.

## Acknowledgments:

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# Bioremediation of a mine effluent by *Lemna minor*

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Good water management practices are urgently needed these days, in parallel with the development of new solutions for problems of environmental contamination in natural aquatic systems, not only because the availability healthy freshwater is decreasing but also because there are goals to be achieved by the year of 2015, based on the Water Framework Directive (Directive 2000/60/EC, of the European Parliament and of the Council, of 23 October 2000), establishing a framework for community action in the field of water policy.

Contamination of water resources, both surface and underground, by mine effluents is a serious environmental problem in a country where metallic ore mining was an important economic activity. This occurs because, in the deactivated mines, mining wastes are frequently left, exposed to atmospheric conditions and subject to oxidation and leaching processes, which contribute for the continuous production of mine drainages that usually join to nearby water resources. An old coal mine, in the north of Portugal (São Pedro da Cova, Gondomar, Portugal) is one of these examples. Forty years after the activity has ended, a mine drainage, rich in iron stills being produced and is continuously released in local streams (Ribeiro de Murta e Rio Ferreira). During the rainy seasons, these streams overflow their banks flooding surrounding lands, affecting the quality of both water and soil resources.

In the last few years, several phytoremediation techniques have been studied to address these mining effluents and different species have been suggested for this purpose. The aquatic plant species *Lemna minor* has been shown to be a good model for ecotoxicological studies, and many authors suggest that it also has the capacity to bioaccumulate metals such as copper, nickel, manganese, among others.

The aim of this work was test the potential of the species *L. minor* to remediate this mine effluent, through the bioaccumulation of Fe. The time required for attaining the maximum removal of Fe, by the plants was also evaluated.

The results have shown that *L. minor* was able to grow and develop in the Fe-rich effluent and to bioaccumulate this element. Throughout the 21 days of testing it was found that there was an increase in the biomass of *L. minor* both in the contaminated and in the non-contaminated water. However, in the case of the mine effluent the growth was stimulated. It was also found that bioaccumulation of iron occurred mainly during the first 7 days of testing. From the data collected throughout the study, we found that *L. minor* has potential for the bioremediation of effluents rich in iron.

## KEY WORDS

Contamination; mine effluent; phytoremediation; bioaccumulation; iron; *Lemna minor*.

# Contribution of soil microorganisms to the phytoremediation of metals

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Salt marshes have an important ecological role; however, they are often sinks of anthropogenic contaminants and their great sensitivity makes them very difficult to clean. Phytoremediation, the use of plants to recover polluted environments, is considered to be a potentially less damaging and cost effective method for the recovery of contaminated areas. Moreover, the potential of plants for phytoremediation of metals seems to be enhanced by the presence of several microorganisms in their rhizosphere. *Phragmites australis* plants have already shown potential for the remediation of cadmium in contaminated salt marshes (e.g [1]). The aim of this work was to assess if the potential of *P. australis* to phytoremediate cadmium can be enhanced by the addition of an autochthonous microbial (AM) consortia resistant to cadmium.

For that, plants were sampled in an estuary together with the sediment involving their roots, placed in vessels and maintained in greenhouses, exposed to natural environmental and light conditions. A nutritive saline solution was added to all vessels through an automated irrigation system to mimic the natural tides and maintain plants at optimum nutritional conditions. Plants were exposed to medium contaminated with two cadmium concentrations, in the presence and in the absence of an enriched AM consortia resistant to cadmium (prepared in the laboratory). After two months of experiment, plants' aboveground tissues were separated from belowground structures, which, in turn, were carefully separated from the sediment. Cadmium content was determined in plants' roots, rhizomes, stems and leafs and in sediments, according to Almeida *et al.* [2]. Moreover, total chlorophylls, carotenoids, lignin and soluble phenolic compounds were determined to assess if toxicity occurred.

The results suggest that *P. australis* plants were able to accumulate considerable amounts of cadmium when exposed to both concentrations of this metal, especially in their belowground tissues, without showing signs of toxicity. Moreover, *P. australis* stems showed increased cadmium concentrations for both cadmium treatments when AM were added, which clearly indicates that inoculated AM increased cadmium translocation. This increase was particularly evident in plants treated with the highest cadmium concentration. Therefore, the addition of AM resistant to cadmium to the rhizosphere seems to be a valuable strategy to potentiate the ability of *P. australis* to phytoremediate cadmium in estuarine systems.

## ***Acknowledgments***

This work was partially funded by Fundação para a Ciência e Tecnologia (FCT), Portugal, through the project PTDC/MAR/099140/2008..To Carla Silva and Carolina Carli for help in sample preparation.

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# Effect of atmospheric pollutants CO, O<sub>3</sub>, SO<sub>2</sub>, and NO<sub>2</sub> in the *Betula* pollen

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*Betula* spp. is a plant used for ornamental purposes in gardens and parks of the cities (Fig. 1). The increase of pollen allergies in urban areas has been attributed to atmospheric pollution.

In this research, *Betula* pollen was fumigated with four air pollutants: CO, O<sub>3</sub>, SO<sub>2</sub> and NO<sub>2</sub>. Two different levels of each pollutant were used: just below and two times the atmospheric hour-limit value acceptable for human health protection in Europe.

Pollen samples were *in vitro* exposed to gases in an environmental chamber, in which the temperature and relative humidity were controlled and sunlight was simulated [1]. Results showed a decrease in the viability and germination rate of exposed in contrast to non-exposed pollen, being higher decrease when exposed to NO<sub>2</sub> and O<sub>3</sub> than in CO and SO<sub>2</sub>.

The protein quantification of pollen fumigated and non-fumigated was made using the UV / Vis spectrophotometer. A general decreasing trend in the total protein content of the fumigated samples when compared with the control was observed. SO<sub>2</sub> and NO<sub>2</sub> caused the mostly well defined decreasing trends.

Soluble pollen proteins were separated by SDS-PAGE. The polypeptide profile of all the pollen samples showed five clusters of bands at about 70, 55, 35, 25 and 15 kDa. No different polypeptide profiles were revealed by SDS-PAGE between exposed and non-exposed pollen.

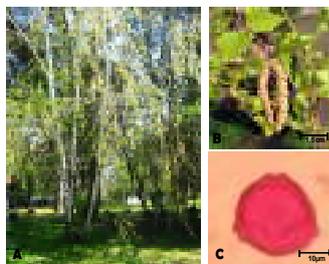


Fig 1. *Betula*: **A.** Plant, **B.** inflorescence and **C.** Pollen

This work was supported by FEDER funds through the COMPETE and National funds through FCT (Ref<sup>o</sup> PTDC/AAC-AMB/102796/2008).

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## **Studies on oxidative stress, fertility, proteins and allergenicity of *Fraxinus floribunda* pollen exposed to ozone**

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The increase in respiratory diseases arising from allergies in industrialized countries is considered to be associated to environmental pollution. Urban air contains several oxidizing gases such as ozone. It is generated by photochemical reactions in the presence of NO<sub>x</sub> emitted by vehicles and industries.

We intended to assess ozone effects on the viability, germination rate, protein content and allergenicity of *Fraxinus floribunda* exposed in comparison to non-exposed control pollen samples. The pollen was exposed to ozone at a concentration far above the standard limit value for the human health protection, during 6 hours in an adapted environmental chamber. The pollen fertility was evaluated by the viability test using Trypan Blue Dye and the germination rate in vitro. Biochemical and immunochemical assays were performed in order to analyze changes in the protein content, polypeptide profile by SDS-PAGE and IgE reactive profiles using sera from sensitized individuals. The results based on the comparison of exposed and non-exposed pollen revealed a decrease on fertility and no differences on protein content. The polypeptide fraction of pollen soluble proteins and the IgE recognition of sensitized patient sera did not show differences on the reactivity.

The oxidative stress was also evaluated through a series of assays as lipid peroxidation (malonyldialdehyde), free proline, hydrogen peroxide content and reactive oxygen species (ROS) fluorescent detection. By assessing the amount of free radical species produced and hydrogen peroxide content no significant differences were observed after ozone exposure. Regarding free proline levels there was a slight increase but no differences on lipid peroxidation and hydrogen peroxide content were observed after ozone exposure. It was also evaluated the amount of ROS and there was an almost 20% increase on exposed *F. floribunda* pollen.

Ozone is one of the most relevant air pollutants mainly due to its oxidative potential, both as an active agent and through ozonolysis-generated compounds that may interfere with pollen metabolism. These results constitute preliminary data that suggest an interaction between ozone and pollen proteins that somehow may interfere with normal pollen metabolism. This interaction may contribute to unbalance the prooxidant-antioxidant equilibrium. This study could be improved by gathering a greater amount of data to test the statistical significance of the observed events.

This work was supported by FEDER funds through the COMPETE and National funds through FCT (PTDC/AAC-AMB/102796/2008).

# Life Cycle Assessment of fish and seafood products: an overview

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The importance of the environmental impacts of the fish sector is revealed in the attention that is being paid by recent research works. Several studies are available concerning the impact assessment associated to the fishing sector. Most of these studies made use of the LCA tool to assess the life cycle impact of the seafood and fish products.

LCA tool considers the material and energy resources associated with the capture, processing and transformation, distribution and consumption of fish and seafood. This tool covers a wide range of activities associated with the fish and seafood products. Due to this complete consideration this tool highlights the critical points of fish and seafood products.

A review of 33 studies focusing on several issues related to the fishing sector is made. The selected publications focus on several categories, namely, on the impact of the fishing fleet; on the processing of fish and seafood products; on the environmental impact of meals and diets and finally on the carbon footprint of the fish sector.

Several methods are being used for the impact assessment, namely CML Baseline 2001, Eco-indicator 99, EDIP and for the assessment of the carbon footprint the IPCC 2007 was used. A number of fishing countries are being in focus, namely, Spain, Iceland, Sweden, Denmark, Norway, Canada and United Kingdom. These studies also focus on economic and mass allocation for several functional units.

The main achievements focus on the analysis of the several methods used, the functional unit, the impact categories identified and in the main life cycle stages that contribute to the overall impact. At the end a sum up on the published LCA studies from the fish sector is made. In addition, opportunities to perform further studies in respect to the fish and seafood national consumption are identified.

# The environmental impacts of dams and the interference in ecosystem services provided by riparian zones

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Water resource is essential to development, endowing economic, social, cultural and ecological values. In view of that their abundance or scarcity directly influences human activities, several techniques aiming water accumulation were developed, and the construction of dams is one global example applied in all over the world.

A dam can be built aiming the water storage for irrigation, industrial or domestic consumption, electrical energy production or flood regulation, for example. Despite the benefits the construction might result in negative impacts in the most different fields.

Among the negative effects, this article focuses in those related to riparian zones, considering the severe consequences for ecosystems relatively to the ability of these areas in provide environmental services.

A literature review regarding environmental impacts, riparian zones and ecosystems services was performed, allowing the description of services specifically provided by riparian forests, seeking to understand how the provision is affected by dams.

Despite the difficulty in enumerating most of the negative effects on riparian forests, studies report that in addition to social and economic impacts, there are ecological impacts [1] and could be mentioned: reduced levels of oxygen in water; high mortality of fish upstream and downstream; changes in habitats; accelerated growth of aquatic plants; and minimization of environmental services which the humanity depends directly and indirectly.

The main environmental services provided by riparian areas were identified in according to production services, regulating, supporting and cultural [2]: hydrological cycle optimization; climate regulation; mitigation of natural disasters; protection against erosion; soil formation; oxygen production; habitats maintenance; supply of genetic material; and opportunity for the formal and informal environmental education.

After identifying the environmental services provided by riparian zones it was concluded that dams construction causes loss on the environmental services provided by these areas. Therefore, recovery strategies, conservation and management [3] must be developed and applied in order to minimize the negative effects.

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Oral Sessions ▶ A4

# **Psychology & Education Sciences I**



# Social and Professional Inclusion of Young People with Disabilities: Promoting Strategies

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The present study aims to characterize the current general model of vocational training for young people with disabilities and, more specifically, to assess whether this encompasses inclusive educational practices and strategies to promote social and professional inclusion along with learners' quality of life.

Four trainees and three trainers participated in this study. Two of the later are responsible for three areas of training that make the theoretical component of the course while another trainer is responsible for the practical component.

To collect the data, in addition to filling out a questionnaire for collecting demographic data relating to trainers and trainees, naturalistic observations were made in the context of training room. Later, the four trainees from the cooking course were interviewed using the Script of Interview for Assessment of Quality of Life for Youth with Disabilities, as well as the 3 trainers using the Script of Semi Structured Interview for Trainers. Both were developed specifically for this study. Finally, we asked the trainers to undertake the functional assessment of trainees through Competency Assessment Checklist, built on the proposal of functional curriculum presented by Costa, Leitão, Pinto and Fino [1,2] .

It was found that regarding the quality of life level there are multiple dimensions that contribute to a quality life. The dimension most reported by trainees to characterize the quality of life is the Personal Development while trainers mention Wellbeing.

In its educational practice trainers integrate some of the features inherent to functional curriculum without, however, reflecting a total adoption of this curriculum model. Regarding educational strategies it was found that trainers only use Cooperative Learning and Cooperative Resolution of Problems. It is noted, however, that the practical component is closer to the functional curriculum perspective than the theoretical one. Regarding the profile of skills displayed by the students it was shown that professional skills emerge as a strong area with the academic and personal skills as deficit areas. The factors that promote effective social and professional inclusion of young people with disabilities relate to the existence of real learning opportunities in a real work environment through access to internships or job opportunities and the existence of quality social policies. The family is also identified as a structuring factor and as facilitator of social inclusion.

The results of this study provide a starting point for the development of relevant investigations and interventions aimed at enhancing the quality of life of young people with disabilities and, consequently, their social and professional inclusion.

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# General and Specific Achievement Goals in sport context: exploratory and longitudinal case study

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The present study was developed within the conceptual framework of Achievement Goal Theory, particularly adopting the 2x2 Elliot's (2003) Model which proposes four types of achievement goals. In sport contexts, within a mastery-approach goal, the athlete pursues the mastery of the game, the desire of learning and improving. Mastery-avoidance goals represent the individual striving to avoid losing capacity. When pursuing performance-approach goals the athlete seeks to achieve better performance than others. Finally, the performance-avoidance goal is characterized by opposition to the previous goal, the target being to avoid performing worse than the others. Generally, whereas mastery goals focus in intrapersonal criteria, performance goals focus in interpersonal evaluation criteria.

The pursuit of these different goals has been associated with different patterns of motivational cognitions, feelings and behaviors. Moreover, besides the more general individual goal orientations, the specific goals that the individuals adopt in concrete situations more directly influence their motivation and behavior in that situation. However, little is known about athlete's specific goals for particular games.

The present study analyzed the achievement goals of the athletes at a Portuguese 1<sup>st</sup> division volleyball team. More specifically it assessed the athletes' specific goals along 15 games as well as their relations with the athletes' game motivation, engagement and achievement.

The study included the assessment of the athlete's general goals orientations (trait-like goals) and of their specific goals (state-like goals, assessed at the beginning each game). The analysis of the relations between the athletes' goals and motivational variables was intended to test the theoretical associations with pre and post-game emotions, post-game attributions, perceived competence, and with engagement and performance perceived by the athlete and by the coach. Results showed that athletes mainly pursue mastery-approach game goals and put relatively lower emphasis in the pursuing of performance-goals. Overall, the results suggested that mastery goals seem to prepare the athletes to game and play a protective role after the game, while performance goals played a contrasting role.

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## **Recruitment at a click away: the candidates' perspective and profile**

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Growing globalization and the use of new technologies have led to changes in the job market. It is in this changing and new technologies growth environment that online recruitment arises, an internet based process, which uses tools that allow job applications' reception, consequent response and online candidacies triage [1].

In order to identify the candidates' profile, namely the ones that resort to this means of recruitment, which remains not very well studied, and their opinion on this process, an exploratory study was conducted. We applied an online questionnaire to a convenience and snowball sample and obtained a total of 74 answers, from subjects with an age average of 28 years old, 32% males and 68% females. Qualitative and quantitative data has been subjected to thematic content analysis and statistical analysis, correspondingly.

Results indicate that 46% of the total sample has already replied to online recruitment adds. When it comes to these participants profile, most of them are female respondents (59%), whereas 79% has higher education degrees. Among the candidates that have obtained responses from the organization's end, 43% have said to have taken them less than a week to receive this feedback. Qualitative analysis, of all respondents' answers, suggests that a positive opinion on this kind of recruitment is dominant (53%). Participants associate to online recruitment positive and negative characteristics, although differentiating more positive ones. The advantages that the respondents have emphasised the most are its swiftly characteristic, as well as its capacity to reach a wider public. As for the disadvantages, the disbelief (or doubttable truth) regarding online recruitment was the most evoked concept.

Organizations' should consider both the advantages and disadvantages associated to this type of recruitment, in order to overcome its disadvantages and optimize the process. Keeping in mind the use of new technologies and the recruitment process significance, the need for more investment in this field is emphasised.

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# Que(er)stioning the Margins: Educational Pathways and Lesbian and Gay Cultures

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The political and civic concerns about the homophobic bullying phenomena produce, in the name of a mere representation, new educational subjects (lesbian and gay young people) who are born essentialized under the homogenous sign of abjection and stigma being impossible to have a positive speech about that youthful segment (Rasmussen, et al 2004). Simultaneously, there is scenery of absence about lesbian and gay educational pathways and their sub/cultures. This study aims to fill the gap promoting the meeting between the school and the youthful practices (Araújo and Willis, 2008) and hear the youth through their own voices and in their own contexts, trying to intersect the margins and to figure out their nature.

Methodologically it is a study situated in a qualitative paradigm, theoretically justified by Sociology of Youth and Education, Cultural Studies, Feminist Pos-structuralism and *queer* theory. Until now we have visited some lesbian and gay points as associations, coffee shops and bars. It is not an ethnography study but we use some ethnographic techniques as participant observation and writing of field notes (Silva, 2010). We still have interviewed 3 young boys and 2 girls (16-24 years old) who identify themselves as lesbian, gay, bisexual or transgender about topics as school, family, work or leisure. We had ethical considerations about anonymity, confidentiality and informed consent.

Being a research under construction, we can assume until now: young people deny being victim of homophobic bullying in spite of admitting that homophobic insult is generalized within school space and it is more related to gender inversion. In their sub/cultures (spaces of security and also exclusion), curiously gay people reproduce the same mechanisms of gender normalization that they criticize in school. For some boys and girls their pathways is more violated and marked by the structure of social class than by their non-heterosexuality. At last, the expression of homosexuality is censured. Some teachers have great difficulty to talk about it in a positive way and when that topic appears, it comes under a bio-ontological approach ignoring that sexuality is a cultural and political dimension as it has been demonstrated by some authors (e.g., Rasmussen et al, 2004). Although, in general, they all agree that “things are getting better”.

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# **The field trip as an interdisciplinary experience in teaching Geography and History**

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Given the new profile of the initial training of History and Geography teachers in Portugal and the need to promote an integral and inclusive education with each student, in the context of a society increasingly specialized, it was considered relevant to fulfil interdisciplinary teaching-learning experience, involving the disciplines of History and Geography.

The theoretical work focuses on the problem of integration of knowledge and interdisciplinarity, regarding to an integral and inclusive education of the students. We believe therefore that is school function to seek to foster the integration of knowledge.

Developed in the context of initiation into the professional practice of the Master's Degree in History and Geography Teaching of the Faculty of Arts of the University of Porto, this case study fulfilled itself by carrying out a field trip, aiming the integration of Geography and History knowledge relative to seventh grade. Thereby, in the experience of integrated teaching, were involved two classes of seventh graders from the school E. B. 2,3 de Santiago – Custóias, where took place the study. It was therefore considered, as a fundamental strategy of operation of interdisciplinarity, the use of the educational potential of the landscape.

For the collection and processing of the data were used instruments and methods of distinctive areas, in order to analyze the development of the learning carried out by the students, as well as the analysis to the content of opinions expressed by the students regarding the field trip, intending to determine which moments stand out from the teaching-learning integrated experience. By analyzing the content of selected answers for the purpose, we tried to analyze more specifically the potential of direct observation of landscapes in teaching.

The analysis of all these data revealed differences in the performance between the two groups, in terms of what they had learned, as well as losses in the learning process, especially in the afternoon period. From the analysis of the content of students' opinions was confirmed their joining to the teaching-learning experience, due to several factors. It was also clear that the landscape presents numerous educational potential, regarding history and geography as a resource for teaching-learning, because it is motivating and raises the development of learning content.

The work reflected in this abstract is the result of the dissertation entitled "Geography, History and Landscape: an educational experience of knowledge integration within the field trip" and presented at the Faculty of Arts of the University of Porto in December 2011.

# Fatigue in Depression and Systemic Lupus Erythematosus: the role of psychological factors

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**Background:** Depressed patients frequently present fatigue as a disabling and severe symptom [1]. Conversely, in several diseases causing fatigue patients also report associated depression. Systemic Lupus Erythematosus (SLE) is an autoimmune disease where fatigue is a common, disabling and resistant symptom and is frequently accompanied by depressive symptoms [2]. Being fatigue present in the two entities, we hypothesized that disturbed sleep, anxiety, depression and immune dysfunction could represent possible mechanisms implicated in this prominent symptom.

**Material and Methods:** Self-reported fatigue was assessed in 29 female depressed patients (mean age 42 years), through Fatigue Severity Scale (FSS). They were screened for sleep quality and general health status, through the Pittsburgh Sleep Quality Index (PSQI) and the SF-36 Health Survey (SF-36v2), respectively. The presence of anxiety and depressive symptoms were also evaluated through the Hospital Anxiety and Depression Scale (HADS). Twenty four aged matched female SLE patients were submitted to the same evaluation procedure.

**Results:** Fatigue was higher in SLE patients ( $p=0,007$ ) and positively correlated with age and poorer quality of sleep ( $p=0,01$   $R=0,508$ ;  $p=0,001$   $R=0,620$ ). Higher fatigue was associated with higher anxiety and depression in all participants. Patients with depression and with SLE presented similar anxiety, depression, mental health and quality of sleep scores (Fig.1). Moreover they showed analogous limitations due to emotional problems and similar impairment in social functioning and vitality.



**Figure 1** –Average score for anxiety, depression, mental health and quality of sleep and significance level for t-test

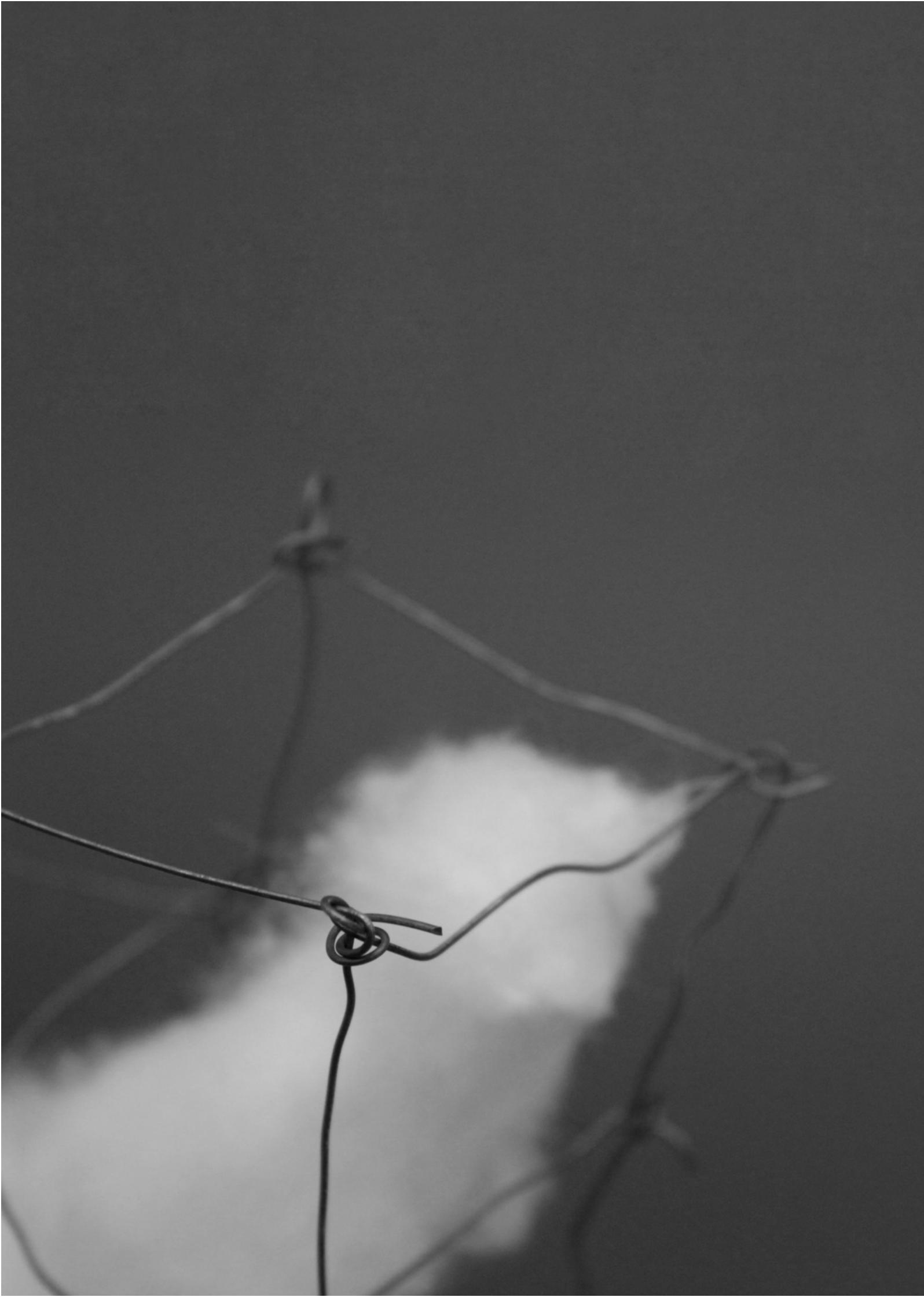
**Discussion and Conclusions:** The association of fatigue with anxiety and depression showed a similar pattern when occurring in psychiatric depressed or SLE patients.

Higher fatigue levels in SLE, associated with poor sleep quality, revealed a particular pattern of fatigue in these patients, confirming the physical burden of the disease. Present results highlight the role of psychological factors related to fatigue occurrence in both entities and the need to characterize common biological determinants.

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**ORAL SESSIONS**

**II**



Oral Sessions ▶ A1

# Biomedicine II



# **In vitro studies on the interaction of proinflammatory cytokines with the anticarcinogenic short-chain fatty acid butyrate at the intestinal epithelium**

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Intestinal inflammation is known to predispose and to play a crucial role in the development of colorectal cancer (CRC), one of the most common cancers worldwide. The short-chain fatty acid butyrate (BT) plays a key role in colonic epithelium homeostasis, being able to prevent/inhibit colon carcinogenesis. BT is transported into colonic epithelial cells by two specific carrier-mediated transport systems, the monocarboxylate transporter 1 (MCT1) and the Na<sup>+</sup>-coupled monocarboxylate transporter 1 (SMCT1), both being proposed to function as tumour suppressors. Recently, the proinflammatory cytokines tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interferon- $\gamma$  (IFN- $\gamma$ ) were reported to decrease MCT1 and SMCT1 mRNA and protein levels at the intestinal epithelium. So, our aim was to characterize the effect of the proinflammatory cytokines TNF- $\alpha$  and IFN- $\gamma$  upon (a) the uptake of BT in tumoral (Caco-2) and non-tumoral (IEC-6) intestinal epithelial cell lines, and upon (b) the effects of BT on cell proliferation and viability.

The uptake of <sup>14</sup>C-BT was measured by liquid scintillometry, and the effects of the proinflammatory cytokines and BT on cell viability and proliferation were accessed with lactate dehydrogenase and sulforhodamine B assays, respectively.

A 24h exposure to TNF- $\alpha$  and IFN- $\gamma$  (100 ng/mL) resulted in a 15-25% inhibition of <sup>14</sup>C-BT uptake in IEC-6 cells, but this inhibition was associated with a marked decrease in cell viability and proliferation. In contrast, a 24h exposure of Caco-2 cells to TNF- $\alpha$  and IFN- $\gamma$  (200 ng/mL) originated in a ~10% inhibition of <sup>14</sup>C-BT uptake, without compromising cellular viability or proliferation.

BT (10 mM; 24h) caused a significant reduction in Caco-2 cell proliferation and viability. Simultaneous treatment with IFN- $\gamma$  (200 ng/mL; 24h) did not modify the effects of BT upon cell proliferation and viability. In contrast, simultaneous treatment with acetylsalicylic acid (0.5mM), a known anti-inflammatory molecule, potentiated the effect of BT upon Caco-2 cell proliferation.

In conclusion, the proinflammatory cytokines TNF- $\alpha$  and IFN- $\gamma$  were able to cause inhibition of BT uptake in Caco-2 cells. Given the role played by BT in the intestine, this mechanism may contribute to the tumour-promoting effect of proinflammatory cytokines at this level.

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# Analysis of the induction of the cytoprotective Nrf2 signalling pathway in reticuloendothelial cells from iron-treated mice and HFE hemochromatosis patients

**F. de Sousa<sup>1</sup>, C. Caldas<sup>1</sup>, J. V. Neves<sup>1</sup>, S. S. Gomes<sup>1</sup>, P. N. Rodrigues<sup>1</sup>, G. Porto<sup>2,3,4</sup>, T. L. Duarte<sup>1</sup>**

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**Background:** HFE-associated hereditary hemochromatosis (HH) is a common genetic disorder that leads to total body iron overload with secondary tissue damage, attributed to oxidative stress. Most HH patients are homozygous for the C282Y mutation in the HFE gene. A major challenge in the HH field is to understand why some patients are at increased risk for developing severe iron overload-associated clinical symptoms while most C282Y homozygotes are apparently healthy<sup>[1]</sup>. We hypothesize that resistance to oxidative stress is a modifier of disease progression in iron overload-related pathologies. Transcription factor Nrf2 is an important modifier of chronic diseases involving oxidative stress, including inflammatory and neurodegenerative diseases, and cancer<sup>[2]</sup>. In theory, Nrf2 could coordinate the transcriptional induction of cytoprotective/antioxidant genes in response to iron overload. **Aims:** The aim of the work was to investigate if Nrf2 is activated by iron overload in reticuloendothelial cells, which play an active role in iron metabolism and clearance. Firstly, we investigated Nrf2 activation in response to iron delivery to spleen/liver macrophages via injection of Fe-dextran in mice. Subsequently, we analyzed the expression of relevant genes in blood monocytes (CD14+) from HFE-HH patients. Whilst the liver is often the most affected organ in these patients, access to liver biopsies would pose ethical issues, so blood monocytes could represent a surrogate tissue. **Results:** Injection of Fe-dextran in mice caused a significant increase in serum iron and a concomitant deposition of iron in splenic macrophages and liver Kupffer cells. Liver and spleen RNA was collected 1d post injection. The expression of 2 cytoprotective genes and of the iron exporter FPN1 was significantly elevated in the liver and spleen of mice injected with Fe-dextran when comparing with animals injected with dextran (control). Notably, no differences were observed in *Nrf2*<sup>-/-</sup> mice, demonstrating that the activation of these genes was Nrf2-dependent. CD14+ cells were isolated from the blood of a group of 7 C282Y HFE homozygous subjects with elevated iron indices followed at Centro Hospitalar do Porto, and 7 apparently healthy controls with normal iron indices and absence of C282Y HFE mutation. We found no significant changes in the steady-state mRNA expression levels of Nrf2 or a panel of Nrf2-regulated cytoprotective genes. **Conclusions:** Nrf2 is activated by iron loading of tissue macrophages in mice treated with Fe-dextran but patient CD14+ cells do not respond to increased serum iron in the same way. **Acknowledgements:** Reitoria da Universidade do Porto (PP-IJUP2011-122), FCT and COMPETE/QREN/UE- project PTDC/SAU-FCF/101177/2008 for financial support.

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# Assessment of cardiovascular risk in hypertensive and normotensive patients: analysis of the correlation among non-invasive clinical indicators

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Systemic arterial hypertension is associated with the high occurrence of vascular diseases and peripheral arterial diseases are among their clinical conditions. Several non-invasive procedures allow assessing the effectiveness of vascular functioning, such as measuring the ankle-brachial index (ABI), determining the pulse pressure (PP), delta brachial-brachial (delta-BB) and delta-ABI. This study aimed to identify the association among ABI, PP, delta-ABI, delta-BB and systemic arterial hypertension, by using automated oscillometric sphygmomanometer.

It is a descriptive and cross-sectioned study, with a quantitative approach, carried out in a Basic Health Unit in a city of São Paulo State. The studied variables were age, sex, blood pressure (BP), abdominal circumference (AC), body mass index (BMI), ABI, PP, delta-BB and delta-ABI. The ABI was determined according to the technique described by Kawamura [1], adopting the reference values established by the Brazilian Guidelines on Hypertension [2]. The measuring and the reference values used for delta-BB and delta-ABI were established according to a study carried out by Kawamura [1]. Regarding PP, which was obtained by the difference between SBP and diastolic BP (DBP) measured at the arm, the normal values used were  $\geq 50$  mmHg for ambulatory measures [3].

The number of participants in this study was 123 (47.7% normotensive and 52.3% hypertensive). The mean age obtained was  $39.74 \pm 1.84$  years old for the normotensive and  $55.62 \pm 1.66$  years old for the hypertensive. When compared to the normotensive group, the hypertensive showed statistically significant alterations on the investigated clinical variables: BMI, SBP, AC, delta-ABI and delta-BB. Although ABI evaluation did not find out significant difference on the average values between the groups, alterations were identified related to slight and moderate obstruction, only among hypertensive (7.2%). When compared to the normotensive, the hypertensive individuals showed higher rates of PP ( $p < 0.0001$ ). It was observed that there is no correlation between the ABI and PP values of normotensive and statistically significant correlation between the values of ABI and PP of hypertensive (Pearson Coefficient = - 0.45,  $p < 0.0001$ ,  $r^2 = 0.21$ ).

By using the cardiovascular risk predictor devices proposed in our study, it was observed that the hypertensive showed higher frequency of changes when compared to the normotensive.

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## Does lack of adrenaline influence $\beta_2$ -adrenoceptor-mediated vasodilation?

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It was suggested that there is a link between adrenaline and the maturation of  $\beta_2$ -adrenoceptor-mediated effects (Guimarães et al 2001). On the other hand, phenylethanolamine-*N*-methyltransferase (PNMT, enzyme that transforms noradrenaline in adrenaline)-knockout mice have higher blood pressure during treadmill exercise than controls (Bao et al 2007), which may be related to the lack of  $\beta_2$ -adrenoceptor-mediated-vasodilation caused by absence of adrenaline.

The aim of this study was to characterize the role of adrenaline on the maturation of postjunctional (smooth muscle relaxation)  $\beta$ -adrenoceptor-mediated effects in PNMT-knockout mice.

Aortas of anesthetized (isoflurane) PNMT-knockout mice (*Mus musculus*; male; 8 to 12 weeks-old) were dissected, collected and cut into rings, which were mounted in a myograph. We evaluated dose-response curves to terbutaline ( $\beta_2$ -adrenoceptor agonist) in the absence or presence of ICI118551 ( $\beta_2$ -adrenoceptor antagonist), and to dobutamine ( $\beta_1$ -adrenoceptor agonist), in rings pre-contracted with phenylephrine (n=5-6 per group). Adrenal glands were collected and immersed in perchloric acid (0.2 M). Adrenaline and noradrenaline were separated by reverse-phase HPLC and quantified by electrochemical detection. Statistical analysis was performed by Student's *t*-test or F-test and  $p < 0.05$  was assumed to denote a significant difference.

We confirmed that adrenaline is absent in the adrenal glands of PNMT-knockout mice. The potency and maximal effect of terbutaline were higher in controls than in PNMT-knockout mice ( $EC_{50} = 5.2 \pm 2.8$  vs  $29.0 \pm 6.1$   $\mu$ M, respectively, and  $E_{max} = 17.7 \pm 3.3$  vs  $7.0 \pm 2.4$  mN/mg, respectively). ICI11855 antagonized the effect of terbutaline in controls but not in PNMT-knockout mice. The potency and the maximal effect of dobutamine were identical among groups ( $EC_{50} = 0.22 \pm 0.07$  vs  $0.18 \pm 0.05$   $\mu$ M, respectively, and  $E_{max} = 20.0 \pm 4.2$  vs  $13.8 \pm 3.0$  mN/mg, respectively).

In conclusion, when adrenaline is absent there is a lack of aortic  $\beta_2$ -adrenoceptor-mediated vasodilation (but not of postjunctional  $\beta_1$ -adrenoceptor-mediated effects) which confirms the link between adrenaline and the maturation of  $\beta_2$ -adrenoceptor-mediated effects (Guimarães et al 2001). The absence of vasodilation mediated by  $\beta_2$ -adrenoceptors may contribute to the observed higher blood pressure during treadmill exercise in PNMT-knockout mouse (Bao et al 2007).

Acknowledgments: This work was supported by Fundação Professor Ernesto Morais (2012, Porto, Portugal) and University of Porto (IJUP2011-219).

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## Role of PKG, Natriuretic Peptides and Nitric Oxide in the Diastolic Response to Acute Myocardial Stretch

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**Introduction:** Acute myocardial stretch induces an adaptive response both at systolic and diastolic levels. The mechanisms responsible for diastolic adaptation remain largely unknown. Therefore, we aimed to evaluate the role of protein kinase G (PKG) and associated signaling pathways in the diastolic adaptive response to acute myocardial stretch under basal and ischemic conditions.

**Methods:** Rabbit papillary muscles (0.2Hz, 30°C) were acutely stretched from 92% to 100% of Lmax in a modified Krebs-Ringer solution (A) under basal conditions and in the presence of (B) Rp-8-Br-PET-cGMPS (an inhibitor of PKG, 10<sup>-6</sup>M, n=7), (C) L-nitro-arginine (an inhibitor of nitric oxide synthase (NOS), 10<sup>-5</sup>, n=8), (D) Hydroxocobalamin (a nitric oxide scavenger, 10<sup>-3</sup>M, n=8), (E) A-71915 (a natriuretic peptide receptor-A (NRPA) antagonist, 10<sup>-6</sup>M, n=9) and (F) A-71915, L-nitro-arginine and Hydroxocobalamin simultaneously (n=10). Group G was stretched during ischemia and other protocols were performed in the ischemic setting in the presence of (H) 8-Bromo-cGMP (an agonist of PKG, 10<sup>-5</sup>M, n=7) and (I) Natriuretic Peptide B (BNP, 10<sup>-6</sup>, n=7). Immediate and delayed responses to muscle stretch were evaluated. Results are presented as mean±standard error of mean (P<0.05).

**Results:** Under basal conditions (group A), after immediate increase in myocardial passive tension (PT) induced by acute myocardial stretch there was a significant and time-dependent decrease in PT of 46.2±1.8% in the 15 minutes following stretch. The presence of an inhibitor of PKG (group B) attenuated the decrease in PT to 26,3±1,1%. Inhibition of NOS (group C), NPR-A receptor (group E) or the use of a NO Scavenger (group D) did not alter the diastolic response to stretch, whereas the simultaneous blockade of NO and Natriuretic Peptides systems (group F) significantly attenuated the PT decrease to 35,9±4,6%. In the ischemic group (group G), diastolic response to acute stretch was completely abolished throughout the 15 minutes of ischemia (increase in PT of 3,5±8,1%). The presence of an agonist of PKG (group H) promoted a decrease in PT of 20,6±3,2% after stretch during ischemia. The presence of BNP (group I) also improved the diastolic adaptation to acute stretch (decrease in PT of 5,6±6,7%).

**Conclusions:** PKG activity is essential to the normal diastolic adaptive response to stretch, and its hypoactivity may be associated with the diastolic dysfunctional adaptation during ischemia.

# Development and validation of a gas chromatography/mass spectrometry method for simultaneous quantification of benzylpiperazine and its metabolites: application to a pilot toxicokinetic study in mice

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N-benzylpiperazine (BZP) is a psychoactive piperazine derivative that has become popular as the major ingredient of “party pills” due to its stimulant and euphoric effects. While legal, these pills were sold as a safe alternative to amphetamines like methylenedioxymethamphetamine (MDMA, *ecstasy*). Results from in vivo and in vitro toxicity studies led to control measures all over the world. Nevertheless, there are only a few published studies on its toxicokinetics, namely on the disposition of BZP and its metabolites in the body. Furthermore, the available toxicokinetic studies show discrepancies between humans and animals (Sprague-Dawley rats), namely in the main free metabolites and type of conjugates. The major metabolites found in plasma, kidneys and liver, in rats are: 4-hydroxy-BZP (4-OH-BZP), 3-hydroxy-BZP (3-OH-BZP), 4-OH-3-OCH<sub>3</sub>-BZP, benzylamine, piperazine, and N-benzylethylenediamine.

In order to assess the toxicokinetics of BZP in mice, a gas chromatography-mass spectrometry (GC-MS) method for simultaneous quantification of BZP and its metabolites was developed and validated.

The method demonstrated selectivity and ability to resolve all the compounds (BZP, 4-OH-BZP, 3-OH-BZP, 4-OH-3-OCH<sub>3</sub>-BZP, benzylamine, piperazine and N-benzylethylenediamine). Furthermore, validation assays showed that the method was linear between 0-5,000 ng/mL ( $R^2 > 0.99$ , except for benzylamine) with limits of detection (LOD) and quantification (LOQ) range between 2-5 ng/mL and 7.5-20 ng/mL, respectively. The precision, inter- and intra-day (CV% between 3.43 and 14.68%), accuracy ( $100 \pm 15\%$ ), and extraction efficiency ( $100 \pm 20\%$ ) were considered acceptable. The applicability of this method was shown in mice plasma samples obtained from a pilot toxicokinetic study after a single BZP dose of 50 mg/kg (i.p.).

Oral Sessions ▶ A2

# Agro Food I



# Detection of walnut allergens in raw and baked products by molecular based approaches

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Walnut (*Juglans regia*) belongs to the tree nut group, which since 1993 has been included in one of the eight groups responsible for the majority of the food-induced allergies [1]. Presently, the prevalence of walnut allergy is estimated to an overall incidence of 2.2%, according to a recent study involving several centers from eleven European countries, USA and Australia [2]. For the allergic patients, food labels must be as informative as possible to allow a correct and safe choice of the foods they purchase. To verify the compliance with food labeling, and most important to provide techniques to food industry for allergen management, the development of adequate methodologies for allergen detection is crucial.

The aim of this work was the development of molecular approaches, namely qualitative polymerase chain reaction (PCR) and real-time PCR for the detection and quantification of walnut allergens in cakes. This work intended also to evaluate the effect of heat processing of foods on the proposed PCR systems. For this purpose and due to the lack of reference/testing materials, model mixtures of dough and baked cakes spiked with walnut (50% to 0.0001%) were prepared. For walnut detection, specific primers and hydrolysis probe were retrieved from the literature for qualitative and real-time PCR amplification [3].

Results of qualitative PCR allowed the relative limit of detection of 10 mg/kg of walnut in dough and 50 mg/kg of walnut in baked cake. The proposed real-time PCR system based on the use of a hydrolysis probe enabled to confirm the previous results of qualitative PCR, with the advantage of obtaining quantitative data of walnut in raw and baked materials. These results highlight the adequacy of the developed PCR approaches for the detection of walnut as a hidden allergen in processed foods.

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# Development of a highly sensitive real-time PCR system for the quantification of soybean as a potential allergenic ingredient

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Soybean is a food ingredient with both techno- and biofunctionality properties, whose use has been increasing considerably in the past decades [1]. Besides its numerous applications, soybean is widely used by the food industry in processed foodstuffs such as sausages, hamburgers or hams. However, since soybean is considered one of the most common foods known to cause allergic reactions in sensitized individuals, the European Union established legislation aiming to protect these patients. According to the Directive 2007/68/EC, soybean plus 13 other groups of foods must always be labeled independently of its amount. For labeling compliance monitoring, the development of adequate methodology for soybean detection is of utmost importance.

In this work, we propose developing a molecular approach based on real-time polymerase chain reaction (PCR) system with adequate sensitivity for the quantitative analysis of soy as a potential allergen in meat products. For this purpose, different model samples of pork meat spiked with known amounts of isolated or concentrated soy protein, ranging from 10% to 0.001%, with and without heat treatment were prepared. The reference mixtures were used to develop a calibration model based on real-time PCR using primers and hydrolysis probes specifically designed to target eukaryotic reference (universal) and lectin (specific for soybean) genes.

The proposed system presented high specificity and sensitivity allowing a relative quantification of 50 mg/kg of isolated or concentrated soybean in pork meat. The performance of the technique demonstrated its appropriateness for quantification by the adequacy of linearity and ( $R^2 > 0.98$ ) and PCR efficiency (~100%) parameters for real-time PCR, similar for both types of protein material in binary mixtures. Heat processing did not affect the performance of the method that allowed reaching the same relative sensitivity. It also enabled amplifying soybean until 2.44 pg (2.2 DNA copies). The normalized technique for the quantification of soybean was successfully validated by its application to blind reference mixtures, indicating a high proximity between the actual and the estimated values. In summary, the proposed normalized system presented adequate sensitivity for the quantification of soybean as a potential hidden allergen in foods.

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# Comparison of DNA extraction methods for the determination of botanical origin of Portuguese honey

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Honey can be classified based on the type of flowers used by the bees to collect pollen as unifloral honey, if arising predominantly from a single botanical origin, which generally represents more than 45% of the total pollen content, or as multifloral honey, if it is produced from various plant species. A single plant origin should assure a product of better quality because it guarantees a specific and well-defined flavor and aroma. For this reason, monofloral honeys generally attain higher commercial value, especially those labeled as PDO (Protected Designation of Origin). Thus, the botanical authentication of honey is of high importance. To date, microscopic analysis of pollen (melissopalynology) is the technique used to assess the origin of honey. However, this type of analysis is time consuming and dependent on the experience and skill of trained analysts. In contrast, DNA-based methods are less dependent on the analyst, highly specific and easily applied to laboratories with the suitable equipment [1].

The aim of this work is to extract pollen DNA from honey samples for further analysis to exploit molecular markers for honey authentication. Considering the complexity of honey matrix, different extraction methods were tested and optimized, namely the commercial kit NucleoSpin Plant (Macherey-Nagel), the classical CTAB-based and the Wizard methods, as described by Mafra *et al.* [2] with modifications. Prior to DNA extraction, three different pretreatments were applied to three unifloral honey samples (*Ericaceae*, *Rosmarinus officinalis* and *Eucalyptus* spp.) and one multifloral honey. The protocols were evaluated by UV spectrophotometry to determine of yield and purity of DNA extracts. The amplifiability was tested by polymerase chain reaction (PCR) targeting *matK* and *rbcL* genes, as candidate loci for barcoding. The obtained honey extracts revealed low DNA yields with all the extraction protocols, but adequate purity for PCR was achieved using the Wizard method. The amplification of both *loci* was successfully attained with the Wizard method when applied to all honey samples, while the other two protocols exhibited lower reproducibility of results. The proposed method proved to be faster, simpler to implement and more robust than classical protocols, and therefore suitable to develop new methodologies based on the use of DNA markers for analyzing the botanical origin of honey.

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# Evolution of antioxidant capacity and nitrate contents of ready-to-eat green leafy vegetables during shelf life

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Ready-to-eat green leafy vegetables used in salads are a growing market that attracts consumers looking for convenient and healthy products. Baby leaf salad is prepared with leaves harvested at a very early stage of maturation and in an active metabolic stage. The leaves suffer minimal processing to ensure freshness and safety of the product, without losing nutritional quality. During shelf life, antioxidant compounds can undergo changes due to the response of the leaves metabolism to storage time and temperature, and the processing methods.

Nitrate plays an important role in the nutrition of plants and is a natural constituent of plant material. Nitrate content depends on a large number of factors, such as the biological properties of the plant, light intensity, temperature, plant maturity, harvesting time, nitrogen source and storage time. Vegetables are one of the major sources of dietary exposure to nitrate. Therefore, the assessment of nitrate contents in fresh vegetables has received a lot of attention. Recently, maximum levels for nitrates in lettuce, spinach and wild rocket have been revised in the European Union (EU) [1].

The purpose of this work was to study the antioxidant capacity (DPPH radical scavenging ability, and the ferric reducing antioxidant power, FRAP) and the nitrate contents (ISO 6635:1984) of 4 baby-leaf green vegetables during their shelf life. Green and ruby red lettuce, wild rocket and spinach fresh-cut baby leaves were packed and stored at refrigeration temperatures ( $3\pm 1^\circ\text{C}$ ). Samples taken after being packed and at the end of the shelf life were freeze dried and stored under a dry and cold environment until analysis.

The samples antioxidant capacity has changed differently, along the shelf life period. The FRAP and the DPPH radical scavenging ability value decreased in the green lettuce and ruby red lettuce leaves, respectively. Wild rocket leaves did not show significant changes in what concerns to antioxidant capacity but presented the highest nitrate levels. Nitrate values respected the EU maximum levels.

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# Ready-to-eat green leafy vegetables: development of an extraction method to evaluate the antioxidant power

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The antioxidant capacity of vegetables is derived from the cumulative synergistic action of a wide variety of phytochemicals such as vitamins, polyphenols, carotenoids, terpenoids, Maillard compounds and trace minerals. Green leafy vegetables are recognized for their high content in antioxidant compounds, especially phenolic compounds, which are ubiquitous and have an active role in the prevention of certain degenerative diseases [1]. Ready-to-eat green leafy vegetables are a convenient and healthy product. They are lightly processed to ensure the product freshness, without losing its nutritional quality. Fresh vegetables continue to breathe after harvesting and have the capacity to respond to minimal processing, increasing the metabolism, and leading to changes in their phytochemical composition [2]. Particularly, polyphenols undergo certain reactions that may cause a decrease in the antioxidant capacity of the foodstuff. These changes could be affected in a different extent by several factors, like storage time, temperature and processing methodology [1]. The objective of this work was to establish the extraction best conditions to obtain the antioxidant compounds and determine the antioxidant capacity of different green leafy vegetables.

Two extraction techniques (ultrasound and heated stirring plate) and two extracting solvents (ammonium acetate 10 mM: MeOH (50:50) and an 80% MeOH solution) were tested. Four different ready-to-eat leafy samples (green and ruby red lettuce, wild rocket and spinach) were submitted to the four conditions of extraction. The samples were freeze dried and reduced to powder in a knife mill before the extraction. The phytochemicals evaluated in the 4 extracts per sample were phenolic (Folin-Ciocalteu method) and flavonoid compounds, and also through the evaluation of radical scavenging ability using the radical DPPH• (2,2 diphenyl-1-picrylhydrazyl) and the ferric reducing antioxidant power (FRAP).

The antioxidant capacity of the extracts obtained with ultrasound extraction and heated stirring plate were similar. The 80% MeOH extracting solvent showed slightly higher antioxidant capacity. Therefore ultrasound extraction using 80% MeOH was applied in on-going analyses to study the evolution of the antioxidant capacity during the shelf-life of the referred green leafy vegetables.

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Oral Sessions ▶ A3

# Engineering I



## Degradation products of paraquat by Fenton's reagent oxidation

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Paraquat (PQ) is a quaternary ammonium compound whose use was forbidden in Europe due to its high risk for human health. However, it is still largely used as pesticide in many countries, leading to the paraquat accumulation in the environment. It thus becomes urgent to find ways that allow an efficient removal of paraquat from contaminated waters. It is also important to assure that degradation products formed are less dangerous than the parent compound itself.

Advanced oxidation processes (AOP's) are, among other technologies, a possibility to be used to remove paraquat from waters. In this work, Fenton's reagent oxidation (a well known AOP) was used, and the experimental conditions were:  $[\text{Fe}^{2+}]_0 = 5,0 \times 10^{-4} \text{ M}$ ;  $[\text{H}_2\text{O}_2]_0 = 1,6 \times 10^{-2} \text{ M}$ ;  $T_0 = 30 \text{ }^\circ\text{C}$ ;  $\text{pH}_0 = 3$ . Such conditions were established upon a preliminary work in a batch reactor [1]; The initial concentration of paraquat was  $100 \text{ mg.L}^{-1}$  and the degradation achieved was around 100%.

Paraquat concentration was quantified by HPLC-DAD and the products of degradation were identified and some of them quantified by LC-MS and HPLC-DAD. The experimental methodology involved the selection of possible degradation products based on bibliographic research, the implementation of an analytical method for their detection by LC-MS and HPLC-DAD, and the identification of these substances in samples taken from Fenton's oxidation of paraquat.

As mentioned above, paraquat was completely removed from the initial solution, but its oxidation was not complete (mineralization, i.e. oxidation up to  $\text{CO}_2$ , was only 60% after 4 hours of reaction). Three compounds were identified as degradation products of paraquat: oxalic acid, isonicotinic acid and 4-carboxy-1-methylpyridinium ion. However, these compounds are responsible for 27% of the 40% of organic carbon remaining in solution after 4 hours of reaction. Five other compounds were detected by LC-MS analysis, whose molecular ions were  $m/z$  201,  $m/z$  265,  $m/z$  267,  $m/z$  283, and  $m/z$  291, which are probably responsible for the remaining 13% of not identified organic carbon. Further work is required to identify and quantify these compounds.

The toxicity values of paraquat, expressed as  $\text{LD}_{50}$ , was compared to that for the three compounds identified as degradation products and it was concluded that they are less toxic than the parent compound.

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# Use of microalgae *Scenedesmus obliquus* for brewery wastewater treatment and biodiesel production

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The microalgae cultivation for obtaining valuable products from their biomass is done since antiquity [1], particularly for food and nutraceutical purposes [2]. Also, under certain conditions, microalgae accumulate lipids that can be used for biodiesel production. They have higher lipid productivity than the terrestrial crops conventionally used for biodiesel production (ex. rapeseed, soybean, sunflower and palm).

Microalgae nutrients requirements for a convenient development and growth include essentially carbon, nitrogen and phosphorus. So in this study a brewery wastewater is used as their source. This procedure has advantages in many aspects as it can reduce the eutrophication of the aquatic environment and can contribute to the lowering of wastewater treatment costs when compared to the traditional methods. Hence, this study aimed to evaluate a brewery wastewater treatment by microalgae and their lipids productivity having in mind its use as feedstock for biodiesel production.

In a former study [3] it was concluded that *Scenedesmus obliquus* can grow in such wastewater, under mixotrophic conditions, with controlled light intensity of 12,000 Lux and a 12 h photoperiod of daily light. The algal biomass growth was monitored, yielding a maximum concentration of 0.9 g dry biomass /L after 9 days and it allowed the effluent treatment, reducing the COD, total nitrogen and total carbon contents by 43%, 14% and 52%, respectively. The lipids extraction was made using a modified Bligh and Dyer's method [4] yielding 26.5% lipids (% dry weight). Biodiesel was produced from the extracted lipids using the Abou-Shanab's method [5] and the obtained biodiesel was analyzed by gas chromatography for determining its esters profile.

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# Tailoring the surface chemistry of multi-walled and single-walled carbon nanotubes by using different oxidizing agents

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Carbon nanotubes (CNTs) are attractive nanostructured carbon materials due to their exceptional structural and electronic properties. CNTs can be divided into single-walled (SWCNT) and multi-walled nanotubes (MWCNT). Oxidation treatments are used to modify the surface chemistry of CNTs, creating different oxygenated surface groups, usually by means of boiling methods with HNO<sub>3</sub> and/or H<sub>2</sub>SO<sub>4</sub>. In our group, a specific HNO<sub>3</sub>-hydrothermal oxidation methodology was recently developed to control the surface chemistry of carbon xerogels and SWCNTs [1, 2]. In the present study, the application of this methodology was extended to the use of H<sub>2</sub>SO<sub>4</sub> (besides HNO<sub>3</sub>) as oxidizing agent for the chemical functionalization of MWCNTs and SWCNTs with different lengths. Different acid concentrations (0.05, 0.10, 0.20 and 0.30 M) were used, and the amounts and types of the oxygenated groups created were determined. The materials were characterized by several techniques, including temperature programmed desorption (TPD) [3], N<sub>2</sub> adsorption at 77 K and pH of point of zero charge (pH<sub>PZC</sub>).

The results of this work indicate that the amounts of oxygenated groups created on the surface of the CNTs can be tuned by selecting the concentration of the oxidizing agent. More acidic CNTs were obtained when H<sub>2</sub>SO<sub>4</sub> was used as oxidizing agent (mainly due to the sulfonic groups created). TPD results indicated that the total amount of oxygenated groups incorporated in CNTs was higher when HNO<sub>3</sub> was employed. The amount the oxygenated groups that are created depends on the kind of CNTs used. Thus, for the same oxidation treatment, the oxygen surface content increased as follows: long-SWCNTs < short-SWCNTs < MWCNTs, which could be related with the different textural and morphologic properties of the CNTs.

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# Biodiesel Production: Improvement in Product Yield and Quality

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The overproduction of greenhouse gases, disrupting the natural balance that was guaranteed by the use of biomass, is now a constant concern. In order to counteract this problem, replacement of fossil fuels with biofuels has been sought [1].

Biodiesel is a biofuel that can be used on compression-ignition engines, being generally produced from vegetable oils or animal fats, presenting many environmental advantages over petrodiesel [2, 3].

In the present work, biodiesel production yield and quality was evaluated by using a two-step transesterification, with glicerol removal and refined sunflower oil as raw-material.

The reaction conditions were 65 °C, 6:1 molar ratio of methanol to oil and 0.8 wt.% NaOH as catalyst. The methanolic solution (containing the alcohol and catalyst) was prepared and added in different proportions (20 to 80 Vol.%) at the beginning of the reaction and after the first step (30 min). The total reaction time was 1 h.

Under the best conditions, biodiesel was produced with waste frying oil and results were compared.

The results showed that the best yield (93.0 wt.%) corresponded to the process which included the addition of 40% of the methanolic solution in the first step of the reaction. Biodiesel production from waste frying oil yielded 89.5 wt.% biodiesel under the same conditions.

Results concerning the viscosity ( $4.62 \text{ mm}^2 \text{ s}^{-1}$ ) and estimation of the purity of the fuel (97.7 wt.%) indicated that the properties of the biodiesel produced agree with the standard EN 14214 on biodiesel quality for use in motor fuels.

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## Selective oxidation of glycerol over CNT supported catalysts

**L.S. Ribeiro<sup>1</sup>, M. Oliveira<sup>1</sup>, E.G. Rodrigues<sup>1</sup>, M. Lipinska<sup>2</sup>, S.L.H. Rebelo<sup>2</sup>, C. Freire<sup>2</sup>, M.F.R. Pereira<sup>1</sup> and J.J.M. Órfão<sup>1</sup>**

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During the last years, the market of biodiesel has continuously increased. Therefore, the conversion of glycerol, a by-product of biodiesel production process, into high-value chemicals is a research area that has received tremendous attention [1]. Liquid phase catalytic oxidation is a promising route to convert glycerol into useful compounds, provided that the catalyst used is sufficiently active and selective for the formation of chemicals such as glyceric acid and/or dihydroxyacetone, potentially useful as chemical intermediates in the fine chemicals industry, especially in pharmaceuticals.

In this work, glycerol catalytic oxidation was studied using metal catalysts supported on carbon nanotubes (Nanocyl-3100), prepared by the incipient wetness impregnation method. Pd, Pt, Rh and Pt-Cu catalysts were prepared and tested in a 350 mL stainless steel reactor equipped with a manometer, a temperature sensor and a sample outlet. In standard tests, 150 mL of a glycerol aqueous solution (0.3 M), NaOH solution (NaOH/glycerol molar ratio = 2) and 700 mg of catalyst were introduced into the reactor under stirring at 1000 rpm. After heating under N<sub>2</sub> to the desired temperature (60 °C), the reaction was initiated by switching from inert gas to O<sub>2</sub> (3 bar). Samples (0.6 mL) were periodically removed for analysis by HPLC. The catalysts were characterized by N<sub>2</sub> adsorption at -196 °C, temperature programmed reduction (TPR), chemisorption and TEM. The influence of several parameters (reaction temperature, thermal treatment and reduction temperatures, O<sub>2</sub> pressure, NaOH/glycerol molar ratio and pH, metal loading) in the reaction was evaluated.

It was found that the distribution of products depends strongly on the basicity of the reaction medium. Catalysts were very selective to glyceric acid (60-70%), in tests carried out under highly alkaline conditions (pH≈13), attaining conversions of approximately 90% after 5 hours of reaction.

Pt-Cu and Pt catalysts were tested in acid medium; under these conditions, they were generally highly selective to glyceraldehyde, also promoting dihydroxyacetone formation. Conversions of 30-60% were obtained after 30 h of reaction with selectivities to glyceric acid, dihydroxyacetone and glyceraldehyde of 30-50, 10-20 and 30-50%, respectively. Best efficiencies were achieved by increasing the loading of Pt from 1 to 5% and by the addition of Cu.

Acknowledgments:

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# Bioethanol from Brewer's Spent Grain: Pretreatment, Hydrolysis and Fermentation

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The main by-product of brewer industry is brewer's spent grain (BSG), a lignocellulosic material rich in cellulose and hemicelluloses that can easily be converted into simple sugars, which can then be fermented into ethanol [1]. Xiros and coworkers [2] estimated that the amount of ethanol that can be produced is 65 g ethanol/ kg dry BSG.

The bioethanol production process begins with the BSG pretreatment [1]. In this work, a chemical pretreatment with hydrochloric, nitric and sulphuric acid was tested. The 2<sup>nd</sup> step is the enzymatic hydrolysis, which was performed with the 1<sup>st</sup> step, to allow for the evaluation of the effectiveness of this step [3]. The enzymes tested were: Viscozyme L, Glucanex 100g, Ultraflo L, and Cellulase and Hemicellulase, both from *Aspergillus niger*. The amount of sugars released after each individual acid pretreatment, combined with each enzymatic hydrolysis was evaluated, concluding that each acid has a different potential on sugars release. Therefore, it was decided to perform the pretreatment with the sequential addition of 2 acids, which effectively conducted to better results. Thus, 100 mL of each of 2 acids, at a concentration of 1% (v/v) were added sequentially (in two process steps, first with HCl and then with HNO<sub>3</sub> or H<sub>2</sub>SO<sub>4</sub>) to 25 g of dry BSG followed by the action of different enzymes added sequentially. The highest sugars release, of 720 g total sugars/ kg dry BSG, was obtained by using Glucanex 100g and Ultraflo L with HCl and HNO<sub>3</sub>. The 3<sup>rd</sup> step of the bioethanol production process is fermentation by yeast. In this study *Saccharomyces cerevisiae* [1,3] was used. For the conditions presented before, the estimated amount of ethanol was 8.3 g (or 10.5 ml) for each 25 g dry BSG. To further improve the process by converting pentoses as well, it was tested the yeast used in the brewery process, the *Saccharomyces pastorianus*, using HCl and HNO<sub>3</sub> with Viscozyme L. Results showed that a lower ethanol content was obtained than when *S. cerevisiae* was used, but the conversion of pentoses was higher. Hence, one can generally conclude that BSG has high potential for bioethanol production but some further research is needed to make the process commercially interesting.

**Acknowledgment:** Financial support from UNICER, project PP-IJUP2011-UNICER-333

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Oral Sessions ▶ A4

# **Psychology & Education Sciences II**



# Promotion of socio-emotional and emergent literacy skills in preschool: an intervention program

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Lately, there has been evidence of the importance of socio-emotional and emergent literacy skills in achieving lifelong success, being preschool years a critical period for the development of these skills. The main objective of this study was to plan, implement and evaluate three modules of the Programa InterLLasos (Leal, Cadima, Peixoto, & Gamelas, 2012), aiming to promote socio-emotional and emergent literacy skills in the context of preschool education comprising 4 and 5 year-old children. Thirty-three 4 to 6-year-old children, attending two different classrooms, and their teachers participate in the study. The program was implemented in one of the classes from February to June 2012. Children of both classes were assessed before and after implementation of the intervention program. To assess the socio-emotional development was used the Social Skills Rating System (Gresham & Elliott, 1990) and the Emotional Situation Knowledge (Denham, McKinley, Couchoud e Holt, 1990). The Peabody Picture Vocabulary Test – PPVT-4 (Dunn & Dunn, 2007), “*Follow Me, Moon*” (Clay, 2000), and the Bateria de Provas Fonológicas (Silva, 2008) were used to assess language and literacy skills. The quality of language and literacy environment of the preschool contexts was evaluated through the Early Language and Literacy Classroom Observation (Smith, Dickinson, Sangeorge & Anastasopoulos, 2002). The results point out a decrease in the number of behavioural problems and a significant evolution in the performance of the children in the experimental group in the areas of emotional knowledge, receptive vocabulary, print awareness and phonological awareness skills. Additionally, there was significant improvement in the quality of the literacy environment in this classroom. These results emphasize the importance of developing systematic interventions embedded in preschool classroom routines and curriculum, stressing the effectiveness of interventions that aim to promote socio-emotional and emergent literacy skills at preschool level in an integrated and coordinated approach.

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# Medical Students and Communication Skills: Relevance and Evolution during Medical Course

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Effective communication is undoubtedly the first step for a successful patient-physician relationship. Teaching clinical communication has become therefore a major concern of medical education. Approaches measuring the evolution of learned skills are needed, since a decline in empathy and patient-centred attitudes has been reported during medical course [1].

Two hundred sixty eight (268) undergraduate students, attending the second year completed a 1.5 hours per week course during 4 months (in a total of 19.5 hours) on basic communication skills. Final students' evaluation consisted in an interview with a standardized simulated patient (SSP), assessed by the teacher using the SEGUE framework [2]. Three years later, when attending the fifth year, 68 students of the same population completed a re-evaluation interview following the same procedure – SSP and SEGUE evaluation.

Mean differences of SEGUE evaluation in 2008 and 2012 are shown on **Figure 1**.

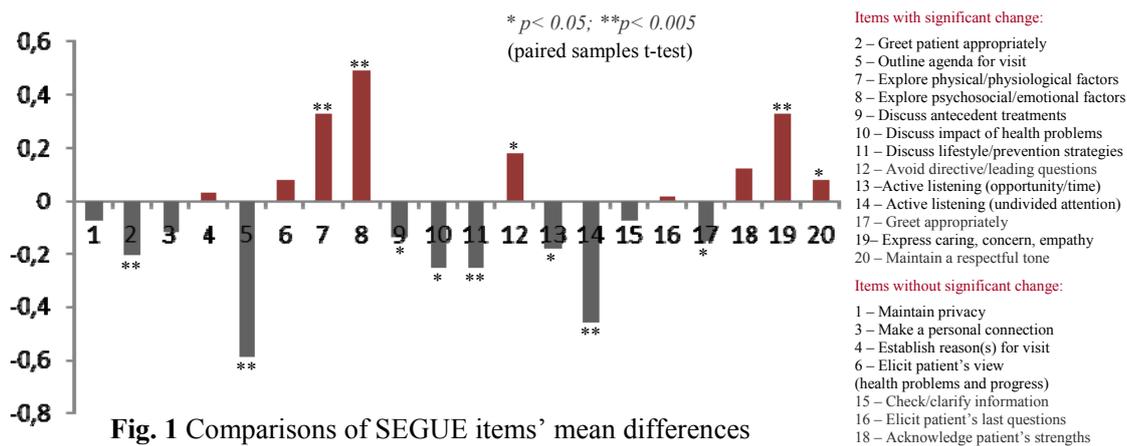


Fig. 1 Comparisons of SEGUE items' mean differences

Fifth year's medical students maintained a communication skills mean level, evaluated by SEGUE framework, similar of post training evaluation at second year. Significant differences between specific communication abilities were detected in this group of students - empathic attitudes and ability to collect information improved, and inversely, interview structure, verbal and non verbal students' behavior showed a decline during clinical practice phase. This could uncover the weight of context and professional influence, also reported by students. Present findings highlight the importance of skills focused training integrated in academic medical curricula. The totality of students interviewed additionally agreed with the importance of communication in the doctor-patient relationship.

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# Playing in the hospital: the professionals' perspective

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This research is part of the list of studies developed by the Research Group Psychology of Childhood (GPPIN). His proposition is justified around the activities of the extension project called "The narrative potential of spaces of childhood: a study of the meanings and practices of social and educational for children". Based on other research group, we started with the assumption that conceptions interfere in adult-child's play in the hospital. Thus, this research appears as a proposal to identify the meanings of professionals working in the pediatric ward of a university hospital, about playing in the hospital, using for this purpose the contributions of Vygotsky<sup>1</sup> (1989) and Sociology of Childhood<sup>2</sup>.

The study included 16 professionals, who were experiencing directly and indirectly to children hospitalized in the pediatric teaching hospital of the city of Cuiaba - MT. The methodology was qualitative, we used participant observation and semi-structured interviews.

Data from the interviews were analyzed using the software Analyse d'Lexicale Contexte one pair of Esemble Segments of Texte (ALCESTE). The corpus analyzed was composed of 16 initial context units (ICU), representing 16 interviews. These descending hierarchical analysis took into account 74% (438) were classified into 4 classes stable, as follows Illustration dendrogram in the figure below.

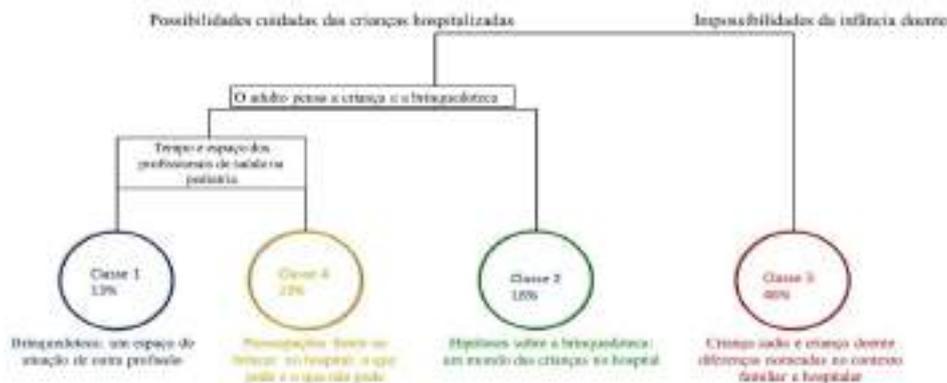


Figura 1 Ilustração Dendrograma

These classes may indicate viewpoints collectively shared, or image field on a given object, or just aspects of the same social representation.

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## **Presentation of a research Project: A questionnaire adjustment and validation for Portuguese language about the perceptions of student teachers about the educational process**

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The purpose of this presentation is to present a project that intent to adjust and validate a questionnaire to Portuguese language, about the perceptions of student teachers concerning the process of teacher education and its contribution to their professional life. The first part includes five aspects of the teaching profession: i) agents of training; ii) components of teacher training; iii) roles of teachers; iv) motivation for teaching; and v) conceptions of teaching and learning process. The second include an open question about the most significant change experienced by novice teachers on their initial training.

In order to answer the purpose, two versions of this questionnaire were already translated by two authors. The two versions were crossed, yielding a final version that was reviewed by another member of the team research. A second revision of this first version was still made by an external collaborator, a recognized expert in the pedagogical area. In sequence, another external element, with great domain of Portuguese and English languages held a back translation of the document [2] regarding a fine cross-cultural conversion. Afterwards, was made the verification between the back translation and the original questionnaire, and the differences were justified one by one and the unjustifiable cases were checked.

The application of the first version of the questionnaire was made to twenty student teachers to test the content intelligibility. After, will be made the confirmatory analysis to ensure the questionnaire validation to the Portuguese reality. An empirical study using the questionnaire is also expected.

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# Item-writing flaws in multiple-choice questions of clinical anatomy: inter-rater agreement

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**Introduction:** Multiple-choice questions (MCQs) are frequently used to assess students in health science disciplines. However, few educators have formal instruction in writing MCQs and MCQ items often have item-writing flaws [1]. The major purpose of this study was to estimate the inter-rater agreement about item classification as either standard or flawed.

**Methods:** 920 test items from 10 examinations were classified as either standard or flawed. If flawed the exact type of item flaw or flaws contained within the question (including options) was recorded. Four judges (2 teacher/2 students), blinded to all item performance data, independently classified each item. A standard item was operationally defined for this study as any item that did not violate one or more of the 31 principles noted in a review article [2] which summarized current educational measurement recommendations concerning item writing. The Fleiss' Kappa was used to evaluate the inter-rater agreement between 4 judges previous the consensus process. Guidelines for interpreting kappa statistics suggest that values between 0.81 - 1.00 indicate almost perfect agreement, 0.61 - 0.80 substantial agreement, 0.41 - 0.60 moderate agreement, 0.21 - 0.40 fair agreement, and values less than 0.21 are poor or slight agreement.

**Results:** The agreement about item classification as either standard or flawed was fair (kappa=0.3). The agreement was moderate/substantial for the following principles: “use positive, no negatives” (kappa=0.7), “use carefully none of the above” (kappa=0.8), “avoid all of the above” (kappa=0.7) and “Choice length equal” (kappa=0.5). All other principles showed poor or slight agreement.

**Discussion:** The results showed many disagreements among judges about item classification, previous the consensus process, however for the more prevalent principles the agreement was substantial, allow assessing the impact of using flawed test items on achievement examinations in medical education.

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# Narizinho

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## **Abstract:**

In this paper the concept of a non-financed volunteering project is presented. The project consists in the creation of a series of events during the year with the single purpose of cheering and entertain the children of the pediatric ward in the Santo Antonio Hospital located in Porto, Portugal. These events are scheduled weekly and are performed by a group of volunteer students in strict collaboration with the health care professionals and teachers assign to this ward.

## **Introduction:**

This project's main purpose is to provide the children in the pediatric ward a few hours every week when they can have different activities and forget their current condition. This project is completely volunteer and has no form of funding. Any materials used in the activities are either recycled materials, materials provided by parents, volunteers or any other person who willingly wishes to help or support the Abel Salazar Biomedical Sciences Institute or the Santo Antonio Hospital.

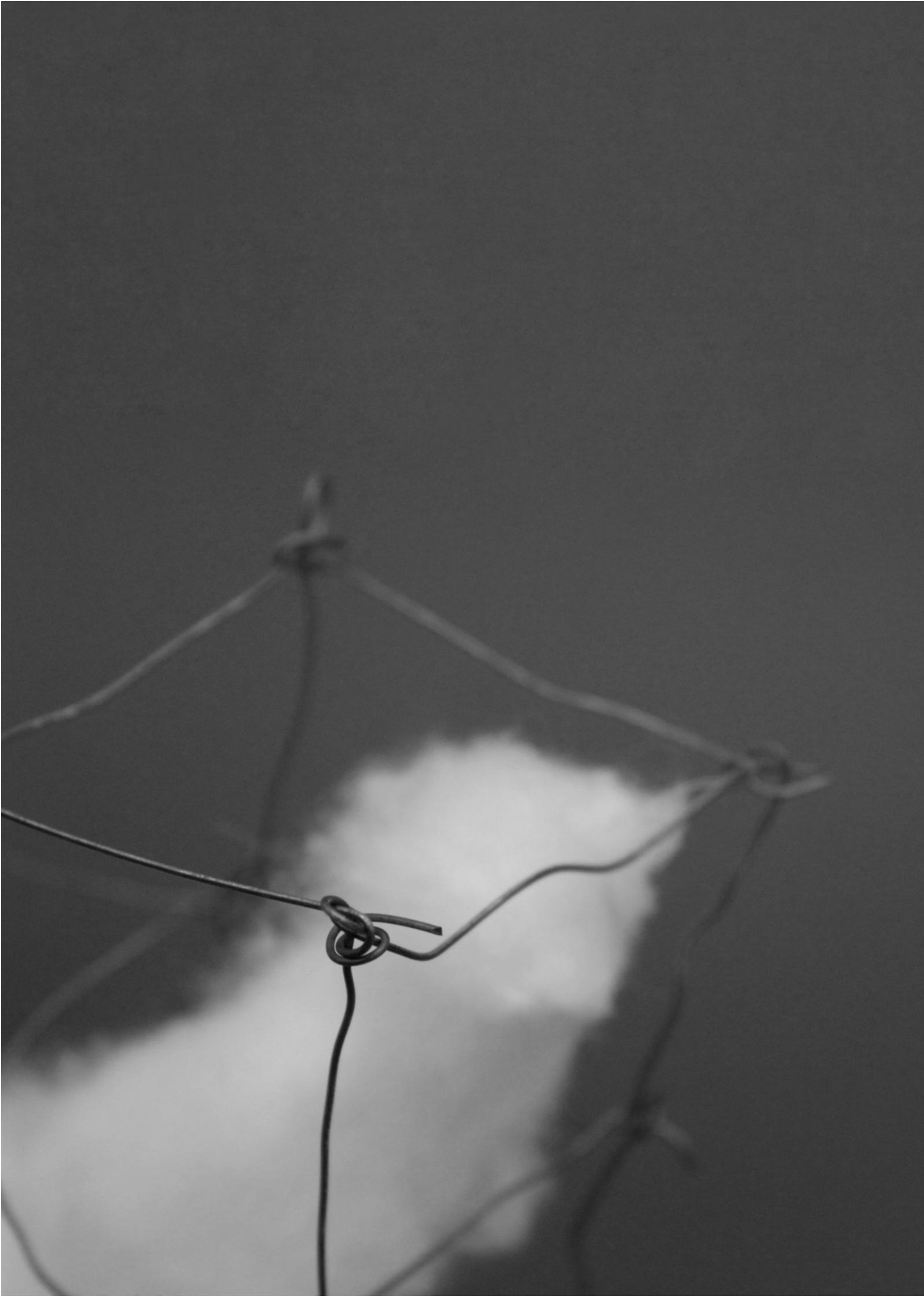
## **Project Description:**

The project consists of a group of volunteers spending a few hours every week in the pediatric ward. Due to space limitations and to avoid unnecessary confusion in the ward, a limitation of six volunteers per session is applied. All volunteers will be rotated in the various sessions according to the needs of the children and to their own availability. Several activities are proposed, focusing in several main objectives: entertain all the children with group games or leisure activities suited to their age and personality, breaking psychological barriers and creating bonds between the majority of them; provide cultural and learning activities to maintain and evolve their education, knowledge and brain activity. Whenever possible, festive days or important dates such as Christmas or Children's Day will be celebrated with special events.

## **Conclusion:**

Children in the pediatric ward often feel lonely and abandoned due to the lack of interaction with other children or school and to the limitations of their parents' working schedule or the hospital's visiting hours. They also tend to get very nervous, anxious or frightened due not only to their own sickness but also to the events surrounding them. It is also scientifically proven that a happy, dynamic environment can cause children to heal and develop more quickly. This project is of an extreme importance for these children since it provides not only fun and diversified moments but also didactic and learning experiences, calming the discomfort any child might feel and converting the pediatric ward in a place more familiar and cozy.





**ORAL SESSIONS**

**III**



Oral Sessions ▶ A1

# **Biomedicine III**



## Exemestane metabolites: Effects on breast cancer cell proliferation and induction of cell death

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Breast cancer is the most common malignancy in women worldwide. Hormone-dependent breast cancer therapy by the inhibition of the enzyme aromatase, responsible for catalysing the last step in the conversion of androgens to estrogens, has been very successful in postmenopausal women. The aromatase inhibitors (AIs) have proven to be good alternatives to the tamoxifen or fulvestrant treatment [1]. Exemestane, a third generation steroidal AI, is an irreversible inactivator of aromatase. Despite its epidemiological studies of clinical safety and toxicity, the metabolic pathways and identification of its metabolites remain to be elucidated. We have previously investigated the biological effects of exemestane in breast cancer cells [2]. In this work, we evaluated the cellular effect of exemestane metabolites, 17-beta-hydroxyexemestane (**17-BHE**), 6 $\beta$ -spirooxiranandrosta-1,4-diene-3,17-dione (**32**) and 1 $\alpha$ ,2 $\alpha$ -epoxy-6-methylenandrosta-4-ene-3,17-dione (**33**), in an estrogen-dependent human breast cancer cell line, that overexpress aromatase (MCF-7aro).

MCF-7aro cells were cultured in steroid-free medium with testosterone and treated with different concentrations (0,1-15  $\mu$ M) of each compound during different time-points (48-144 hours). Cell proliferation and cell viability were evaluated by <sup>3</sup>[H] thymidine incorporation, MTT and LDH assays. Morphological alterations were evaluated by Giemsa and Hoechst staining and the presence of acid vesicles (AVOs) was observed by acridine orange staining. The effect on cell cycle progression was evaluated by flow cytometry.

Exemestane metabolites induce chromatin condensation, membrane blebs and the presence of acidic vesicles (AVOs) for the higher concentrations. This was accompanied by a decline in cell proliferation and viability, being compound C33 the most potent. Moreover, exemestane metabolites induced cell cycle arrest in G0/G1 phase. However, further studies are needed to elucidate cell death mechanisms in cancer cells that occur following exemestane treatment.

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# **A link between TGF- $\beta$ signaling pathways and nucleolus function in *Drosophila melanogaster***

**A. Correia<sup>1</sup>, T. Martins<sup>1</sup>, J. Marinho<sup>1</sup>, P. Pereira<sup>1</sup>**

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Animal organogenesis requires the establishment of a highly regulated interplay between cell growth, proliferation and differentiation. Cell growth and proliferation are critically dependent on an efficient ribosome production, to sustain high protein-synthesis levels. Ribosome biogenesis and maturation takes place in the nucleolus, a dynamic subnuclear organelle that has also been characterized as a regulatory compartment involved in important cellular processes as cell-cycle control, apoptosis and cellular stress response.

Previous studies in our laboratory have shown that the fruit fly Noll2 homologue Viriato (Vito) is a key determinant of nucleolar structure that is required for tissue growth and cell survival during *Drosophila* development<sup>1</sup>. Moreover, we have identified a strong genetic interaction between Vito and TGF- $\beta$  signalling pathway members, and demonstrated that Vito is required for TGF $\beta$ -dependent tissue growth and photoreceptor neuronal differentiation<sup>2</sup>. These results strongly support a novel signalling branch where nucleolar events contribute positively in the transmission of TGF- $\beta$  signalling.

The main aim of this work is to understand if the described function of TGF- $\beta$  in tissue growth is based on a possible role of this pathway in nucleolar function. We observed that expression of a strong RNAi targeting Put, the shared type II receptor for both branches of TGF- $\beta$  signalling, affects nucleolar retention of structural proteins, Fibrillarin and Nopp140, which are also crucial in rRNA processing. In addition, ribosomal proteins involved in ribosome biogenesis were found to change its nucleolar localization and levels. We also observed a strong increase in nucleolar rRNA levels, suggesting that pre-rRNA processing and thus ribosome biogenesis may be compromised. In fact, *put*RNAi-expressing salivary glands have decreased amounts of mature rRNA subunits, and a more detailed analysis by transmission electron microscopy also showed that those nucleoli display nuclear accumulation of groups of small circular structures that might represent retained pre-ribosomes. Importantly, alteration of Put levels directly affects the nucleolar architecture and salivary glands growth, therefore supporting a regulation of nucleolar function by TGF- $\beta$  signalling.

Putting all together, our results unveil a new and unexpected growth mechanism, in which TGF- $\beta$  signalling regulates nucleolar structure and function, opening new perspectives in the understanding of the developmental networks that govern animal organogenesis.

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# Neuregulin-1 treatment reduces the severity of pulmonary arterial hypertension

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Neuregulin (NRG)-1 is implicated in the preservation of left ventricular function in pathophysiological conditions [1]. However, the role of NRG-1 in right ventricular (RV) failure is still unknown. We analysed the effects of NRG-1 treatment in an animal model of pulmonary arterial hypertension (PAH) and RV heart failure (HF).

Male Wistar rats (180-200g) randomly received monocrotaline (MCT, 60mg/Kg,sc) or vehicle. After 14 days, animals randomly received NRG-1 (40µg/Kg/day,ip) or vehicle, resulting in 4 groups: ctrl (n=10); ctrl+NRG (n=10); MCT (n=10); MCT+NRG (n=10). RV hemodynamics and sample collection for vascular, morphometric, histologic and molecular studies were performed 25-28 days after MCT administration. Only significant results (mean±SEM, p<0.05) are given.

MCT group developed PAH, as shown by increased RV maximum pressure (MCT vs ctrl: 63±3 vs 34±3mmHg) and by decreased cardiac output (MCT vs ctrl: 34±4 vs 65±4mL/min) which were both attenuated in the MCT+NRG group (53±3mmHg and 52±2mL/min). Animals from the MCT group developed RV hypertrophy (RV weight/tibia length ratio MCT vs ctrl: 0.08±0.002 vs 0.05±0.003g/cm) and pulmonary congestion (lung weight/tibia length ratio MCT vs ctrl: 0.7±0.03 vs 0.4±0.03g/cm), both changes were minimized by the NRG-1 treatment (0.06±0.002 g/cm and 0.6±0.03 g/cm, respectively). Histological analysis also revealed a decrease of RV cardiomyocyte hypertrophy and fibrosis in the MCT+NRG group vs MCT group. The RV of MCT group presented increased expression of brain natriuretic peptide (BNP) and endothelin (ET)-1 (17.5 and 5.0 times vs ctrl, respectively). These changes were attenuated or reversed in the MCT+NRG group (BNP expression increased only 5.6 times vs ctrl, and ET-1 expression did not change). The MCT group presented endothelial pulmonary dysfunction (35±2% vs ctrl 86±2% of relaxation in response to acetylcholine), which was attenuated in the MCT+NRG group (48±3%).

NRG-1 chronic treatment significantly reduced the severity of PAH and RV hypertrophy, as well as the expression of genes associated with overload and ventricular hypertrophy. These findings suggest that the NRG-1 pathway has a relevant role on the pathophysiology of PAH and RVHF, representing a potential therapeutical target.

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# REFERENCE VALUES OF HEART RATE VARIABILITY PARAMETERS IN ADOLESCENTS

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Heart rate variability (HRV) is a noninvasive tool that provides indicators of the autonomic nervous system integrity. HRV has been considered an early marker of cardiovascular risk in different populations and clinical conditions. However, there are no normative values of HRV parameters for adolescents. The present study aimed to establish reference values of the parameters of HRV in adolescents. The sample included 1152 male adolescents (14-19 years) from high school public. HRV was obtained for 10 minutes using heart rate monitor with the adolescents in the supine position. The variables of time domain (standard deviation of all RR intervals [SDNN], square root of the average of the squared differences between adjacent normal RR intervals [RMSSD] and percentage of adjacent intervals with more than 50 ms [pNN50]) and the frequency domain (high bands (HF) and low (LF) frequency and the sympathovagal balance [LF/HF]) were obtained. For description of percentiles (5<sup>th</sup> and 95<sup>th</sup>, respectively), mean and standard deviation were used. SDNN had an average of  $61.9 \pm 23.5$  ms, and changes in percentiles were 29.4 to 103.7 ms. RMSSD had an average of  $54.5 \pm 29.4$  ms, with variation between percentiles from 18.2 to 113.0 ms. The average pNN50 was  $29.4 \pm 20.4\%$  and a range from 1.1 to 66.2%. LF and HF bands were averages, respectively,  $53.0 \pm 15.6$  and  $47.0 \pm 15.6$  nu. Moreover, the variation of the LF band was from 26.7 to 77.7 nu while the HF band ranged from 22.3 to 73.7 nu. Finally, the ratio LF/HF had an average of 1.44 and a range from 0.36 to 3.49. In conclusion, the results of this study provided indicators that assist in interpreting the results of HRV in adolescents.

## Effects of voluntary physical activity and endurance training on cardiac mitochondrial function of sub-chronic Doxorubicin-treated rats.

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Doxorubicin (Dox) is a potent antibiotic widely used to treat several types of cancer, although with limited use as it induces a dose-related cardiac toxicity [2]. Dox-induced cardiomyocyte dysfunction involves the disruption of mitochondrial function [2, 3]. To counteract Dox-induced cardiotoxicity, several adjunct strategies, including physical exercise, have been studied [1]. We aimed to analyze the effects of endurance training (ET) and voluntary physical activity (VPA) against mitochondrial dysfunction induced by sub-chronic treatment of Dox.

Male Sprague-Dawley rats were divided in six groups (n=6 *per* group; 15wk old): saline sedentary (Sal+Sed), saline ET (Sal+ET; 12wks of 1h/d endurance treadmill running), Sal+VPA (12wks of voluntary free wheel running), Dox+Sed (7 bolus of 2mg.kg<sup>-1</sup> per wk), Dox+ET and Dox+VPA. Heart mitochondria were isolated by differential centrifugation and *in vitro* endpoints of mitochondrial function [oxygen consumption, transmembrane electric potential ( $\Delta\Psi$ ) and susceptibility to mitochondrial permeability transition pore (mPTP)] were evaluated.

Dox treatment (Dox+Sed vs. Sal+Sed) affected heart mitochondrial oxygen consumption,  $\Delta\Psi$  endpoints and susceptibility to mPTP induction. ET *per se* (Sal+ET vs. Sal+Sed) increased respiratory control ratio (RCR, a measure of coupling between oxygen consumption and ADP phosphorylation), decreased ADP lag phase, i.e. the time that mediates ADP-induced depolarization and repolarization post-ATP synthesis, and increased mitochondrial tolerance against calcium-induced mPTP opening, thus improving mitochondrial bioenergetics. Dox-induced impairments in RCR, ADP lag phase and mPTP resistance were reverted by both ET and VPA.

It can be concluded that the cardiac mitochondrial adaptations induced by physical exercise can be translated into a functional phenotype that antagonizes DOX-induced cardiac mitochondriopathy.

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# Long-term endurance exercise training provides a cardioprotective phenotype against age-induced cardiac dysfunction

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Aging is characterized by a progressive impairment in cardiac maximal function, with a more incidence of diastolic dysfunction in contrast to a relatively well-preserved systolic function. Exercise training is thought to mitigate several age-related biological changes, preserving the overall functional capacity. Our purpose was to determine whether one year of exercise training could modulate the cardiac functional decline observed in sedentary aging.

Female Wistar rats ( $n = 16$ ; weight= $130 \pm 2.4$ g), were randomly displaced into the following two groups: sedentary (SED;  $n=8$ ; with restricted movement to the cage space for 52 weeks) and exercise (EX;  $n=8$ ; submitted to treadmill exercise training for 52 weeks, 5 days/week, 60 min/day at 20 m/min). Twenty-four hours after ending the training protocol, all the animals were anesthetized, tracheostomized for mechanical ventilation, the right jugular vein was cannulated and the heart exposed by median sternotomy. After, a pressure-volume catheter was inserted in the left ventricle (LV) for hemodynamic evaluation in baseline conditions and under acute occlusion of the ascending aorta (isovolumetric heartbeats).

EX animals presented a lower HR (EX: $294.3 \pm 4.887$  vs. SED: $313.9 \pm 6.669$  bpm) and a decreased EDP (EX: $6.288 \pm 0.4982$  vs. SED: $9.023 \pm 1.219$  mmHg), while MaxP (EX: $161.2 \pm 2.714$  vs. SED: $145.1 \pm 4.050$  mmHg) and ESP (EX: $158.8 \pm 2.653$  vs. SED: $141.9 \pm 3.771$  mmHg) were increased in comparison to the SED group ( $P < 0.05$ ). The training program did not promote significant alterations ( $P > 0.05$  vs. SED) in the dP/dt max (EX: $9968 \pm 178.9$  vs. SED: $9324 \pm 538.7$ ) and dP/dt min (EX: $-11383 \pm 114.5$  vs. SED: $-10175 \pm 591.9$ ). Under isovolumetric heartbeats, the EX group exhibited an improved response ( $P < 0.05$ ) as shown by the increased dP/dt max (EX: $9846 \pm 208.8$  vs SED: $8720 \pm 316.4$ ), decreased EDP (EX: $6.969 \pm 0.354$  vs SED: $10.41 \pm 1.347$  mmHg) and faster Tau (EX: $13.55 \pm 0.472$  vs SED: $16.32 \pm 1.149$ ).

Our data suggest that long-term endurance exercise training of moderate intensity provides a cardioprotective phenotype against biological aging, manifested by improved cardiac function in baseline and against acute afterload elevations.

## Angiotensin 1-7 attenuates Angiotensin II vasoconstrictor effect in human internal mammary arteries: underlying mechanisms and its modulation by statins

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The internal mammary artery (IMA) is becoming the preferred conduit to be used in coronary revascularization surgery (CRS). Cardiovascular history and therapeutics of patients submitted to this surgery can interfere with IMA function. The renin-angiotensin system has a critical function in cardiovascular pathophysiology. The final effect of this system depends on the balance between two main axis: 1) the ACE/AngII/ AT<sub>1</sub> receptor 2) the counter-regulator action of ACE2/Ang 1-7/ Mas receptor axis. In this context, the main goal of this work is to evaluate the vascular effects of Ang1-7 on the vasoconstrictor effect of Ang II in the IMA of patients submitted to CRS. Furthermore, the influence of patients' clinical characteristics on this response will be studied.

Supranumerary IMA segments were cut in 2 mm vascular rings and mounted in a myograph (*Multi Wire Myograph System Model 620M - DMT*). In the first protocol, after the pre-contraction with phenylephrin ( $10^{-5}$  M), the rings were treated with isotonic saline solution (vehicle) or Ang 1-7 ( $10^{-5}$  M) and incubated with increasing concentrations of Ang II ( $10^{-9}$ – $10^{-5}$  M). In the second protocol, the segments were divided in 4 groups with different pre-treatments: I) selective Mas receptor antagonist (A-779,  $10^{-5}$  M); II) selective AT<sub>2</sub> receptor antagonist (PD123177,  $10^{-6}$  M); III) selective AT<sub>1</sub> receptor antagonist (losartan,  $10^{-6}$  M) and IV) mechanical removal of endothelium. After that, the rings were incubated with Ang 1-7 ( $10^{-5}$  M) or vehicle and, in the end, with increasing concentrations of Ang II. At last, we compared the inhibitory effect of Ang 1-7 according to a previous categorization of patients in the following groups: with diabetes, with arterial hypertension, smokers, therapeutic with statins and therapeutic with renin-angiotensin inhibitors.

The vasoconstrictor response mediated by Ang II was significantly inhibited in the presence of Ang 1-7 ( $10^{-5}$  M) compared to vehicle (TA<sub>max</sub>:  $16.1 \pm 2.4\%$ ; TA<sub>max</sub>:  $46.2 \pm 3.3\%$  ( $p < 0.05$ ), respectively). A-779, PD123177 and endothelium removal did not statistically change Ang 1-7 effect on the Ang II actions (TA<sub>max</sub>:  $15.41 \pm 2.34\%$ ,  $29.65 \pm 6.35\%$ ,  $28.19 \pm 5.76\%$ , respectively). In the presence of losartan, no difference was observed between the group treated with Ang 1-7 and the control group (TA<sub>max</sub>:  $13.97 \pm 2.91\%$  vs  $12.15 \pm 1.98\%$ , respectively). Ang 1-7 had a significant higher inhibitory effect on patients chronically treated with statins than those who were not. (Inib<sub>Ang1-7</sub>:  $69.21 \pm 3.53\%$  vs  $53.5 \pm 3.90\%$ ,  $p < 0.05$ ). There were no statistically significant differences among the other groups.

In conclusion, this work shows that Ang 1-7 significantly attenuates Ang II vasoconstrictor response in the internal mammary artery of patients submitted to coronary revascularization. This effect does not seem to depend on Mas receptor, AT<sub>2</sub> receptor and endothelium. This modulation of Ang II actions by Ang 1-7 is significantly increased in patients treated with statins.

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Oral Sessions ▶ A2

# Agro Food II



# Screening of hydroxymethylfurfural and furfural in different types of beer

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Furan derivatives are actually receiving increased attention. On one hand, these compounds contribute to the flavor of foods and beverages, but on the other hand, they are associated to potential harmful effects on human health. During brewing, Maillard reaction and caramelization occur as roasting proceeds at high temperatures up to 250 °C leading to the formation of hydroxymethylfurfural (HMF) and furfural [1]. However, some preliminary experiments indicate that HMF levels in beers are relatively low (<5 mg/L) even though significantly higher levels (>1000 mg/kg) may form in dark roasted malts [2].

Around the world different types of beer are produced. There is no universally agreed list of beer styles as different countries and organizations have different sets of criteria. In general they are categorized into one of three different categories: lagers, ales, and the rest fall into specialty beers. Specialty beers are either ales, lagers, or a hybrid of the two that will contain other ingredients that cause it to not fit into a true ale or lager style. Due to different malt composition, addition of adjuncts, rich in sugars and brewing conditions a wide variety of beer styles are produced. Considering that it is expected variation of the content of HMF and furfural. Thus, the goal of this work was to screen the content of HMF and furfural in different types of beer commercially available in Portuguese market.

Reversed-phase HPLC with Diode Array detection was used to determine the contents of HMF and furfural in 50 samples of beers randomly purchased from market including pilsner, stout, weiss, dunkel, among others. Some samples were alcohol free beers. Prior furan determination, beer samples were filtered through 0, 45 µm syringe filters and degassed by sonication. The quantification was performed by external standard calibration method. HMF was detected in the 50 beers analyzed, however, low variation was observed, because content ranged between 2.07 and 7.79 mg/L and the average value was 5.34±1.73 mg/L. Furfural was not detected or detected in very low concentrations.

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# Quantification of hydroxymethylfurfural and furfural in bread by high performance liquid chromatography

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Baking is a complex process that involves physical, chemical, and biochemical changes, which are essential for the development of the aroma, taste and color surface in the baked products. Colored compounds that result from Maillard reactions produced during the baking process are also related with nutritional aspects and formation of undesirable compounds. Hydroxymethylfuraldehyde (HMF) and furfural are furanic compounds generated during the advanced stages of Maillard reaction, commonly measured as quality parameters to evaluate the severity of the heat treatment. The objectives of this work were optimization and validation of an efficient extraction methodology for high performance liquid chromatography (HPLC) analyses of HMF and furfural in bread.

Extraction of HMF and furfural before quantification by HPLC was performed with oxalic acid and with water [1] followed by clarification with TCA [2], and extraction with water and clarification with Carrez I and II reagents [3]. Modifications of this last procedure were tried, namely, replacement of water extraction by different mixtures of water/methanol - 60/40; 70/30, 80/20 - followed by clarification with Carrez I and II reagents. The solvent mixture methanol:water (30:70) allows the highest yield.

The compounds under study were not detected in 100g of bread dough baked in microwave during 1 min and 30 sec, being an adequate matrix of baked dough free of HMF and furfural. Thus, it was used to study the matrix effect. Comparison was performed between the slopes of the regression lines obtained for pure standard calibration curve and for the sample curve calibration by least-squares analysis, and statistical differences were found ( $p = 0.95$ ,  $n = 12$  Student's t - test for paired samples), which points out the matrix effect and the choice of sample curve calibration for quantification of HMF and furfural in bread. Good quality parameters were obtained and the method was successfully applied to the analyses of different types of bread.

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## Influence of the addition of $\beta$ -glucans in the volatiles profile in homemade bread

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The quality of bread is normally defined according to its volume, texture, color, and flavor. However, the characteristic aroma of bread is undoubtedly one of the most important parameters influencing its acceptance by consumers. The volatile and nonvolatile compounds that contribute to the flavor of bread include acids, alcohols, aldehydes, esters, ethers, ketones, furans, hydrocarbons, lactones, pyrazines, pyrroles, and sulfur compounds [1].

There is currently a growing search on the incorporation of new ingredients in bread with the aim of improving its flavor, nutritional quality and health properties.  $\beta$ -glucans improve cardiovascular health through a decrease the cholesterol of lipoproteins of low density (cLDL) and glycemic response. Also, they may have a powerful immunomodulating effect and show anti-inflammatory and antitumor effects and promote a greater stimulation of the immune system against infections [2]. The objective of this work was to evaluate the impact of the incorporation of  $\beta$ -glucans from cell wall in the volatile profile of bread.

Breads supplemented with  $\beta$ -glucans (0,5; 1; 1,5 and 2 g/500g flour) were analyzed and compared with bread without supplementation. In all cases, the assessment was performed in triplicate. For the Headspace analysis by Solid Phase Micro Extraction (HS-SPME), 2 g of bread, crumb and crust, was crushed and weighed into a 50-mL vial. Then, 10 mL of a 20% NaCl solution (pH = 3 with 0.05M citric acid) were added to the vial and the vial immediately sealed at once and kept at -4 °C during 10 min. Afterwards, the vial was placed into an ultrasonic cleaner during 15 min. To extract volatile compounds were used a CAR-PDMS SPME fibre (75  $\mu$ m thickness, Supelco Co., Bellefonte, PA, USA) inserted into the sample vial through the septum and exposed to the HS for 60 min at 50 °C under constant agitation (600 rpm). Thereafter, the SPME fibre was inserted in GC-MS in the split-less mode.

The analysis of volatiles in the bread shows that predominant groups of compounds, expressed as relative percentage of area, are alcohols and aldehydes. The most important compounds in each category correspond to ethanol, hexanol, isoamylalcohol and butanal, 3-methyl-butanal, 2-methyl-butanal; hexanal and heptanal. Although, the  $\beta$ -glucans addition does not generate a significant change in the total area of volatile compounds found in different bread samples ( $p > 0,05$ ). However,  $\beta$ -glucans addition reduced significantly ( $p < 0,05$ ) the number of volatile compounds identified.

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# Influence of $\beta$ -glucans addition on texture and color of homemade bread

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$\beta$ -glucans are soluble and fermentable fibers that, once in the intestine can compose highly viscous solutions, presenting benefits for insulin resistance, dyslipidemia, hypertension, and obesity [1]. Thus,  $\beta$ -glucans can be useful in the food industry, increasing the fiber content of food products and enhancing their health properties. According to EFSA, the amount of yeast  $\beta$ -glucans in conventional foods must range between 50 and 200 mg per serving [2]. However, the influence of  $\beta$ -glucans addition on bread sensory characteristics was not understood.

Bread texture and color affect consumer preference. Sensory evaluation is not simple, nor objective. Thus, instrumental measurement is crucial. The objective of this work was to study the effect of adding different levels of  $\beta$ -glucans extracted from yeast cell wall to improve the bread's characteristics.

Breads were made from 500g of flour mixture of NACIONAL - CEREALIS appropriate for homemade bread “pão caseiro” 320 ml water and variable amounts of  $\beta$ -glucans (0; 0,5; 1; 1,5 and 2g). Weight and volume were evaluated. The characteristics of texture and color were measured. A Texture Analyser was used for evaluation of Hardness, Cohesiveness, Elasticity, Adhesiveness and Masticability. Colour, lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ) were determined using a Minolta colorimeter. The bread displaying the largest volume was that with a dose of 1,5g and the one with the smallest volume was that with a dose of 0g of  $\beta$ -glucan extract. For Hardness, the highest value corresponded to the 0g dose and the lowest to the 1,5g one; whereas for Elasticity, the highest was for 1,5g and the lowest for 0g and 0,5g; the highest value for Masticability corresponded to 0,5g and the lowest one to 1,5g. In respect to color, the  $L^*$  parameter was the highest for the 1,5g dose and the lowest for the 1g one; the  $a^*$  parameter was the highest for 0g and the lowest for 1,5g, the  $b^*$  parameter was the highest for 0g and the lowest for 1,5g. More studies are needed to confirm the influence of  $\beta$ -glucans addition to homemade bread using different baking conditions.

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# Reducing salt in bread using natural flavour enhancers: consumer's acceptability and saltiness perception

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Cardiovascular diseases (CVD) are the leading cause of death worldwide. Hypertension is considered as the greatest modifiable risk factor for CVD, and excessive intake of salt its main cause. Processed foods are responsible for 75% of total salt intake, especially among cereals as being the group with the highest individual contribution. Thus, reduction of salt content in bread may substantially reduce these risks to a population level; several strategies have been developed with this purpose. In this work, we intend to verify whether the introduction of natural flavour enhancers may be a means to significantly reduce the salt content of white wheat pan bread, without significantly affecting its final quality and acceptance by the consumer. Following an exploratory study with seven flavour enhancers and three different levels of added salt [1] the formulations using olive oil, lemon juice, thyme infusion and garlic powder as natural flavour enhancers were selected and reproduced considering medium and low levels of added salt (8 and 4 g / kg flour) and a control, with medium level of added salt was also considered, totalling 9 formulations. These were evaluated by an untrained panel of consumers (n = 30) for the overall acceptability using a 9-points hedonic scale [2] and perceived saltiness with a 5-point JAR scale, aiming for a penalty analysis [3]. It was found that samples with olive oil, garlic powder and lemon juice had an overall acceptance equal or superior than the control, even with half the amount of salt, and that the perceived lack of saltiness, observed in the control loaf, was slightly improved by the addition of flavour enhancers with reduced salt content and significantly improved when the salt content was the same as the control. Available sodium in these 9 loaf formulations was quantified by atomic absorption spectrometry [4]. Results show that the final NaCl content of bread, as determined analytically, was similar to the calculated level considering the amount of added salt and final bread weight, 5.3 versus 5.5 g NaCl/kg bread (medium salt added) and 2.6 versus 2.7 g NaCl/kg bread (low salt added), respectively. It was concluded that the flavour enhancers do not act by adding sodium. The results of this study indicate that the use of lemon juice or olive oil may be a relevant strategy to reduce the salt content in bread loafs, without significantly affecting consumer acceptability.

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Oral Sessions ▶ A3

# Engineering II



# Power System Reliability Evaluation Using Monte Carlo Simulation and Evolutionary Programming

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Monte Carlo Simulation has been, for many years, the reference technique for Power Systems Reliability Evaluation. Although the Chronological approach allows a better understanding of the phenomena related to the behavior of components in power systems, it requires very significant computation resources. An alternative is the use of the Non-chronological approach that allows faster and accurate results, although in a much less extension that the ones obtained by the chronological approach.

Recently, meta-heuristic techniques such as Evolutionary Programming have been successfully applied to solve many engineering problems, including Reliability evaluation and start to appear as serious competitors of Monte Carlo-based techniques, due to the ability of search meaningful states more effectively and the necessity of lower computation resources.

This paper presents the results of the reliability evaluation of an electric power system composed by 20 generators. Results for reliability indexes from the analytical approach are compared with the ones obtained by Monte Carlo Simulation and the same indexes are also determined using Evolutionary Programming. The results are compared according to the accuracy provided by each technique. To allow the direct comparison of the results obtained by Monte Carlo Simulation and Evolutionary Programming, the temporal evolution of reliability indexes determined by these techniques are also presented and conclusions are drawn.

The main conclusions are that Evolutionary Programming allows a faster calculation of reliability indexes without compromising the accuracy on these results, when compared with the ones obtained by Monte Carlo Simulation and obtained by the analytical model. Due to the nature of Evolutionary Programming, the results obtained cannot be inserted in a confidence interval like Monte Carlo Simulation. However the results obtained by Evolutionary Programming are contained in the confidence interval defined for the calculated reliability indexes by the Monte Carlo Simulation, long before the interval limits are established.

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# Monitoring of bedridden patients: Development of a fall detection tool

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The falls of patients are an important issue in hospitals, it causes severe injuries to the patients and it is progressively capturing the attention of the scientific community. The low rate of nurses per patient give rise to great technical complications on patients' control, and frequently patients fall from the hospital bed. Although it is difficult to predict a fall from a bed, its detection in time would provide faster rescue to the patient, preventing injuries, reduction of patients' length of stay at the hospital as well as costs to the hospital and to patients. It would also allow enhancing organization and effectiveness of the hospital nursing staff, saving valuable time. To our best knowledge, no solutions have yet been developed with the required accurate results of time and location, to alert the nursing staff that the patient has fallen out of the hospital bed.

The MovinSense® is an electronic device designed for monitoring patients to prevent pressure sores, and the main goal of this work was to develop a new tool for this device, with the purpose of giving feedback to the nursing staff if the patient has fallen from the hospital bed, without changing any of the device original features - portability, light-weight, safety and ease of usage. These bring some advantages when comparing the MovinSense® to other technologies. It is one single device attached to the patients' chest that wirelessly transmits information to a receiver at the nursing staff workstation, making easier its acceptance by the responsible care personnel.

Experiments for gathering data samples of inertial signals of falling from the bed were obtained using the device, signal processing was performed in Matlab, and the algorithm was developed. For fall detection a sensitivity of 72% and specificity of 100% were reached. Other algorithm was developed to detect if the patient got out of his/her bed which may result in a fall from the bed, or while walking around; injury; as well as patients' loss.

This work was performed under the scope of the first author's Dissertation Project for the degree of Master in Bioengineering, and it was developed in association with TOMORROW OPTIONS, Microelectronics S.A..

## Preparation processes for the recycling of electrical and electronic waste

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The present work performed an evaluation of the processes involved in processing of electrical and electronic equipment (EEE) when they reach their end of life, giving a special emphasis to recycling. This kind of waste presents a diverse typology and has components with dangerous features for the environment and human health, therefore, a correct management is essential [1, 2].

At a national level, ERP and AMB3e are licensed to perform an integrated management of the waste from electrical and electronic equipment (WEEE). Although in legal terms this type of waste is divided into 10 categories, in operational term it occurs a division into 5 different flows (large equipment, cooling appliances and refrigeration equipment, fluorescent and discharge lamps, monitors and televisions). Each flow presents different types of treatment, recovery options (including reuse / recycling) and disposal.

From the analysis performed, some gaps were found. One of the most relevant is the fact that most operations occur out of the Country, meaning that there is an evident lack of supporting technologies regarding the management of WEEE in Portugal.

Ultimately, a case study was selected for intervention at the level of preparation for recycling. The study was conducted in two different samples of waste from electrical cables from a company (one obtained on site and another prepared from grinding the original material), consisting of a mixture of plastics, which current destination was the landfilling due to the presence of plastic contaminants that could not be recycled.

Through experimental tests, the plastic components were subjected to gravity separation and thermal treatment to evaluate deformation. The separation procedure adopted was effective, but only in the laboratory prepared mixture, meaning that the source of the waste and the grinding procedures at the company should be carefully studied aiming to implement this procedure.

It was possible to separate the non-recyclable plastic fraction, which showed potential as a pre-treatment in the future; further studies are however required to allow implementation in the company.

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## Methodology to recover metals from waste printed circuit boards

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The problems related with the incorrect management of electronic waste are very important, due to their potential impacts on environment. In particular, waste printed circuit boards (WPCB) are produced in great amount, being harmful for the environment if not properly managed, and presenting high metals recovery potential, not properly explored. <sup>[1]</sup>

Up to date, current alternatives still fail to provide a simple and cost effective pre-treatment of WPCB aiming metal recovery and proper management. <sup>[2, 3]</sup> Accordingly, the present work aimed to study this subject using a random sample of a set of waste printed circuit boards, from computers collected at the Faculty of Engineering.

The methodology consisted on grinding the boards in a mill (blades), followed by the classification of the different fractions obtained (by particle size) and further characterization regarding their metal content.

The classification method yielded six fractions (fraction 1 > 6.3 mm, 5.0 mm < fraction 2 < 6.3 mm, 4.0 mm < fraction 3 < 5.0 mm, 2.8 mm < fraction 4 < 4.0 mm, 0.85 mm < fraction 5 < 2.8 mm and fraction 6 < 0.85 mm).

The metal content was assessed in each fraction using magnetic and gravity separation. The fractions 5 and 6 presented the highest metal content. In such high metal content fractions, up to 23 wt.% of ferrous metallic compounds and up to 21 wt.% of other metallic compounds were found.

According to the results, in order to prepare this type of waste to maximize its metal recovery potential, WPCB should be finely grinded; therefore, grinding methods that can maximize the production of fine fractions have potential as pre-treatment technologies.

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# Modulation of constructive process of the Central Sub-viaduct of the Corgo Viaduct

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This work focuses on the structural analysis of the constructive process of the Central Sub-viaduct of Corgo River Viaduct, having as main goal a comparative analysis between numeric and the in site measurements made by of the Structural Health Monitoring (SHM) system. This viaduct, which is now under construction, has a stay cable prestressed concrete deck with a central span of 300 meters built using the balanced-cantilever method. The numerical model, that was elaborated in software *Evolution* [1], reproduces not only the geometry of the structure but also all of its loading history and constructive process. The considered structural material behavior uses the concrete rheology and steel relaxation laws presented in Eurocode 2 adjusted using the results of laboratory tests and in site measurements..

The Fig. 1 presents the measured and numeric estimated extensions in the superior extensometers on deck's section T-P19c located near one of the masts. The close relation between the two presented series indicates that the elaborated numeric model represents accurately the structure behavior.

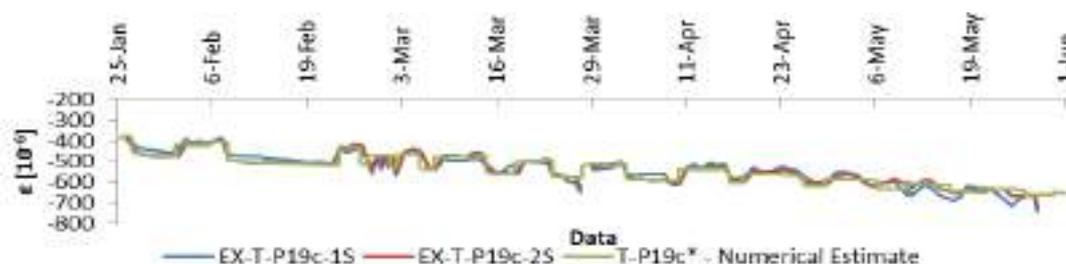


Fig. 1 – Measured and numeric estimated extensions in the superior extensometers on deck's section T-P19c in the period between January and June of 2012 [2].

By the accomplish comparative analysis between the SHM system measurements and the correspondent numeric predictions of strains, forces and displacements it can be conclude that:

- ! The Corgo Viaduct has the expected behavior in the analyzed structural parameters;
- ! Numeric models calibrated with results of SHM systems are very useful to understand in a more efficient way the behavior of the structures. Also, this models can permit the detection of structural damage, the elaboration of predictions in different scenarios and long term analysis of structural behavior;
- ! It was also realized the importance of the constructive phase on the structures design because in this structures live phase there are conditioning stresses.

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# Crack opening path with a meshless method

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The present work extends a recent developed meshless method to the fracture mechanics analysis of structures. The objective of this work was to develop a numerical tool capable of predicting the fracture crack opening path.

The finite element method (FEM) has been extensively used to predict the crack path since the 1980s [1]. However the FEM is particularly unsuited to problems with complex geometries since it is difficult to align the element edges with the edges of the domain of the problem, which can be cracks. Additionally, the domain remeshing due the crack evolution is a very burdensome and costly task in the FEM. In meshless methods the finite element concept of connectivity between elements is irrelevant, since the discretization is purely nodal. The test functions are constructed on a given nodal arrangement, which discretize numerically the problem domain. Because meshless method analysis is based on nodes or particles, it demonstrates flexibility in modelling complex discontinuities. It also avoids the distortion of mesh in the presence of large deformation, and provides an efficient mean for addressing high gradient problems, such as high strain/stress concentration problems. Fracture problems typically demonstrate high strain/stress concentrations, due the singularities incited by the discontinuities on the crack tip zone. Since it is very important to determine accurately the stress field around the crack in order to choose the correct path for the propagation of the crack tip, meshless methods are the proper numerical tool to study the fracture mechanics problem.

In this work, the numerical method used in the analysis was the Natural Neighbour Radial Point Interpolator Method (NNRPIM), an efficient and flexible meshless method [2], presenting various numerical advantages when compared with other numerical methods such as the Finite Element Method (FEM). The NNRPIM uses the Voronoï concept to force the nodal connectivity and to construct an integration mesh, both completely node-dependent. It is this node-dependency that gives the NNRPIM the advantage comparing with the FEM. The interpolation functions possess the delta Kronecker property, which simplifies the imposition of the boundary conditions.

Considering the maximum stress criterion, an algorithm was developed to predict the fracture crack opening path. Several fracture mechanics benchmark examples were solved with the NNRPIM and the obtained results demonstrate efficiency and accuracy of the developed algorithm.

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Oral Sessions ▶ A4

# **Psychology & Education Sciences III**



# High School Bullying: Adaptation of a new assessment tool to better understand its patterns and effects

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School violence is not a recent concern. The growing attention given by society, impersonated, mainly, by the media, and the frequency and/or severity increase keep it contemporary. However, it remains an underestimated public health issue that overcomes the school context.

The present study aimed at assessing the status of the bullying phenomenon at high school level, since none of the literature review mentioned research concerning these subjects. To achieve this, the option was to translate and adapt Dr<sup>a</sup> Susan Swearer's North American assessment tool: *Bully Survey – Student Version*, due to gaps noticed in other assessment tools (distinct appraisal of target-behavior and concept definition; main focus in aggression among peers, without regard for other agents, etc). The original version was subject to some alterations, through adding, cutting and modifying items, intended to make it closer to the literature status, and the needs of the study. It's a five-part survey: A) directed at victimization B) bystander and C) aggression behaviors; D) assessment of general thoughts about bullying and coping mechanisms and E) social-demographic data, with the final title of QBVE (Questionário do *Bullying* – Versão dos Estudantes).

The administration was made at 3 public and 1 private schools, in a total of 598 students, arranged in a fairly equal way by sex, age and school grade.

The results show that there aren't statistical differences in the frequency of bullying experiences, considering age, type of school, and social class. The remaining hypothesis were partially confirmed, showing a relationship between the type of bullying involvement and the coping mechanism used, just as an association between an increase in the violence exposure, as a bystander and bully, and a more positive attitude towards it.

The data, along with other information more qualitative in nature, which emerged in the observation of the institutional contexts and responses to open questions, raise awareness to the need to have a creative and attentive posture to mend the teenager's difficulty to communicate in a healthy and assertive way, making a choice for non-aggressive solutions.

# The experience of occupational stress in Portuguese financial managers

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Occupational stress can be seen as the adaptive reaction of individual at work environment demands, when these exceed his capabilities [1]. However, there has been proof that occupational stress isn't something exclusively negative, so there is the need to differentiate distress and eustress [2]. In this study, to approach this theme, the chosen population was the Portuguese Financial Managers because, despite recognition of managers as agents of change [3], and their contribution the success of organizations [4], the investigation in Portugal, for this functional group is still scarce [5].

So, we proposed ourselves to explore and understand the experience of occupational stress among the Portuguese financial managers through a qualitative, non-experimental, transversal study, of exploratory and descriptive type research, [6] and we will answer to the following questions considering Portuguese financial managers: (a) What is the occupational stress level they perceive? (b) What are the main factors of stress associated with this function? (c) What are the physical, psychological and behavioral consequences associated to the experience of stress? (d) What are the main coping strategies identified by these managers? Information was collected on six case studies using semi-structured interviews later submitted to content analysis [7].

The results show that Financial Management is perceived as a stressful activity and the workload, the deadlines, the economic crisis and interpersonal relationships are the main sources of stress detected. The coping strategies are emotional and problem focused and the detected consequences are majorly psychological. The evidences stating the positive impact of stress, made us conclude that the experience of eustress is a reality and that has to be taken into account in the holistic analysis of occupational stress in this profession. We suggest further studies on eustress in financial managers in view of better understanding of occupational stress in this occupational group which will allow most appropriate interventions.

Key-Words: *Occupational Stress, Distress, Eustress, Financial Management, Coping.*

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# Targeting Prosody: A Case Study of a Child with Asperger Syndrome

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Prosody is defined as the suprasegmental features of the speech that enhance and modulate its meaning in human communication. Atypical prosodic patterns commonly result in serious barriers to communication and may affect the process of language acquisition such as occur in speakers with Autism Spectrum Disorders (ASD) [1]. Considering these findings, it's essential to study prosodic acquisition and prosodic impairments to understand how prosodic delays or disorders affect child's development. In order to do so, it is critical to develop specific assessment tools to evaluate these abilities.

It is currently in progress a project which goal is to computerize the Portuguese Version of the *Profiling Elements of Prosodic Systems-Communication, PEPS-C* [2], with the purpose of simplify and accelerate the processes of data collection, coding and analysis in normative and clinical populations. The PEPS-C is an instrument that assesses receptive and expressive prosodic skills at two levels: form (acoustical parameters) and function (communicative functions: turn-end, affect, chunking and focus).

The present study attempts to build a comprehensive analysis of the prosodic profile of a child diagnosed with Asperger Syndrome (AS). To address this goal, the prosodic skills of the child with AS were compared to those of a typically developing speaker matched in age, nonverbal intelligence, receptive vocabulary, language and socioeconomic status. None of the 2 children reported visual or hearing deficits and the following skills were assessed: Prosody, nonverbal intelligence, language, grammar, vocabulary, phonological awareness, pragmatics, attention, socialization and executive functions.

Results revealed that the child with AS had more difficulty comprehending and producing prosody than the typically developing child. Although at the formal level the prosodic skills were similar, the differences were clearly evident at the functional level: the child with AS presented significantly lower performance on turn-end and focus abilities, evaluated with the PEPS-C, than the typical child. Moreover, the major problem of the child with AS was regarding the knowledge about language rules which seems to be incomplete in its linguistic foundations.

These patterns of impairment are very common in children diagnosed with ASD. As such, it is essential to create and develop interventions that address these domains, with regard to enhance the prosodic and pragmatics abilities in these children discourse which, in turn, will show benefits in its social interaction. 4

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# The exercise of parenting by mothers victims of domestic violence

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Domestic violence is a violation of human rights which, according to statistics, affects mainly women and children. Some studies have shown that children raised in these contexts are more likely to become victims or abusers when they reach adulthood. An important factor that may protect these children and, therefore, prevent the perpetuation of violence is the parenting practices and styles used by the mother. With that in mind, the aim of this study was to analyze the impact of marital violence on the parenting of victimized women, not only in terms of their beliefs and practices, but also in terms of their parenting practices, particularly in what concerns the use of physical punishment, comparing them with women who are not victims of domestic violence.

We used a quantitative method and applied three inventories to a sample of 64 participants. The inventories used were: “Inventário de Violência Conjugal (I.V.C.)” (Machado, Matos & Gonçalves, 2000b) [1], “Inventário de Práticas Educativas Parentais (I.P.E.)” (Machado, Gonçalves & Matos, 2000) [2] and “Escala de Crenças sobre Punição Física (E.C.P.F.)” (Machado, Gonçalves & Matos, 2000) [3] in order to assess: a) the degree of physical and emotional violence perpetrated against them within the marital relationship, b) the parenting practices and c) the beliefs underlying the tolerance of the use of violence as an educational practice. This sample was divided into two distinct groups: women victims of domestic violence (n = 32) and women non-victims of domestic violence (n = 32).

Overall, the comparative analysis of data collected from the two groups of participants, showed significant differences in relation to the practices and beliefs that guide the parenting performance. Specifically, it was found that victims of domestic violence use more physical punishment than mothers not victims of domestic violence. It was also found that mothers who are victims of domestic violence differ from mothers who are not victims of domestic violence by the presence of some specific beliefs, particularly about the normality and efficiency of the use of violence. We discuss both the results and implications of these findings for the practice of preventive psychological intervention with victims of domestic violence.

Keywords: Domestic violence; parenting ; physical punishment ; beliefs ; behaviors.

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# The Erasmus students at FLUP: experiences and perceptions

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This research focuses on the theme of the Erasmus students, aiming to analyze their practices and perceptions during the exchange program. The study of these experiences is a sociological challenge, since these students face a completely different cultural setting or, in Henslin's words, face a *cultural chock* [1]. Thus, this investigation tackles with such concepts as *globalization* [2], *social integration* or *cultural diversity* [3].

The methodology of this investigation was based on a mixed qualitative and quantitative approach, although positioned on a hypothetical-deductive logic. First, at an exploratory basis, we performed a set of direct observation sessions to our target audience: the students doing an Erasmus program at FLUP during the school year of 2010-2011. Still at an exploratory level we conducted three interviews. On a second moment we applied an inquiry to 57 Erasmus students at FLUP during the second semester of the school year in analysis. Lastly, we conducted a set of five more extensive interviews, to deepen the conclusions of the inquiry.

Our results showed that the sample was feminized and mainly from European countries. Relatively to their involvement in classes, results shown that these students usually do the assignments, but lack in oral participation, which is a direct result of only a few of them learning Portuguese during their semester abroad. Also, when having to work in-group, Erasmus students choose more often to not work with Portuguese students. In terms of social support, these students feel more support from other Erasmus students and from the teachers, in comparison to Portuguese students. On a cultural level, they didn't feel incompatibilities or discriminations, usually attended parties or sport events, but at the same time usually created intra-group friendships with other Erasmus students. Their overall perception of the experience was very good.

In regards to the main conclusions, it was notorious that the mother-language and the birth country of the Erasmus student didn't create significant differences in-between these students in regards to academic and social integration. On the other hand, we were able to realize that the general perception of the exchange program wasn't significantly influenced by the social, educational, economical and cultural capital of the students.

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# Beliefs and representations in dentistry: A small study with children and elderly people

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Our aim developing this study was to examine and better understand the beliefs and representations that children and elderly people have towards dentistry.

On the one hand, beliefs are preconceived ideas in which people believe that shape their own attitudes and actions. On the other hand, representations are reality interpretation schemes which organize the relationship between the individuals and the world. Taken together, they both form a psychological meaning system capable of orientating peoples' actions in society and influencing their feelings, thoughts, and behaviours [1,2].

Accordingly, previous studies [3,4] showed that beliefs and representations about oral health and oral health care are strongly related to the patients' perception about their own experience in previous dental treatments, and also that these perceptions contribute to the establishment of certain attitudes towards dental care, which in turn influence treatments' results. Also, some studies suggested that those beliefs and representations change across the life-span [4,5]. So, it is important to recognize that, for instance, children's beliefs and representations about dentistry differ from those of older people.

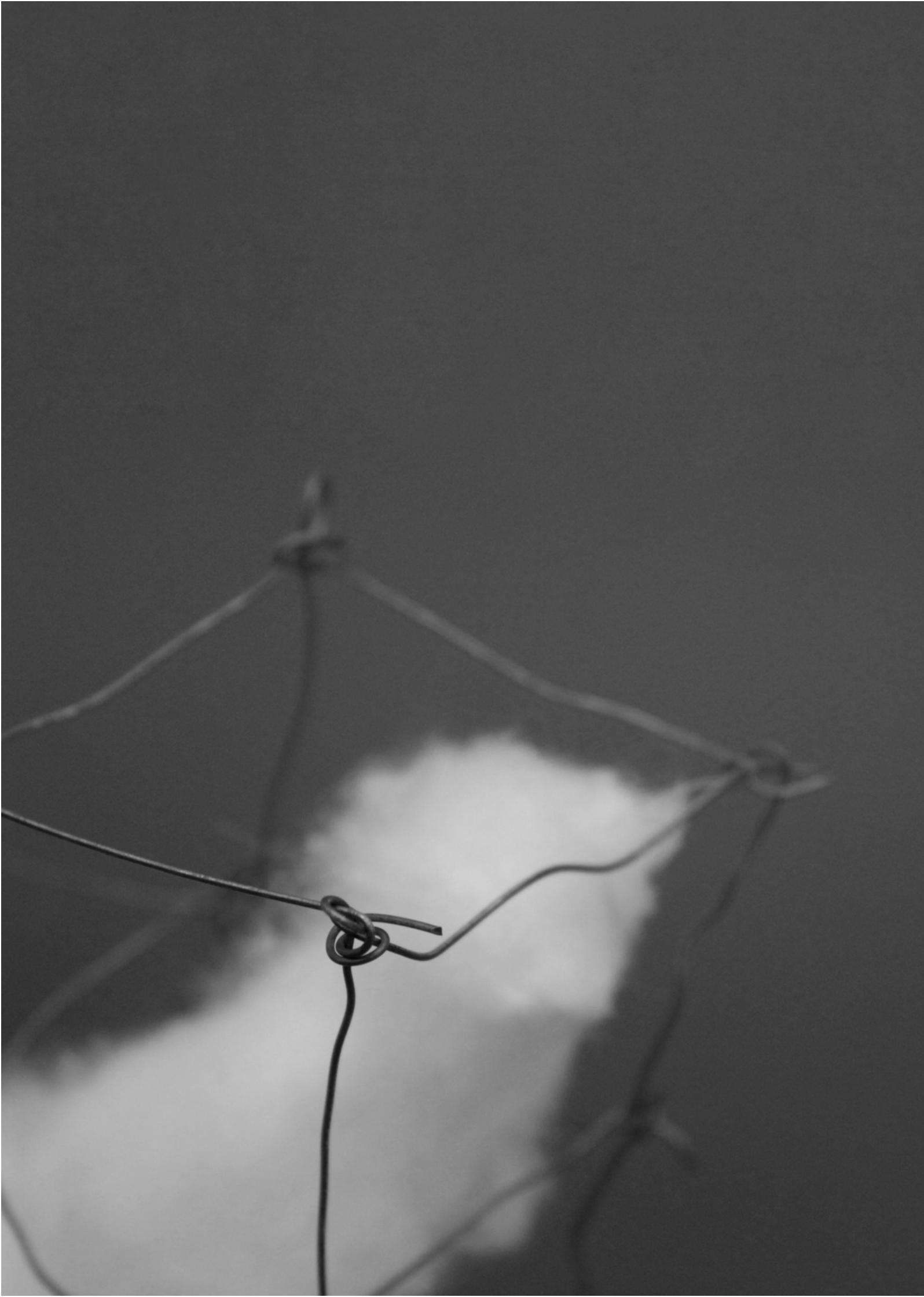
Participants were 30 children aged between 6 and 12 years-old and 30 adults aged more than 56 years-old. Beliefs and representations were assessed using a questionnaire.

The main results showed that all participants believed that it is important to visit the dentist regularly and that their dentist treats them well. Most of them had sometimes felt pain, but they did not think that going to the dentist was painful. Overall, they trusted their dentist and did not fear dental treatments, but the older participants reported more negative experiences in the past compared to children and they were more concerned with aspects such as the possibility of feeling pain again. Our discussion suggests that sociocultural factors, personal experiences and life events are important determinants for the construction of beliefs and representations related to dentistry, while they may also explain the differences found between children and elderly people.

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**ORAL SESSIONS**

**IV**



Oral Sessions ▶ A1

# Biomedicine IV



# Effects on the host cell cycle induced by *Listeria monocytogenes* infection

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*Listeria monocytogenes* (*Lm*) is Gram-positive human foodborne pathogen that causes illness in high-risk groups, including elderly, immunocompromized individuals, pregnant women and neonates. This intracellular facultative bacterium is able to invade, survive and multiply inside phagocytic and non-phagocytic cells [1,3-5], leading to the development of *listeriosis*, a disease characterized by septicemias, meningitis, meningo-encephalitis and abortions [1,2].

To promote cellular infection *Lm* is able to interfere with and manipulate a number of biological processes. It explores the functions of cellular receptors to induce its internalization, escapes autophagy, controls the expression of the host genome and uses the actin cytoskeleton polymerization machinery to disseminate. However, *Lm* capacity to interfere with the host cell cycle was never been reported, as it was to other bacteria like *Escherichia coli*, *Helicobacter pylori* or *Chlamydia trachomatis*.

We thus investigate whether *Lm* is able to manipulate the cell cycle progression during infection. Two different cell lines, Caco-2 and Jeg-3 cells, were infected with *Lm* during 17 h. Cells were fixed with 1% paraformaldehyde followed 70% ethanol and cellular DNA was stained with propidium iodide. The percentage of cells in each phase of the cell cycle was assessed by flow cytometric DNA histogram analysis. Data resulting from infected and non-infected cells were compared.

Analysis of DNA histograms of infected Caco-2 and Jeg-3 cells, revealed a significant increase in the percentage cells in S-phase as compared to non-infected cells, and a concomitant decrease in the percentage of cells in G1/G0.

These results suggest that *Lm* interferes with the host cell cycle probably retarding infected cells in S-phase. We hypothesized that this delay on cell cycle during this phase is important to create the suitable conditions for colonizing the host and favors the overall *Lm* infection capacity.

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# **Chronic food restriction increases the number of NPY-containing neurons in the hippocampal dentate gyrus of aged rats**

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It is well known that prolonged dietary caloric restriction increases both the mean and maximum lifespan and retards the development of the metabolic, structural, and physiological alterations that are normally associated with aging in several animal species. The restriction of calories has also been demonstrated to reduce or delay the incidence of several neurodegenerative diseases in adulthood and senescence and to protect neurons after metabolic and ischemic insults. In the present work, we intended to analyze the effects of prolonged food restriction on the NPY expression in the dentate gyrus of aged rats. NPY was chosen because it has important roles in the regulation of various functions including circadian rhythms, feeding behavior, blood pressure, emotion and memory and also because there is evidence that the expression of this neuropeptide undergoes a dramatic reduction with aging in several brain regions, including the hippocampus.

Male Wistar rats were individually housed and maintained throughout the experiment under room temperature and a daily photoperiod of 12 h. Young control rats (2-month old) were fed for 6 months ad libitum with standard rodent laboratory chow. Aged rats (18-month old) were randomly assigned to an aged control group and to an aged food-restricted group. Aged control rats were fed for 6 months ad libitum with standard rodent laboratory chow and food-restricted rats were fed 60% of the calories consumed by control animals every day for 6 months. All rats had free access to water throughout the experimental period and were weighted at 2-week intervals. After treatments the animals were perfused and processed for immunocytochemistry. The number of NPY-immunoreactive neurons was estimated using stereological methods. The handling and care of the animals followed the Principles of Laboratory Animal Care (NIH Publication No. 86-23, revised 1985) and the European Communities Council Guidelines in Animal Research (86/609/UE). All efforts were made to minimize the number of animals used and their suffering.

It was found that the total number of NPY-immunoreactive neurons in the hilus of the dentate gyrus was dramatically decreased in old rats when compared to young rats. Interestingly, it was also found that the reduction of the number of NPY-immunoreactive neurons was significantly less severe in food-restricted old rats when compared to old control rats.

Our results corroborate previous works demonstrating that aging induces dramatic reduction of the number of NPY-containing neurons in the hippocampus. Furthermore, the present results also show that food restriction appear to have an important role in the prevention of aging-related neuronal degeneration of NPY-containing neurons.

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## Early neurite outgrowth and mitochondrial dynamics in cortical and striatal neurons: effect of epigenetic modulators

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Lysine deacetylase (KDAC) inhibitors are epigenetic modulatory drugs, with potential therapeutic application in neurological disorders [1]. Still, their efficacy and neuroprotective mechanisms remain controversial. In the present study we compare two neuronal populations with epigenetically encoded differences: cortical vs. striatal neurons. We assess morphological differences in early neurodevelopment and mitochondrial distribution and test how selective KDAC inhibitors affect these parameters.

Primary rat cortical and striatal neurons assayed at 1 day in vitro following 24h exposure to selective KDAC inhibitors (AGK2, EX527, and tubastatin A). Neuronal branching and mitochondrial distribution were assessed by epifluorescence microscopy in neurons labelled with Fura-2 and TMRM<sup>+</sup>.

The following parameters were significantly higher in cortical vs. striatal neurons: Neurite density (summed neurite length (μm)/soma); Total number of primary neurites/soma; Total number of secondary neurites/soma; Ratio of secondary/primary neurite number; Average primary neurite size; and the Summed primary neurite length.

The SIRT1 inhibitor EX527 decreased the primary neurite size in striatal neurons. The SIRT2 inhibitor AGK2 decreased the distance of secondary branching points to soma in striatal neurons. The HDAC6 inhibitor tubastatin decreased neurite density in striatal neurons and the total number of secondary neurites/soma. Preliminary data suggests that tubastatin may increase mitochondrial fractional occupation (summed neuritic mitochondrial length/neurite length) in striatal neurons.

In summary, young cortical neurons display more extensive and complex branching than striatal neurons. Treatment with AGK2, EX527 or tubastatin decreased neuronal branching in striatal neurons. Possible effects of tubastatin on mitochondrial dynamics may explain an increase in mitochondrial fractional occupation.

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# Genotyping of *APOE* gene in patients with Alzheimer's Disease and with Frontotemporal Lobar Degeneration

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For over 20 years, the  $\epsilon 4$  allele of the gene *APOE* has been reported as a risk factor for Alzheimer's disease (AD) and related to early onset of dementia associated with amyloid plaques.<sup>1</sup> Some studies have suggested that  $\epsilon 4$  might also be a risk factor in frontotemporal lobar degeneration (FTLD). A recent meta-analysis demonstrated an increased susceptibility in  $\epsilon 2$  carriers to develop FTLD, however, the role for *APOE* alleles in FTLD is still unclear.<sup>2</sup>

The objective of this study was to investigate the presence of *APOE* alleles  $\epsilon 2$  and  $\epsilon 4$  in patients with FTLD (n=47) and AD (n=30) and compare this data with meta-analysis of Brazilian studies for controls (n=360).

FTLD and AD were diagnosed by Neary<sup>3</sup> criteria and NINCDS/ADRDA criteria, respectively. Genomic DNA was extracted from peripheral lymphocytes. *APOE* polymorphism genotyping was based on differential amplification by means of Real Time PCR.<sup>4</sup>

The presence of a copy of the  $\epsilon 2$  and  $\epsilon 4$  alleles were calculated in patients with FTLD ( $\epsilon 2$  =23.4%;  $\epsilon 4$  =25.5%), patients with AD ( $\epsilon 2$  =6.7%;  $\epsilon 4$  =33.3%) and controls ( $\epsilon 2$  =13.3%;  $\epsilon 4$  =24.7%), however, statistical differences (chi-square) were not still obtained due to small sample.

Preliminary data reasserts the role of the  $\epsilon 4$  allele in AD and indicates that the presence of a copy of  $\epsilon 2$  allele may have an implication in FTLD.

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# Prevailing inhibitory A<sub>1</sub> over facilitatory A<sub>2A</sub> adenosine tonus contributes to neuromuscular tetanic failure in rats with Experimental Autoimmune Myasthenia Gravis (EAMG)

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*Myasthenia gravis* (MG) is an autoimmune disease that affects the neuromuscular transmission due to autoantibodies raised against muscle-type nicotinic acetylcholine receptors (nAChRs) at the motor endplate. At the neuromuscular junction (NMJ), adenosine plays a dual neuromodulatory role via the activation of presynaptic inhibitory A<sub>1</sub> and excitatory A<sub>2A</sub> receptors [1], which activity is highly dependent on the nerve stimulation pattern [2]. We demonstrated that tonic activation of A<sub>2A</sub> receptors on motor nerve terminals may overcome neuromuscular tetanic fade during high stimulation frequencies [3]. Hence, we hypothesized that activation of excitatory A<sub>2A</sub> receptors could be a relevant therapeutic strategy to overcome neuromuscular transmission deficits observed in MG. In this study, we used a model of experimental autoimmune myasthenia gravis (EAMG), which was generated by immunizing Wistar rats with the R97-116 peptide, a synthetic peptide corresponding to a specific region on the  $\alpha$  subunit of the rat nicotinic AChR, made up in a solution containing the Complete Freund's Adjuvant (CFA) [4]. Thirty days after the first inoculation, the animals were boosted with the R97-116 peptide made up in the Inactive Freund's Adjuvant (IFA). Control animals received the CFA emulsion without the peptide; animals from the naive group were not submitted to any kind of treatment. Clinical scoring was based on the presence of tremor, hunched posture and fatigue; muscle strength was assessed by the grip strength test (BIOSEB, France) (see e.g. [4]). EAMG animals were screened for signs of immunological and neuronal imbalance and compared with control (only injected with CFA) and naive (no treatment) littermates. As expected, EAMG animals exhibited (1) an increase in the activity of adenosine deaminase (ADA) in the serum; (2) a reduction of FOXP3 expression in CD4<sup>+</sup>CD25<sup>+</sup>T-cell populations (Treg) isolated from lymph nodes (flow cytometry analysis); (3) tetanic failure (fatigue) of diaphragm muscle contractions produced by indirect phrenic nerve stimulation with intermittent 50 Hz-bursts, and (4) synaptic disorganization of motor endplates detected by immunofluorescence confocal microscopy. In this respect, fluorescence labeling of motor endplates of EAMG rats showed abnormal swelling of axons and myelin, atypical tortuosity and branching of motor nerves, enlargement of motor endplates and widening of synaptic clefts, as compared with control and naive animals. Despite the fact that we observed no changes in the A<sub>2A</sub> receptor immunolabeling of motor nerve terminals of EAMG animals, activation of the A<sub>2A</sub> receptor with the selective agonist, CGS21680C (3 nM, -20±9%, n=3) and inhibition of ADA activity with EHNA (50  $\mu$ M, -15±13%, n=3) failed to modify [<sup>3</sup>H]-ACh release from stimulated motor nerve terminals in EAMG animals. These findings contrast with the facilitatory effects observed with the two compounds in control and naive rats. In the absence of effects mediated by excitatory A<sub>2A</sub> receptors, we observed that the adenosine tonus in myasthenic animals was predominantly inhibitory via the activation of A<sub>1</sub> receptors. This was inferred since inactivation of endogenous adenosine with ADA (0,5 U/mL, +38±11%, n=2) mimicked the facilitatory effect observed upon blocking selectively the A<sub>1</sub> receptor with DPCPX (10 nM, +28±22%, n=2) in EAMG rats. In conclusion, data indicate that functional impairment of adenosine A<sub>2A</sub> receptor-mediated facilitation of ACh release may contribute to neuromuscular transmission failure in EAMG animals by shifting adenosine tonus to a prevailing inhibitory response operated by presynaptic A<sub>1</sub> receptors.

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# Green tea catechins prevent detrimental changes in the dendritic trees of aging hippocampal neurons – a Golgi study

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**Introduction:** Aging is associated with oxidative stress-related biochemical alterations that cause progressive neuronal damage. The hippocampal formation is particularly vulnerable to these degenerative events resulting in deleterious morphological modifications accompanied by behavioral impairments. Catechins, present in beverages such as the green tea, display strong anti-oxidant and anti-inflammatory properties that we have previously found to protect the hippocampal formation from oxidative stress damage and behavioral deterioration. Epigallocatechin-3-gallate (EGCG) is the major catechin in green tea, although it also contains other catechins and bioactive compounds such as theanine and caffeine. In the present work, we intended to analyze the effects of the prolonged consumption of green tea and a purified green tea extract without EGCG on the structure of the dendritic trees of hippocampal CA1 pyramidal cells.

**Methods:** Male Wistar rats aged 12 months were treated with green tea (n=4) or a catechin extract without EGCG (n=5) until they were 19 months old and compared with age-matched controls (n=4). Ten Golgi-impregnated CA1 pyramidal cells were sampled and the apical and basal dendritic trees were drawn, with the aid of camera lucida. The dendritic segments were allocated according to their different length distributions, i.e. terminal and intermediate segments. The length of each type of segment was measured and the total dendritic length was calculated as sum of the length of all dendritic segments. The mean segment length of the terminal and intermediate segments was also calculated. The number of each type of segments was counted, as well as the total number of segments per cell. Sholl analysis (concentric circles method) was also performed to characterize the dendritic density of the apical and basal dendritic trees.

**Results:** The apical dendritic trees of the catechin extract group of rats presented a statistically significant higher total dendritic length when compared to age-matched controls. The total number of segments of the basal dendritic trees was also significantly higher when compared to controls. Also, the total length of the terminal segments in the apical trees of the animals that consumed catechin extract was higher when compared to controls. Furthermore, the rats that consumed green tea presented favourable changes in the structure of the dendritic tree. Sholl analysis also revealed that the density of dendritic segments of the apical and basal trees was higher in rats that consumed the catechin extract.

**Conclusion:** Our results demonstrate that the consumption of green tea catechins protected the basal and apical dendritic trees of CA1 pyramidal cells from aging-related declines. Furthermore, EGCG is not essential for the neuroprotective effects of green tea on the hippocampal formation of aging rats.

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# TRAIN : Trace element in human brain: age-related changes and anatomic region specific differences

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Although its causes are not yet well defined, neurodegenerative diseases have been subject of intensive research in last decades in an attempt to understand the mechanisms underlying the neurodegeneration processes. The etiology of these diseases is multifactorial, being assumed that it involves a complex interaction between the (natural) aging, genetic predisposition and environmental factors. One of the factors identified as responsible for their development is the alteration of homeostasis of some trace elements (TE), namely, copper, iron, manganese and zinc in certain areas of the brain. The goals of this work were to study, directly in human brain samples: 1) the regional anatomic differences of TE levels within the brain; 2) the changes on TE levels in relation to age; and 3) the differences in TE levels between individuals with and without evidence of ND.

From each neurologically and psychiatrically healthy individual submitted to autopsy ( $n=44$ ) the following 14 areas were sampled: (1) frontal cortex; (2A) superior and (2B) middle temporal; (3A) caudate nucleus, (3B) putamen, (3C) globus pallidus; (4) cingulated gyrus; (5) hippocampus; (6) inferior parietal lobule; (7) occipital lobe; (8) midbrain; (9) pons; (10) medulla; and (11) cerebellum. Samples from individuals with previous diagnosis of Alzheimer's ( $n=2$ ) and Parkinson's disease ( $n=1$ ) were also collected. After microwave-assisted acid digestion of the samples, copper, manganese and zinc levels were determined by Inductively Coupled Plasma Mass Spectrometry and iron levels by Graphite Furnace Atomic Absorption Spectrometry.

Iron (mean  $\pm$  sd:  $343 \pm 65$   $\mu\text{g/g}$ ) was the most abundant metal, followed by zinc ( $53 \pm 4$   $\mu\text{g/g}$ ), copper ( $21 \pm 5$   $\mu\text{g/g}$ ) and manganese ( $1.3 \pm 0.4$   $\mu\text{g/g}$ ). It was found that distribution of TE within brain tissue is not homogeneous. Zinc highest levels were found in hippocampus and middle temporal region; highest levels of the other three TE were found in basal ganglia. Pons and medulla were the regions with lowest levels of the four TE studied. In specific areas, TE levels seem to be age-related: it was observed a positive correlation for iron, manganese and zinc but a negative trend for copper.

When compared with non-diseased people of the same age sub-group, individuals affected by Alzheimer's disease showed significantly altered TE levels in regions mainly related to memory and learning. In a Parkinson's disease patient high iron levels in brain areas related to motor and movement control functions were observed.

Since no updated and comprehensive data about TE in human brain are available, particularly with this detail regarding to the anatomical regions sampled, this study is a relevant scientific contribution for establishing "normal" human brain levels, allowing a comparison with the levels found in neurodegenerative diseases in an attempt to clarify TE role in the disease process.

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Oral Sessions ▶ A2

# **Biological Sciences I**



# Biodistribution and toxicity of gold nanoparticles following intravenous injection in Wistar rats

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Gold nanoparticles (AuNPs) offer great biomedical potential as immunodiagnostic, drug/gene delivery and contrast agents [1]. A significant effort has been done to develop surface coatings in order to increase AuNPs stability and biocompatibility, which are crucial for their successful implementation into the clinical setting. The pentapeptide CALNN (cysteine-alanine-leucine-asparagine-asparagine) has been found to effectively convert citrate-stabilized AuNPs (Cit-AuNPs) into stable, water-soluble AuNPs, with some chemical properties analogous to those of proteins [2]. We have recently reported that CALNN capping significantly increased hepatic accumulation of AuNPs comparing with Cit-AuNPs, at 24 h after a single i.v. injection in the rat [3]. Therefore, this study aimed at providing further insight into the kinetics and toxicity effects of Cit-AuNPs vs CALNN-AuNPs (16 nm), after a single i.v. injection (0.7 mg Au/Kg) in the rat.

Male Wistar rats (150-300g; n=5/group) were divided into control (0.9% NaCl), Cit-AuNPs and CALNN-AuNPs groups. At 30 min and 28 days after injection, the distribution of AuNPs was evaluated based on the Au tissue content measured by Graphite Furnace Atomic Spectroscopy. The animals were kept in metabolic cages and animal weight, food and water intake were recorded daily. Also, feces were collected and assayed for Au excretion. At the end of the study, the rats were sacrificed and blood samples collected for hematology and serum ionogram analysis. Toxicity of the AuNPs was also investigated by determining the organ indexes.

No changes in body weight, food and water intake were detected in Cit- and CALNN-AuNPs-treated animals comparing with control rats. The pattern of AuNPs distribution was very similar in the two assessed time-points, either in Cit- or CALNN-AuNPs-treated rats. Both AuNPs were quickly removed from the bloodstream and preferentially accumulated in the liver. However, Au blood levels detected 30 min post-injection of CALNN-AuNPs were significantly lower comparing with Cit-AuNPs injected rats, suggesting that CALNN capping may not be effective in increasing the plasma half-life of the AuNPs. Twenty eight days post-injection, the liver remained the main accumulation site but at significantly lower levels compared to those found at 30 min after injection, suggesting elimination of the AuNPs. This hypothesis was confirmed by the presence of Au in 5-day fecal samples of AuNPs injected animals. In addition, the spleen index of CALNN-injected rats was significantly lower comparing with control rats, at 28 days post-injection. The hematological findings revealed signs of slight anaemia in CALNN-AuNPs rats. On the other hand, no changes in the ionogram were found in Cit- and CALNN-treated rats. Our results confirmed the liver as the preferential organ for accumulation of Cit-AuNPs and CALNN-AuNPs.

Under our experimental conditions, surface coating seems to have more impact on the toxicity rather than on the biodistribution of the NPs. Nevertheless, further investigation is necessary to better assess and understand the potential long-term effects of these AuNPs.

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# Comparing the response of the brown shrimp *Crangon crangon* (Linnaeus, 1758) to prolonged deprivation of food in two seasons

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The brown shrimp *Crangon crangon* (Linnaeus, 1758) is a highly abundant epibenthic crustacean along European coastal waters from Norway to Morocco and throughout the Mediterranean and Black Seas [1,2]. With such large geographic distribution and being a species that spends most of the life cycle in estuarine environments, *C. crangon* is exposed to great range of abiotic conditions and food availability. Food availability changes seasonally with the system's productivity and thus it often occurs periods of food scarcity like during winter time. Additionally crustaceans often undergo periods of starvation due to physiological processes like moulting [3,4]. This study evaluates how the brown shrimp *Crangon crangon* (L.) responds to prolonged deprivation of food in two seasons of the year, and how this species mobilizes its energetic reserves. Shrimps caught in June (summer) and October (autumn) 2010 in Minho estuary (North of Portugal) were placed in individual cages in experimental aquaria and kept in starvation until the last shrimp died or was sacrificed (six shrimps per aquarium every week). The caloric content, total lipids and total proteins were compared between seasons, sacrificed and naturally dead shrimps, and weeks of starvation. Summer shrimps were proven to be better prepared to endure stressful situations than those caught in autumn: they survived 2.5 times longer, had a higher Fulton's condition factor and higher caloric, lipid and protein content at the beginning of the experiments. During the first week of starvation the percentage of total proteins decreased significantly and stabilized in the next four weeks to decrease again abruptly in the fifth week. The percentage of total lipids only started to decrease after four weeks. This suggests that, on one hand, *C. crangon* probably uses stored proteins as a first energetic recourse and after that carbohydrates and eventually lipids, but at much lesser extent; and on the other hand, that after four weeks under starvation a critical point is reached when structural components might be mobilized to pay for maintenance costs.

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# Cannibalism in the brown shrimp *Crangon Crangon*

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The brown shrimp *Crangon crangon* (Linnaeus, 1758) is a very common crustacean in the European shallow coast from Norway to Morocco and in the Mediterranean and Black Seas. Due to its high abundance the species is a very important component in the ecosystem's trophic web, both as prey and predator. The brown shrimp is considered an opportunistic predator, feeding on the most available prey species within a list of more than 20 organisms [1]. However, cannibalism is very common in this species and might account to up to 20% of its diet [2]. In fact, cannibalism might be a result of the species' opportunistic behavior. Nevertheless, no information exists on size selectivity i.e. till which size proportion can a cannibal-shrimp feed on a conspecific.

The objective of the present project was then to study the size relationship between the potential cannibal-shrimp and its "prey-shrimp". For this an experimental design was set with two observation aquaria, each divided in 32 compartments by an acrylic structure. The temperature (13 to 15°C) and water salinity (26 to 30) were controlled on a daily basis. The photoperiod during the observations was 12/12h. Shrimps of known size were divided into 16 sets of two groups: a single "cannibal-shrimp" and five "prey-shrimps" of the same size but smaller than the "cannibal", each group placed separately in one compartment. The predator was kept in starvation during 48h before the observations. After this 48h period, the "cannibal" was transferred into the preys' compartment along with an extra dead shrimp with the size of the other preys. After a 24 hour period, the animals were separated, measured in case of moult, and the sex of the "cannibal" was identified. The number of the remaining "prey" shrimps was also registered.

Preliminary results suggest that when cannibalism occurs the dead animal is the first to be eaten, confirming the opportunistic behavior of brown shrimps. Only 5,43% of the "cannibal" shrimps did not eat the dead prey. It seems that "cannibals" much larger than the prey might eat more than one or two smaller shrimps. However, some additional observations are missing to cover all size classes in order to conclude on the size relationship between "cannibal" and its prey.

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## How do reproductive hormones affect the early oocyte stages in zebrafish?

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Oogenesis in zebrafish comprises five stages. Oocytes are strictly connected to endocrine cells that form two layers (granulosa and thecal layers), which have a crucial role in the oocyte development. Most of the studies in this area focused on the later stages of development, namely the vitellogenic (III) and matured (IV-V) oocytes.

The present work aimed to get further information about the early stages of oocytes, the ones in the folliculogenesis (I) and cortical (II) phase. Three reproductive hormones, E2 (17 $\beta$ -estradiol), 11-KT (11-ketotestosterone), DHP (17,20  $\beta$ -dihydroxy-4-pregnen-3-one) and a secondary messenger cAMP (cyclic adenosine monophosphate) were studied by exposure to oocytes of stages I and II in an *in vitro* system for 24h. The ultrastructural analysis was performed by transmission electron microscopy and it was observed that E2, cAMP increased the appearance of the endoplasmic reticulum in the ooplasm. The stereological quantifications showed that 11KT, DHP and cAMP decreased the nuclear volume of granulosa cells. Furthermore, qPCR results demonstrated a significant increase of expression of cyclin E, a protein present at the transition from G1 to S phase of the cell cycle. In conclusion, reproductive hormones in the early oocyte stages stimulated the intracellular activity of the ooplasm and inhibited possibly the proliferation of granulosa cells, which would suggest a dual role of locally produced hormones.

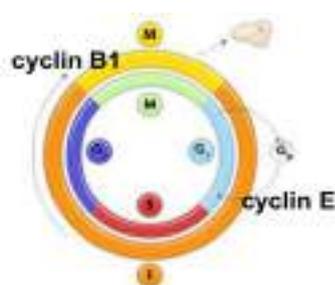


Fig.1 – Representation of the different steps of the cell cycle and places where cyclin B1 and E have a role. M – mitosis, S- synthesis, I- interphase, G0- quiescent phase, G1 – phase “gap 1”, G2- phase “gap2”.

# Review on the impact of great cormorants on European fisheries

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In Europe, the population of great cormorants *Phalacrocorax carbo* (Aves: Phalacrocoracidae) is rising since 1980 [1]; thus, becoming one of the most abundant fish predators in several aquatic ecosystems. Cormorants are mainly found near the coast and in estuaries almost all over Europe. This species reproduces in northern Europe and migrate south during winter. They gather at sunset in night roosts, leaving at dawn to their fishing grounds.

In an Estonian estuary, the number of cormorants increased from 300 to 2800 individuals in eight years, coinciding with a 100 fold decrease of natural fish populations [2]. In Finland, their Baltic Sea coast was colonized by cormorants in 1996, and since then cormorants' population increased up to 32000 individuals in 2009. Despite this tremendous increase, the stock of the main fish species was not affected [3]. In Minho estuary (NW-Iberian Peninsula) cormorants' population varies between 150 and 350 individuals, reaching higher densities during the wintering period. In this estuary, it was estimated that cormorants consumed 70% of the flounder reported to be captured by fishermen. However, since fishermen do not declare all their catches, the impact that cormorants might exert on flounder population is expected to be lower than estimated [4]. However, Minho fishermen blame cormorants for the lack of fish and urge local authorities to establish measures to control their reduced population, rather investigating other potential stressors. In England and in Wales, fishermen also have the perception that cormorants are responsible for major economic impact on fisheries, however scientists state that their complaints might be correct in very particular cases, but overall the density of cormorants does not affect fishermen catches [5].

In conclusion, the impact of cormorants on fish stocks varies markedly between aquatic ecosystems, even if they are very few kilometers apart. So, management measures on cormorants' population must rely on scientific data rather than on public perception.

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# Variation in cranial size in the Red Fox (*Vulpes vulpes*)

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The Red Fox (*Vulpes vulpes*) has one of the most wide geographical ranges among Carnivores; it is also an abundant specie in Portugal, where it is not considered threatened. Sexual dimorphism of the species is well known and is frequently evidenced by body measurements, both with body mass and linear dimensions/proportions; such variation is probably one of fox most significant characteristic [1]. Craniometrical analysis is one important tool because it can be an abundant source of information; skull morphology reflects many population/individual characteristics, genetic distance, environmental effects and factors affecting growth. The aim of the present study is to analyze fox skull dimensions and also use them in order to appraise, for the first time, sex, age and geographic variation effects in Portugal.

A total of 60 fox skulls (26 males and 34 females) were obtained during the 2010-11 hunting season, in Portugal. The twelve craniometrical measures considered were measured with a Mitutoyo digimatic caliper (0.02 mm precision for measurements of 0-100 mm, and 0.03 mm precision for measurements of 100-150 mm) or with a ruler (1 mm precision for all measurements exceeding 150 mm). Skull data were analyzed with the IBM SPSS Statistics 19. One-way ANOVA or non-parametric tests were used according to the results of the normality tests. STATISTICA 10 was used to perform a PCA analysis.

Sexual dimorphism is evident, as most craniometrical measures are bigger in males (differences being statistically significant). Only two measures showed to be bigger in females but only one showed a statistically significant difference (lyre breath) as referred for other fox subspecies (*V. vulpes abietorum* and *V. vulpes cascadenis*) [2]; bigger size in post-orbital constriction is supported by literature, in Pampean fox [3]. No significant statistical differences were found when geographical origin was considered but the boundaries used (rivers Douro and Tagus) might not be appropriate; sample size is also a probable bias source as southern sample was very small. The lack of statistical differences was also found when age was considered, although with two exceptions (inter-orbital width, post-orbital constriction and lyre breadth). PCA analysis confirmed the sexual dimorphism (the first two axis explained 71.12% of the variation).

Our results confirm the sexual dimorphism of the species but brought no evidence to any geographical variation (Bergman rule) in Portugal. It must be stressed however that our sample size might be insufficient, so further research seems necessary.

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Oral Sessions ▶ A3

# Engineering III



# Automatic Debugging of Android Applications

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In the past years, the market of smart-phones and tablets has been growing exponentially, and the number of devices of this types shipped in 2011 exceeded the number of PCs. This represents a big shift in some technological areas and implies changes and adjustments, some of them in the software development area. Although the process used in mobile applications development is barely the same, there are some resource restrictions and particularities, inherent to embedded nature of this devices, that have to be taken into account. Locating software components which are responsible for observed failures is the most expensive, error-prone phase in the software development life cycle[1], and although there are some automatic fault localization techniques that have been proven effective, their application to mobile devices represents a big challenge because of the restrictions mentioned.

Spectrum-base fault localization (SFL) is one of this techniques, and it is considered to be a lightweight approach[2], which can be a plus when coping with such restrictions. SFL is already applied in some automated diagnosis tools, like Zoltar[3], GZoltar[1] and Tarantula[4]. This tools rely on the instrumentation of the source-code in order to obtain code coverage traces that, after analyzed, provide a list of potential faulty locations. Nevertheless, no tools have been developed regarding the automated diagnosis of mobile applications, and so it still is a manual, exhaustive, time-consuming task. However, for this techniques to scale to the mobile applications scope, maintaining their efficiency and effectiveness, it is essential to optimize them to be the less resource-consuming possible.

This work aims to develop a tool dubbed MZoltar to be integrated with the Gzoltar Eclipse[5] plug-in. This tool should be capable of scaling to the Android mobile devices maintaining both performance and effectiveness of diagnosis. Simultaneously it should be capable of providing device specific diagnosis through a set of new visualizations similar to the ones provided by GZoltar[1]. It is expected to verify if it is possible to scale such automated diagnosis techniques to resource-constrained devices and the utility of the diagnosis offered by the tool in the development of mobile applications.

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# Support Software Fault Isolation with HTML5 Visualizations

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Visualizations make all the difference when applied to certain situations. For example in the representation of a data sample, a good visualization is able to pass a clear and concise picture of the results obtained. It allows the extraction of value and a good interpretation of the data [1].

In the last years, the association between visualizations and software projects is growing up [2], and in this case the interconnection is made between some visualizations and the debugging software process, with the main objective to facilitate and speed up the process.

This project identifies two main phases: (i) an adaptation of technologies and (ii) a human study. The debugging tool explored is an eclipse plugin [3] called GZoltar[4]. GZoltar is an innovative tool, developed at the University of Porto, for automatic testing and debugging using spectrum-based fault localization [5] and providing visual support.

Currently, an OpenGL module with two visualizations is implemented to aid and supplement the debugging process. The aim is to re-implement these visualizations using HTML5 and Javascript. This replacement is justified with the problems around the use of OpenGL technology. For instance, some incompatibilities with certain devices and graphics drivers and the fact that it is slow and heavy on more complex scenes.

The use of HTML5 is due to its universality and current use and expansion, and the Javascript will be dependent on the D3[6] library which is a powerful tool for building visualizations, and currently is very active.

The most important stage of this project arises after this adaptation of technologies, which will be made a human study of usability, to really understand how beneficial is the inclusion of visualizations in the process of debugging software. Emerging at this point is the possibility of extracting a good theoretical basis and complement for this scientific area.

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# Generating Asserts for Test Cases Efficiently

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When developing software applications, tests are needed to assure the product quality. These are mostly human made and, in the most cases, made by the same people that produced the code. This is error-prone and coping with errors in the operational phase is rather important. In other words, software systems have to be capable of detecting failures in order to recover from them. Aimed at total automation of the detection-diagnose process, there have been some studies on the utility of low-cost, generic invariants (“screeners”) in their capacity to act as error detectors[1]. Fault Screeners are simple software (or hardware) constructs that detect variable value errors based on unary invariant checking[2].

Spectrum-based fault location (SFL) is a technique used to shorten the test-diagnose-repair cycle by reducing the debug effort[3]. This technique is already applied in some automated diagnosis tools, like GZoltar[4], which will be the main focus of this work. GZoltar is an Eclipse<sup>1</sup> plug-in used to obtain a list of possible faulty locations in the source code, that after analyzed can be of great value to reduce the time and money invested in identifying this problems.

The aim of this work is to investigate and answer some main questions, such as: In the context of SFL, can we obtain better performance using fault screeners? Can we automatically reduce the number of instrumented program points (such as memory loads/stores, function arguments and return values)? In addition, an automatic framework for automatic error detection should be developed, which will be later integrate in the GZoltar toolset [4].

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# Dynamic QoS Management of an Ethernet-based Video Surveillance System

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The use of multimedia applications within industrial systems has become commonplace. An example is video surveillance which incorporates multimedia transmission and processing in a distributed environment. Normally, industrial systems rely on real time network protocols, particularly Real-Time Ethernet (RTE), to provide guaranteed low latency communication services that correspond to Constant Bit Rate channels with static QoS. However, the traffic generated by multimedia encoders is of Variable Bit Rate nature, which makes the adaptation difficult and increases bandwidth inefficiency.

In this work we address the situation described in [1], in which a dynamic number of cameras is displayed on one or more surveillance consoles. The bandwidth of the link to each of these consoles becomes a scarce resource that needs to be adequately allocated to maximize the number of visualized cameras with minimum QoS penalty. For the network we selected the FTT-SE protocol that provides adaptable real time communication channels, which operational parameters can be changed at run time.

The developed application has two main modules, one at each multimedia generator (cameras) and another one at the multimedia sink (the console). The sink computes the available bandwidth and distributes it to each channel associated with a multimedia generator. At the generator side, the compression is adapted online to make the best use of the bandwidth made available to that node. This adaptation is made using a rate-quantization model,  $R(q)$  [1], which predicts a value of bandwidth based on the video quality factor.

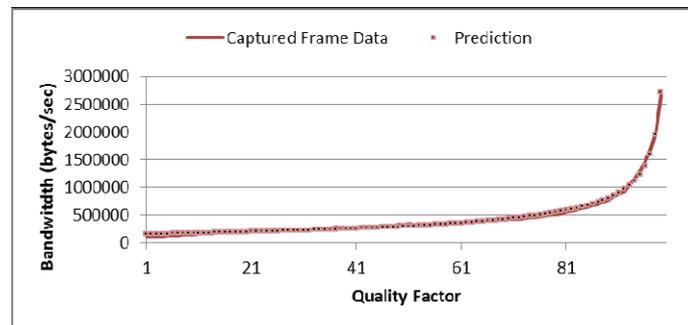


Fig. 1 -  $R(q)$  model approximation and real values

Currently, we have identified the  $R(q)$  model capturing several frames of video with different quality factors and determining the generated bandwidth using a transmission period of 60ms. The predicted bandwidth values differ from the real ones by less than 80KB/s across the whole range (Fig 1) making this model a suitable choice for the compression adaptation. The presentation will cover the whole system with more detail and disclose more experimental data on the global bandwidth efficiency.

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# Indoor Navigation Using Wi-Fi Signals Lateration

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Nowadays localization is needed for a vast range of applications, e.g. robot orientation, navigation on public buildings and geographical routing in sensors networks [1]. Some of these applications need localization indoors, where the GPS signal does not reach.

In this work we assess lateration of Wi-Fi signals [2] for indoors localization. We first developed an Android application that uses a map and collects signals information from reference points, while estimating the position of all access points in a building. With such information, a user application running on a smartphone can estimate its current position in the building based on a WPS (Wireless Positioning System) [3].

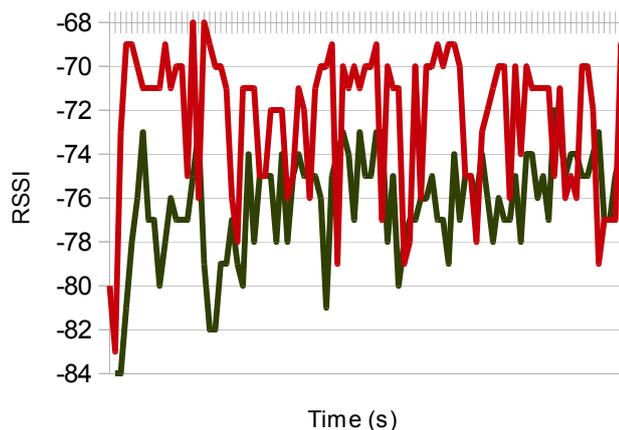


Fig. 2 – RSSI 100s traces from 2 access points

For each reference point we have captured one hundred samples of the surrounding access points to mitigate the variations caused by crossing walls and doors and by moving people [4]. Fig. 1 shows two traces of our RSSI measurements inside a building, from two distinct access points, during one hundred seconds at a rate of one sample per second.

In spite of the strong Wi-Fi signal variations inside buildings, if the environment is well populated with access points, averaging RSSI and selecting just the strongest and most stable signals can lead to acceptable lateration positioning errors that can be tolerated by a wide range of applications. We are currently carrying out a quantitative characterization of such errors within the buildings of the FEUP campus to confirm our conjecture.

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# Interfacing Vehicles Management with Smart Houses

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**Abstract:** Nowadays, the number of intelligent houses integrating smart systems and functionalities is steadily increasing, being able to provide information about the house itself as well as other important information to the residents. In this work we propose a system able to support a wireless communication channel between a smart house and a vehicle, profiting from all of the available capabilities and services in home automation. Our approach is to create a small module that is able to ensure a bidirectional communication between a vehicle and a smart house in order to extract some features and variables from the vehicle (e.g. fuel consumption, mileage, available fuel).

**Introduction:** Imagine yourself resting at home planning a trip and you want to know if the available fuel in your car will be enough. Today the solution is to get into your car and get the information directly from the car's dashboard.

The idea of this project is to create a small and affordable device which both reads all the desired parameters in the vehicle (using a connection to the EOBD/OBDII port – *On-Board Diagnostics* port [1]) and set up a low power wireless communication channel between your smart house and the vehicle, transmitting all the required data [2]. With this system, the user will be able to get the desired information using a simple handled device connected to the home network, such as a smartphone or a tablet. All the vehicle's information can be easily accessed, also providing a way to configure some of the car's parameters (GPS system, radio, etc.)

**System Description:** The system of this concept is based on a small device connected to a vehicle - providing a data extractor module and establishing the outer communication channel - and a house that integrates a home automation system with wireless capabilities. The device features a microcontroller and a communication module based on the same standard of the automation system, interfacing the OBD-II bus and the smart house communication network. The house should integrate a home automation system with wireless capabilities based on the same standard of the module. A software layer should read and interpret all the desired information, and provide an easy and simple interface to the end-user.

**Conclusion:** We set out to create an affordable system to establish a communication channel between vehicles and smart houses that can be used to transmit and receive information. This system should seal the existing gap in the communication between vehicles and home automation solutions, providing a complete, multi-platform and easy to use information system.

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Oral Sessions ▶ A4

# **Geography, Health & Crime**



# Management & Design: Synonym Sustainability in Urban Areas? Serralves Garden & Urban Park West of City of Porto

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## Abstract

Green spaces, in special take on a strategy on urban management policies of cities, since they constitute a living space of most populations, also as benefits as biological and cultural diversity. For this, becomes crucial to promoting a more efficient and rational use of its natural resources and the promotion of its aesthetic beauty.

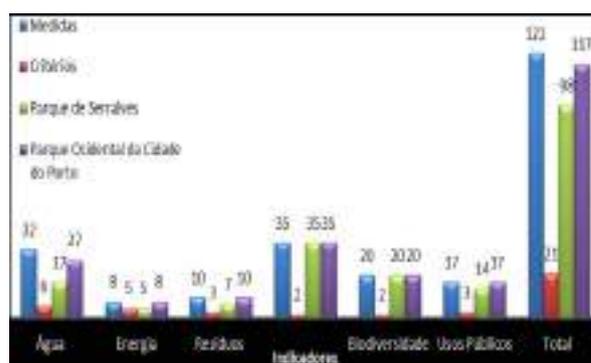
**Keywords:** Sustainability, Urban Design, Green Spaces

## Methodology

Having as main objective comparative the analysis of management in the private domain and the public domain of urban green spaces, we used as case studies the Serralves garden and the Urban Park West of City of Porto. For this, our methodology was based on three stages, particularly in developing of a grid of analysis, translated by publishing “*Criterios para una jardineria sostenible en la Ciudad de Madrid-Área de Gobierno de Media Ambiente y servicios a la Ciudad*”, encompassing twenty-one criteria, one hundred twenty-two steps and six indicators (water, energy, waste, natural resources, biodiversity and public use). We conducted fieldwork, requested meetings to obtain assistance in responding measures here referred and a survey on-line was conducted, with 82 valid responses in order to compare the final results as well as understanding the interaction of society with the parks and gardens in the study and the importance of these, hold in Oporto.

## Results/Conclusions

As a final conclusion, according to figure 1 and surveys conducted, it follows that Urban Park West of City of Porto by having an area of public management and be a public free access to the community can "contribute" to a good and balanced urban sustainability, a particular note, that it



would be interesting the applicability of the referred grid in urban management of urban green spaces in the country, more concretely, at the department of urban and green spaces in each municipality, since it becomes fundamental to attract population of different ages for this common green spaces, providing socializing and leisure activities, able to improve the mobility of people, their health and well-being as well as break the loneliness of the elderly population.

Figure 1 - Results from the environment indicators of the study case's

## References

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# Effects of oak woodland fragmentation on epiphytic lichens spatial pattern (species richness and composition) in Aboboreira region

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Nowadays, landscape fragmentation is a major concern for the conservation of biodiversity in forested landscapes because heterogeneous habitat fragments affect population dynamics and species establishment.

Lichens, being poikilohydric organisms, are sensitive to increases in light intensity and consequently they are sensitive to changes on forest ecosystems. Therefore there is an urgent need to understand how lichens species persist in highly dynamic habitats. Persistence and abundance of lichens species are determined by their dispersal and establishment ability. Species dispersal can be limited by distance and reproduction mode, and establishment can be dependent on habitat quality.

In this study we hypothesized that changes in lichens spatial pattern (species richness and composition) varies under oak woodland due to differences in patch properties. We examined 12 different sites located in the southeast region of the Marão Natural Park in Aboboreira. These sites were chosen and studied using Geographic Information Systems (ArcGIS, Map Comparison Kit, Fragstats and Conefor Sensinode). To verify the hypothesis we calculated correlation coefficients, factor and cluster analysis.

Results showed that species richness and abundance are highly correlated with core area and with the related circumscribing circle of the patches. Our study also indicated that especially the best preserved forests present well established sciophytic communities of epiphytic lichens. Nitrophytic species were found to be associated with small and more fragmented patches.

We also modelled the potential distributions of a set of selected species using maximum entropy modelling. The predictive model allowed the identification of areas that deserve further surveys.

In summary, this study points out the presence in Aboboreira of a lichen community that has suffered general decline throughout Europe and particularly of some rare species such as *Lobaria amplissima*, stressing the need to develop management actions for this area. Aboboreira oakwoods emerge as sites of high importance for epiphytic lichen preservation.

# **Maternal healthcare in Migrants: Equity and Social Determinants of Health**

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**Background:** One of the noblest challenges to developed countries is the provision of universal and equitable healthcare to migrants, regardless of gender, ethnicity or country of origin. The concept of health as a universal right is one of the keystones for social inclusion of immigrants, and a primary route of access to citizenship and civil rights.

**Objectives:** Considering pregnancy and motherhood as periods of increased vulnerability in migrant women, in order to characterize the health care provided to this population collective regarding maternal and newborn care, we considered its most important factors: access, utilization and quality of care received during the defined period, validated by the perspective of users, allowing the construction of a holistic and integrated view of this phenomenon.

**Methods:** The present research is following a mixed methodology for collecting and analyzing data (quantitative and qualitative interface). Main participants are immigrant from Eastern European countries, Brazil and PALOP (cases) and Portuguese (comparison group) pregnant women / recent mothers, contacted through civic associations and referral hospitals serving the metropolitan area of Porto. It's intended to assess whether the qualitative data collected (interviews) converge with numerical indicators (gathered through a case-control study) using triangulation techniques. A comprehensive interpretation of the resulting information will be made (content analysis of information emerged), and confronted with quantitative data contiguously collected and statistically analyzed (maternal health outcomes).

**Results:** Migration is known to have varying effects on health. Despite the existence of a risk profile associated with migrant women, some perinatal outcomes were benefited. Ongoing research can already point out that most complaints concern aspects not covered by legislation, which facilitate the interpretability of the law and its usurpation by those who firstly receive immigrants. Growing bureaucracy from the Portuguese government institutions available to supervise and regulate the entry and integration of immigrants in the country has been extremely damaging, increasing difficulties and vulnerabilities arising from the migration process. Misinformation about rights and inappropriate clarification during medical appointments often interacts with social determinants of poor prognosis among some population groups, resulting in lack of adjusted public health policies.

**Conclusions:** Special attention must be provided to immigrants' health literacy in order to improve utilization and appropriate seek of care so their needs can be addressed. New challenges in public health tend to reside no longer in assuring access, but mostly in promoting equity further than equality, endeavoring for superior quality of care for all.

# Development and validation of an individual social capital measurement tool in Portugal

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**Background:** Social capital, commonly described as being implied in norms and social networks, reveals social relationships within communities or among social groups grounded on different amounts of trust and reciprocity. The study of social capital has been showing a growing interest on health research due to its potential as determinant of health and wellbeing. Different tools for empirical measurement of social capital have been developed, but there are insufficient validated instruments for assessing social capital at an individual level.

**Objective:** To develop and validate a tool for measure individual social capital in youngsters.

**Methods:** The study was developed within a population-based cohort of urban adolescents born in 1990 and assembled at 13 years in public and private schools of Porto, Portugal (EPITeen). The present analysis included cross-sectional information of 2446 participants, collected using a self-administered questionnaire when they have completed 17 years old. The social capital scale comprised 16 items regarding formal and informal networks, civic participation, social support, trust, reciprocity and social control. The items were assessed using a four-point likert scale. Exploratory factor analysis was used to evaluate the dimensionality of the items and GEOMIN rotation was applied. Cronbach alpha was used to measure the reliability. The global goodness of fit of the underlying structure with five factors was evaluated. To assert construct validity, scale dimensions were tested according to gender, parents' education and school failure.

**Results:** Explanatory factor analysis suggested 5 factors: informal network items, formal network and civic participation dimensions, institutional social support, family social support and trust, reciprocity and social control items, explaining 61.2% of variance. Excluding the last, there was significant correlation between the factors. The items presented consistent item-total correlation (values ranged from 0.20 to 0.51). Estimated Cronbach's alpha ranged from 0.43 to 0.67. In construct validity, no significant associations were found according to gender, except for trust, reciprocity and social control factors in which boys presented a lower mean value. School failure implied lower mean values, except for family social support factor. Mean values increased with parents' education, except for family social support factor.

**Conclusion:** The present tool showed to be a reliable and valid instrument for assessing individual social capital in Portuguese youngsters.

# Typologies of intimate partner offenders

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Many authors have been studied typologies of male domestic violence offenders in heterosexual relationships (Gottman et al., 1995; Holtzworth-Munroe and Stuart, 1994; Johnson, 1995). These typologies should be considered when adapting treatment interventions to offenders' characteristics.

Holtzworth-Munroe and Stuart (1994) suggest that these offenders are heterogeneous. They reviewed fifteen previous studies about batterer typologies and regrouped them into three groups: family-only, dysphoric-borderline and generally violent-antisocial. Holtzworth-Munroe et al. (2000) tested and validated their typology empirically. Other recent studies found similar results (Johnson, 2006; Thijssen, & De Ruiter, 2011).

Currently, we are conducting a study with intimate partner male offenders condemned for domestic violence crime and complying a community sanction (n= 63). The objective is to establish a typology with different groups of intimate partner male offenders, and then distinguish each other.

We collected data through documental analysis of criminal process and we will use questionnaires (that measures frequency, intensity and severity of domestic violence, with questions about alcohol and drug use, beliefs about marital violence, emotional expressivity and regulation, social desirability and early maladaptive schemas).

To construct a typology we only we will only consider three descriptive dimensions: severity and frequency of violence, generality of violence (limited to family or outside family), and psychopathology or personality disorders. Other dimensions will be considered only to distinguish the groups among them.

We expected to find the three groups presented above and examine their different profiles in several dimensions.

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## Composite Sketch - Drawing the absent identity

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Composite Sketch - Drawing the absent identity is an essay on the problem of representation in composite imagery, found in the exercise and strategies of drawing. This study, divided in four distinct and related chapters, begins with an analysis on the history and understanding of the face, on an effort to clarify the essence of identity and the way it is perceived by society. Thus, the problem of identification is introduced in this investigation, with a particular emphasis on facial recognition and the chain of procedures that make possible the authentication of the criminal in the extent of criminal investigation. To capture the face, learn, remember and describe it, is therefore an implicit process in the making of a composite sketch (Fig. 1). For this reason, it's clear that the study of this particular drawing is not solely based on graphic representation, but on a connection between distinct fields of knowledge and different players that ultimately grapple the subject of drawing itself. The generative substance of this portrait comes to reality in the networking between the information given by who describes the face and the graphic output from the sketch artist. It's in the presence of these two elements that the identity-aiming portrait is prepared, this being only finished with the exercise of drawing or the support on the strategies of this discipline.



Fig. 1 – Interview by Flora Lichtman, 2010. Video screenshots revealing the process of making a composite sketch (drawing by Stephen Mancusi)

Oral Sessions ▶ A5

# Architecture I



# Brain and Space – The Production of Perceptual Space

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This dissertation is focused on the study of the relation between space and the self, and of the underlying mental and physical processes that allow this interaction to occur. It is intended to make the chain of events responsible for the creation of perceptual space explicit, describing the whole process of being in space and being aware of it. From the basic physical ways of perceptions to the construction of subjective places, it is proposed to link space with the self.

For this research to be possible it is necessary to reach several other fields of knowledge besides architecture. Through philosophy and psychoanalysis it is possible to refer to the theories that surround space in his basic form and the place where consciousness takes part. With the fields of biology and the cognitive sciences we can describe accurately (as they are sciences) how the natural system works with the brain.

Following a materialist theory of the philosophies of the mind, it is believed that mind has a physical support, the brain. Through the communication of brain-cells we gain conscious-awareness and the ability to act, feel and think. It gives us also the awareness of time, mental imaging and the capacity to create dreams and emotions.

Nevertheless, the brain doesn't work alone; it depends on a big nervous system that allows it to receive stimuli coming from the outside world through nervous impulses. This kind of information is responsible for our perception of the physical environment and thus the creation of mental space.

But the process of space-awareness does not come directly from this impulses. There's a whole variety of mental processes, defined by the cognitive sciences, that allow the provided (and already treated) information to be organized. Only after the perceptual space can reach the place of awareness.

“... at levels below its atomic and molecular materiality, nature is without the status of certainty; the complementarity of particle and wave, matter and spirit, provides only for indeterminate outcomes. Non locality and non-linearity are qualities at the very foundations of life, the specificity of all quantum events depending on our active participation as observers.”

Following this chain of thoughts, it is extremely important to be aware of the uncertainty of the field in question. We are not even near to reach a conclusion in this matter but happily the neurosciences are recently involved in the pursuit of more answers.

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# Parametric Urbanism: methodology for urban design

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Evolution and innovation in architecture, regarding architectural and urban ideals, is intensely related to the rise and developments in technology. The continuing demand for progress in buildings, cities or territories motivates the development in the architecture that is a result of addition and transformation of knowledge subsequently leading to an evolution in architecture in ideological and technological field. Therefore it is possible to say that recent decades produced opportunities for the development of new methodologies for the development of urban design and architectural, like the Parameterization.

This paper is focused on the subject of Parametric Urbanism: discussing / questioning about the emergence of this new architectural methodology; addressing the factors that contributed to their appearance; and seeking to understand whether this approach has benefits and whether they may or may not be a solution to urban plans.

This paper is organized into three parts: the first part provides a brief literature review on the parametric urbanism, which presents historical contextualization and technological Parameterization, its application in architecture, as well as development of Parametric Urbanism.

The second part is characterized by a practical case study of parametric urbanism of an Urban Plan in Beijing, developed in an academic context, at the University École Polytechnique Fédérale de Lausanne in 2012 under the supervision of Dr. Jeffrey Huang. From examples of parametric urbanism it is tried to identify / justify decisions defining principles and application guidance for this project.

In the conclusion, the third part of the paper, it is presented the final considerations on opposing the case study with the theoretical approach, presented in the beginning of the paper, seeking to clarify whether this new form of urban design, Parametric Urbanism, is or not a viable methodology. It is not a intent of this academic research project to endorse a use of Parametric Urbanism as single or exclusive method for developing / obtaining quality urban plans, it is however intent to address a critical view to this new practice of urban design, increasingly present in offices around the world, investigating about their ideals and goals.

## People establishment along the *Entre-Douro-e-Minho* territory in the 19<sup>th</sup> century – a case study

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The *corpus* of this research is the form of the place as a territorial unit with specific conditions of autonomy and, simultaneously, part of a complex ‘urban set’ [1] in the Iberia northwest case and, specifically, in between Douro and Minho rivers case.

The problem of city limits extension becomes a fact during the 19<sup>th</sup> century and is one of the most lasting products of the Industrial Revolution. In fact, Urbanism itself, as an autonomous subject, developed inside the intense discussion about what to do with the uncontrolled intensity growth of the city and urban influence area. But did this urbanization process overcome the local differences regionally based? [2]

Extensive and diffuse urbanization process theory has been trying to explain the social and real forms of transformation that was printed in *Low-Minho* lands, along the last decades of the 20<sup>th</sup> century. Nevertheless, it is, at least, debatable the idea that these last decades distorted the ancient pattern, which we will call a recognizable old organized system of small places sited outside the urban limit. Therefore, we are forced to tag as *influenced area* those places, which are not included in the formal city or sufficiently distant to be considered outside the system of influence.

Some of these places seem to offer us the central condition and old local autonomy (with its own way of life and regional role) anchored in an informal and non-administrative establishment of people [3]: the form and the reason of the phenomenon are key points of this discussion.

We are focusing on a logical structure of trivial units placed in Porto regional limit during the 19<sup>th</sup> century, which has to be considered as a part of an economic cluster system, at that time, mainly connected to the primary sector, i.e. agriculture.

The resources we used in this research can be located in a pair of spheres: firstly, we had to appeal to geophysical and natural influences, and secondly, we tried to decode the cultural and formal epochal circumstances, such as mobility and its respective infrastructures, social-economic activities, lands use, etc.

The knowledge of ancient practices of settlement in loose land as a justification of the current form of establishment of places can make this work be a strong contribution to the contemporary project in the space that is outside the formal city.

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# **The Levels of Architecture: Reconnection, Reflection and Redefinition**

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The subject of my research focuses on the relationship between architecture and social sciences, having as a primary goal the re-establishment of connection between the common citizen and the production of collective spaces. Contradicting the idea of architecture as a closed and unilateral discipline, that delivers a final product to its users, this research intends to demonstrate the benefits of changing the idea of 'city' from a static, consumable product into an alphabet of self-expression, mean to be used and appropriated by its inhabitants.

In order to demonstrate it, I divided my attention into three main points: the relationship between people; the relationship between people and public space and, finally, the learning process, applied not only within the architectural field, but as transmission of knowledge based in an open-source methodology.

I start by analyzing the contemporary reality of consumption and the way it has shaped the habits and beliefs of the men. The progressive separation between public and private, work and free time, aligned with the development of virtual means of communication, has increased the distance between people, and diminished our ability to communicate and relate within the city, discussing common problems and acting as a collective in order to solve them.

I intend to demonstrate the value of the critical perspective of the common citizens, with their skills, tools and points of view, but mainly to include them within a collective force, comprised by a multiplicity of values, interests and priorities that need to be discussed and reflected about, when developing an architectural project.

The second point reinforces public space as an external reflection of a collective group and intends to stimulate a sense of belonging between people and the places they inhabit. As for contemporary architecture, the results are commonly presented to the public, unilaterally, and the reaction is a subsequent bi-product, having minimum effect on the development of the city.

The role of the architect comes finally into question, as well as his skills and tools. Assuming his social role as the most important, the architect needs to understand a certain reality in its specificities, benefiting from the vision of the inhabitants as the most real and critical of all. Therefore, it becomes essential for the architect to develop, not only his specific set of skills, but also social skills, as a way to understand his role in the world, and his duties towards the development of better places, to which we all want, and inevitably, belong.

As a result, I defend a participatory vision of architecture, based on a multidisciplinary approach. Without the intention of creating a method (in itself, mutable or endless), for it would be impossible to apply it in a generalized manner, I defined a set of strategic actions that would facilitate the process, deconstructing and demystifying the design method. The citizen would, therefore, become part of it and be able to use it, in order to facilitate subsequent change and stimulate collective and positive transformations.

# **Towards a condition of *new* in architecture: of the conflict between being or not being modern starting from Álvaro Siza**

**Nuno Sousa Gomes**

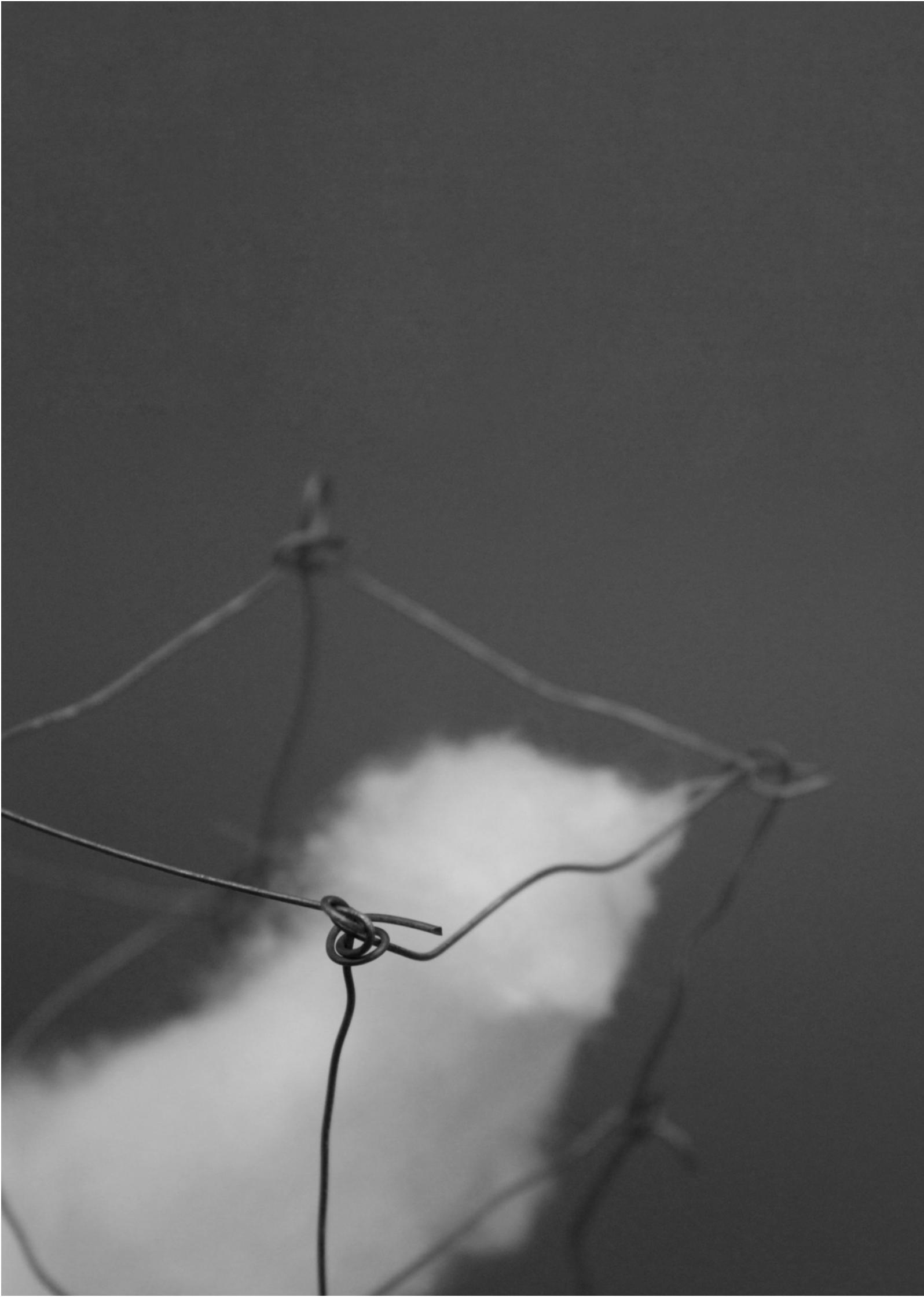
Faculty of Architecture, University of Porto, Portugal.

The work of Álvaro Siza has been widely studied and normally associated to the modern, which continues to mark in various ways the discussion within the field of architecture theory and critics. This work aims to formulate the problem of the condition of *new* in architecture, by constructing a critical discourse on Álvaro Siza's thought as it is translated into his writings, projects and built work. The aforementioned condition belongs to the scope of a necessity, a requirement, a limitation of a new existence to which architecture must provide an answer. The relevance of this subject is apparent due to the fundamental dimension of the *new* within the universe of architecture. This is particularly evident in the XX century that has been profoundly marked by a condition of rupture. In fact, the modern in general, constitutes a reference catalogue for numerous authors of the second half of the former century. But this legacy, besides being heterogeneous is understood and worked upon in rather dissimilar ways. In Álvaro Siza we find a reflection and a reference field far beyond the modern experience.

The work begins with a broad investigation focused on the issues of modernity and its subsequent critic, the Portuguese culture and architecture, the conflict between the modern and the vernacular in Portugal, "Porto School" and Fernando Távora. The problem and specific object of the present study then emerged from the aforementioned framework and therefore included a systematic analysis of all of Álvaro Siza's writings published between 1963 and 2008, his projects and built work as published by various authors.

The present study allowed to conclude that, on one hand, we can effectively find in Álvaro Siza's work references to some XX century authors that are considered to be modern. But on the other hand, we identify in this same work and written thoughts a conception which is essentially the opposite of what the modern has of its own. To Siza the *new* is not quite a problem to be framed within the historical domain because it is not a requirement belonging to a timeframe but above all it is a requirement belonging to a place. This is an idea of architecture that makes an option for the more concrete and objective reality of the programme and the territory, implicating a fundamental relationship with nature. Therefore, the present work relocates the conventional discussion between the modern and the vernacular to a confrontation between the modern and the classical, as concepts that are mutually excluding even though they may often be materialized as juxtaposed or even as coincident. Simultaneously, both extend beyond the established historical boundaries and therefore a critical contribution for the contemporary debate is proposed.

Acknowledgements: This work is based on a master dissertation made under the supervision of Prof. Manuel Mendes.



**ORAL SESSIONS**

**V**



Oral Sessions ▶ A1

# **Public Health & Epidemiology I**



# Developing a Food and Nutrition Quality Stamp for Public Restaurants in Portugal

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The creation of a food and nutrition quality stamp that enables restaurants to advertise the use of good nutritional practices and consumers to identify establishments that supply healthy dietary choices may be a potential strategy to reduce the association between inadequate dietary intake outside home and development of obesity, and increased nutritional risk [1, 2]. The objective of this work was to develop a food and nutrition quality stamp as well as the eligibility criteria, allowing their assignment to catering establishments with a view to creating a healthy restaurants network.

It was defined as working methodology the selection of information that would allow the determination of eligibility criteria for applications to the quality stamp.

The following criteria were established: prerequisites, food quality and balanced menus, portion sizes, employees' training and nutritional composition of meals.

In fact, with the growing public concern for nutrition, this stamp could lead to a competitive advantage for adherent restaurants, allowing generating a value chain, dignity and constant improvement.

Nowadays catering occupies an important role in European society, in economic and social terms. This concept is closely related to social responsibility, since the traders' awareness in relation to health issues is an essential part of the process [3].

This is a quality stamp that recognizes good food and restaurants' adequate nutritional practices in Portugal, and motivates them to continue with the commitment to always do more and better for the health of their clients, offering healthy options on their menus.

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# Ready-to-eat salads a vehicle of bacteria and clinically relevant antibiotic resistance genes

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**Background.** The increase demand for fresh vegetables is causing an expansion of the market for minimally processed vegetables along with new recognized food safety problems. The aim of this study was to analyse the microbiological quality of Portuguese ready-to-eat salads (RTS) and their role in the spread of bacteria carrying antibiotic resistance (ABR) genes.

**Methods.** RTS (n=50; 7 brands; split or mixed leaves, carrot, cornmeal) were collected in 5 supermarkets in Porto (2010). We screened for *Salmonella*, *Listeria monocytogenes* and aerobic mesophilic counts, coliforms and *Escherichia coli* (standard methods). Samples were also plated in different selective media with/without antibiotics before and after enrichment. ABR was studied by agar diffusion/E-test (CLSI/EUCAST) and detection of extended-spectrum  $\beta$ -lactamase (ESBL) by double disk synergy test and sequencing. Species were identified by PCR (Gram positive), API ID32GN/16S PCR (Gram negative). ABR genes, integron types and *E. coli* phylogenetic groups (PHG) by PCR, clonality by MLST/PFGE in *E. coli* and conjugation assays in specific isolates were performed.

**Results.** A high number of RTS presented poor microbiological quality (86% for aerobic mesophilic, 74%-coliforms, 4%-*E. coli*), despite the absence of pathogens. *E. coli* was detected in 13 samples (n=78; all types and 4 brands; PHG A, B1, B2, D) with resistance to tetracycline (72%; *tetA* and/or *tetB*), streptomycin (56%; *aadA* and/or *strA-strB*), sulfamethoxazole (50%; *sul1* and/or *sul2*), trimethoprim (50%; *dfrA1* or *dfrA12*), ampicillin (47%; *bla*<sub>TEM</sub>), nalidixic acid (36%), ciprofloxacin (5%) or chloramphenicol (3%; *catA*). Two integron types (*dfrA1-aadA*, *dfrA12-aadA*) were detected in 12 multidrug resistant isolates (MDR), which includes *E. coli* (n=2; D) belonged to the widespread clonal lineage ST69. Among coliforms, were detected 2 *Raoutella* sp (2 samples) carrying an ESBL SHV-2 and 1 *Citrobacter freundii* with a *qnrB* gene. Among *Enterococcus* (n=108; 35 samples; *E. casseliflavus*-40, *E. faecalis*-20, *E. faecium*-18, *E. hirae*-9, *E. gallinarum*-5, and *Enterococcus spp*-16) ABR was detected for tetracyclines (6%; *tetM* and/or *tetL*), erythromycin (3%; *ermB*), nitrofurantoin (1%) or ciprofloxacin (1%).

**Conclusions.** The present study positions RTS within the spectrum of ecological niches that may be reservoirs/vehicles for antibiotic resistance bacteria/genes with clinical interest (e.g. *E. coli*-D-ST69; *bla*<sub>SHV-2</sub>; *qnrB*) being these findings worthy of attention as their spread to humans by ingestion cannot be dismissed.

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# **A review of the availability and consumption of fish and seafood in Portugal**

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Fish consumption has been associated to a range of health benefits. Fish is low in fat, high in protein and an excellent source of omega 3 fatty acids. With a huge seaside border, Portugal has a privileged position for fish and seafood access. In Portugal, eating fish has not only an important dietary value but also an important role in cultural heritage. The aim of this work was to review the available data on fish and seafood supply/consumption in Portugal, evaluating both time trends and self-sufficiency.

The searched data sources included national or regional representative studies: FAO food balance sheets (FBS) (1961 to 2009), households budget surveys (HBS) (1990 to 2005) and individual consumption obtained through the EpiPorto study circa 2000, the National Health Survey (INS) (1995 to 2006) and the SPCNA/Nestlé survey in 2009. The first two sources used provide data about the availability of fish and seafood: the FBS at national level, focusing on the major food groups and the HBS at household level, listing the fish and seafood species; the individual data translates the real consumption of fish and seafood either in frequency or quantity.

In 1961 the fish and seafood self-sufficiency ratio (production/(production+imports-exports)) for Portugal was 1.01, which indicated the independence of the country for this group of products. Along time this ratio has decreased and in 2009 its value was 0.37, which is explained for the decrease in production and the large increase in the imports. However, Portugal still has a high supply of fish and seafood. In 2009 FBS, a value of 167g/capita/day was registered for Portugal, ranking the country in the 4<sup>th</sup> position among the world countries with largest supply and the 1<sup>st</sup> position among the Mediterranean countries. By contrast, the EU-27 registered only 63g/capita/day in 2009. From the 2005 HBS it was observed that around 70% of the household acquisitions of fish and seafood rely on cod fish (18.6%), hake (13.7%), jack-fish/mackerel (13.8%), sardines (9.8%), tuna (4.3%), squid/cuttlefish (4.2%), octopus (3.9%) and swordfish (2.4%). Data from the INS revealed that the proportion of people consuming fish has decreased from 55% in 1995 to 49% in 2006. The 2009 SPCNA/Nestlé survey found out similar results, a value of 49% for fish and of 5.5% for seafood. Additionally, data from the EpiPorto study reveals a daily consumption of fish and seafood of 75.6(±34.3)g/day for women and 78.6(±37.0)g/day for men.

The work to be presented will allow concluding that fish plays an important role in the Portuguese diet. However, as the characterization of food patterns is an essential tool for building shaped nutritional policies and to assess the associated food consumption environmental impacts, efforts to keep the data updated and comparable along time are necessary.

## Rainbow trouts containing *Enterococcus* with virulence factors, antibiotic and biocide resistance genes: Can your dinner be a matter of concern?

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Portuguese marketed trouts (*Oncorhynchus mykiss*) for human consumption are mostly of aquaculture origin and studies showing their contribution to the human spread of potential clinical relevant bacteria through the food chain are unavailable. Our goal was to study the occurrence of *Enterococcus* carrying virulence and antibiotic (AB)/biocide resistance genes in rainbow trouts marketed in Portugal.

Trout samples of aquaculture origin (n=27; 3 trouts/sample; muscle and viscera) were collected from 8 supermarkets (n=25) and an aquaculture facility (n=2) (May-July 2012). Samples were enriched in peptone water (1:10) and plated in Slanetz-Bartley agar with/without antibiotics. Species identification and search of genes coding for AB<sup>R</sup> (*vanA*, *vanB*, *vanC*; *tetM*, *tetL*, *tetS*, *tetK*, *tetO*; *ermA*, *ermB*, *ermC*; *aadE*), copper (3 multicopper oxidases, 2 copper export ATPases), mercury (4 *merA* sequences) and virulence (*esp*, *hyl*, *acm*, *gel*, *asa*) were done by PCR. Susceptibility to 13 AB was tested by disk diffusion (CLSI).

*Enterococcus* were detected in 81% of the samples corresponding to 7 supermarkets and one aquaculture (27 *E. faecium*-Efm, 9 *E. faecalis*-Efl, 17 *E. durans*, 2 *E. hirae*, 6 *Enterococcus* spp). They were resistant to tetracycline [43%; *tet(M)*-92%, *tet(L)*-35%, *tet(S)*-12%], erythromycin [20%; *erm(B)*-83%], chloramphenicol (7%), HLR-streptomycin (5%; *aadE*-100%), ciprofloxacin (8%), trimethoprim-2% or nitrofurantoin (3%). Multidrug resistance was detected in 7% of the isolates. Genes encoding for copper (*tcpB*-5%, *cueO*-11%) and mercury (*merA1*-2%; *merA2*-2%; *merA3*-7%) resistance were detected only in Efm and those coding for virulence in different species (*gel*-13%, Efl; *asa*-3%, Efl; *acm*-39%, Efm). AB and copper or mercury resistance genes were co-detected in 11% and 7% of the isolates, respectively.

Market trouts are vehicles of *Enterococcus* spp carrying virulence (e.g. associated with adhesion), AB and biocide resistance, which might colonize human after ingestion. Studies in aquaculture production setting are needed to better understand the source of bacteria and genetic determinants contamination found in trout samples.

# Assessment of knowledge and behaviours of nutrition, hygiene and food safety of non-teaching staff at nursery schools.

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Pre-school is the first stage of basic education in the process of training and learning of a child's life. At the nursery school, children contact daily with a group of adults that symbolize role-models. Within this set, non-teaching staff: including sociocultural animators, education assistants and cooks, which mainly provide assistance at mealtime (lunch and morning and afternoon snacks), transmit concepts of food education to children [1]. The main objective of this study was to assess knowledge, attitudes and behaviours that these professionals apply in the context of daily food, to evaluate if both knowledge and behaviour are at an acceptable level, considering the role those professionals represent to children. This cross-sectional study consisted on the application of a questionnaire, which include a Portuguese version [2] of the *Nutritional Knowledge Questionnaire*, and a set of questions from the *Questionnaire for food-service staff* [3,4]., to 270 people

A sample of 270 non-teaching staff from nursery schools at the Municipality of Vila Nova de Gaia, Portugal, was obtained. Participants, with age between 20 and 66 years ( $39 \pm 9.3$  years), shown that only basic nutrition messages are well understood, failing to understand more complex ones. More than 90% of participants know they should eat more fruit and vegetables, however, only 23.2% know the recommended amounts.

As the nutritional knowledge is low, there is no proportional relationship on translating of messages to food choices, and on associating health problems (such as cancer, hypertension and obesity) with intake of fruits and vegetables, salt and fat. The knowledge level of hygiene and food safety is good and higher than reported behaviours. The results show that for these professional, knowledge isn't transformed into behaviour, and they fail to apply it in their daily work. The assistants present more knowledge than cooks. However, cooks are the ones that receive more training; concluding that training isn't effective or misdirected.

The level of knowledge acquired is reasonable but should be improved, aiming for a structured training to fill the gaps in nutrition, hygiene and food safety knowledge, assessing constantly the transformation of the knowledge into behaviour.

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# Dental materials disinfection: knowledge and practices among Portuguese dentists and dental prosthetic technicians

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The risk of cross-infection in dental clinics and laboratories is undeniable when effective infection control procedures are ignored. Many pathogens can be transmitted in dental environment, such as streptococci, staphylococci, mycobacterium, influenza virus, human immunodeficiency virus (HIV), Hepatitis B and C virus (HBV and HCV)[1,2]. The aim of the present study was to evaluate the infection control knowledge and practices of dentists (D) and dental technicians (DT), including dental impressions disinfection, communication between dental laboratory staff and the dentist, HBV immunization and occupational vulnerability and contamination risk awareness.

The infection control practices and knowledge were evaluated by a national survey sent via email to more than 1000 D and 320 DT. Ninety-five D and 25 of DT completed the survey.

Regarding disinfection habits, only 58.5% of D always disinfect the dental impression in every situation. In contrast, 20.2% never disinfect, while 33.3% mention that the prosthetics laboratory carries out the disinfection. In relation to the DT, 62.3% always disinfect the impression although only 33.3% of these refer being afraid of contamination. On the other hand, 12.5% never disinfect the impression either by employer directive or because they do not believe it is relevant. It is also noteworthy that 82.6% of DT don't know if the D disinfected previously the dental impression. This result is in agreement with the information obtained from D in which 58.9% of the inquired reported not to inform the prosthetics laboratory about the disinfection status of the impression. Regarding the disinfection procedure for the alginate, polyether and silicone (addition- or condensation-cured) impressions, 32% of D washed the material with water followed by disinfection with glutaraldehyde or sodium hypochlorite, while 29% only washed with water. For the DT, 33% wash and disinfect the material while 6% only wash with water. Despite the different importance regarding contamination risk, 22% of D and 36% of DT consider HIV, HBV, *Candida albicans*, *Escherichia coli* and others microorganisms equally relevant in cross-infection events. Only 3% of D and 8% of DT didn't have their vaccinations up to date. Although also recommended by the current guidelines, only 23% of D and 20% of DT verified their immunization status regarding HBV.

The present study shows that there is some lack of knowledge regarding the cross-infection risk, dental impression disinfection protocols and infection control guidelines. Therefore, infection control procedures should be more rigorously applied in order to guarantee the safety and security of the health professionals and the patients.

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# **Biological Sciences II**



# Effect of fluoroquinolones on osteoclastic cell cultures performed over nano- and micro-structured hydroxyapatite

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Bone defects represent a medical and socioeconomic challenge. Autologous bone grafts are still considered as the gold standard for reconstruction of extended bone defects. The generation of bioartificial bone tissues may help to overcome the problems related to donor site morbidity and size limitation. Hydroxyapatite (HA) has been widely used as a biocompatible ceramic in many areas of medicine, mainly for contact with bone tissue, due to its resemblance to mineral bone. Due to the nanofeatures of bone tissue, new nano-HA based materials are certainly among the most promising challenges in bioactive ceramics. Recently, it was observed that fluoroquinolones have the ability to interfere with osteoclastogenesis, in standard polystyrene cell culture plates. The aim of this work is to go one step further in this issue. For that, the osteoclastogenic-modulation properties of different fluoroquinolones were addressed in cell cultures performed over HA surfaces with nano- and micro-structured topography (nHA and mHA, respectively).

The first part of this work included the validation of the cell culture model and, also, the preparation of HA samples in the form of disks. Osteoclast precursor cells were isolated from human peripheral blood, and were cultured for 21 days in the absence or presence of exogenous osteoclastogenic factors (M-CSF and RANKL); cultures were characterized through the quantification of tartrate-resistant acid phosphatase (TRAP) activity and staining. For the preparation of HA disks, the sinterization temperature used was 830 °C (nHA) and 1300 °C (mHA). The disks were analysed by scanning electron microscopy (SEM).

Results showed that the osteoclastogenic behaviour was significantly higher in cell cultures performed in the presence of M-CSF and RANKL. Regarding the characterization of the HA disks, SEM analysis revealed an average grain size of  $69.6 \pm 23.1$  nm (nHA) and  $3895.2 \pm 1343.1$  nm (mHA). In the second part of this study, the effect of different fluoroquinolones on osteoclastogenesis will be addressed in osteoclastic cell cultures performed over both nHA and mHA.

In conclusion, it is expected that the present work may contribute to a better understanding of the potential effects of fluoroquinolones on bone tissue, particularly in contexts where it is important to ensure a proper bone tissue regeneration.

## Genotoxicity of PCBs and PAHs in the germ cells of zebrafish (*Danio rerio*)

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In the last century we witnessed a huge industrialization leading to new types of pollution. Persistent organic pollutants are very stable chemical compounds that resist to photolytic, biological and chemical degradation. Therefore they can remain intact in the environment for long periods and exert a harmful effect. Studies made in the Douro River reported the bioaccumulation of PCBs in flounder (*Platichthys flesus*) and mullet (*Mugil cephalus*) and the occurrence of PAHs in marine and estuarine sediments.

In this work, we addressed the genotoxicity of two major persistent organic pollutant groups (polychlorinated biphenyl (PCB) and polycyclic aromatic hydrocarbon (PAH)) on the germ cells (stage II oocytes) of zebrafish (*Danio rerio*) using the comet assay technique. DNA damage in germ cells may be directly transferred to the coming generations, which can potentially have harmful effects. The knowledge of consequences of PCBs and PAHs exposure on these specialized cells in teleost fish species is still scarce.

In this first experiment, we analyzed two water samples from the Douro River estuary (Freixo and Fluvial) and two from the coast of Porto (Leça and Matosinhos) for their genotoxicity at the same concentrations as found in the environment. A solvent control, ethanol, and a positive group, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), were included.

Statistical differences were observed in our preliminary data for the tail moment in all four locations, as well in the H<sub>2</sub>O<sub>2</sub> group, when compared to the ethanol group, which indicated induced genotoxicity.

However, we cannot assume that the genotoxicity originates exclusively from these types of compounds, since there are further, undefined substances in the extracts of the water samples. In a second experiment, oocytes were exposed to a standard mixture consisting of two PCBs and five PAHs in different concentrations. Induced genotoxicity of this standard mixture was observed, which led us to suspect that these substances at least contributed to the overall observed genotoxicity in the analyzed water samples.

Until now, these are preliminary results and further experiments are planned; we intend to analyze by real-time PCR whether DNA repair or apoptotic mRNA expression is altered.

## Bioactivity of bacteria isolated from the marine sponge *Erylus deficiens* from Berlengas

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Oceans are the source of a large number of structurally unique natural products present in microorganisms and accumulated in invertebrates like sponges. Oceans are, thus, a potential treasure box of bioactive substances useful for the treatment of human diseases. Most biological marine resources are still unexplored contrarily to the terrestrial ones. Portugal is surrounded at east and south by the Atlantic Ocean which harbors rich biological diversity, namely around the small Berlengas islands, a habitat protected by UNESCO where the samples for this work were taken.

The aim of this study was to evaluate the potential of heterotrophic bacteria as producers of bioactive compounds isolated from the marine sponge *Erylus cf. deficiens*. The search for bioactive compounds in 264 bacteria was assayed at Fundación MEDINA. Salinity tests were performed revealing that 73% of the bacteria are strictly saline. Several screening methodologies that included different test plates were performed. In a double-faced microbial culturing pre-screening assay using Janus plates, none of the bacteria showed activity against *Candida albicans* and *Acinetobacter baumannii*, but 87.3% were active against *Bacillus subtilis*, 11.11% against *B. subtilis* and methicillin-resistant *Staphylococcus aureus* (MRSA) and 1.58% against only *S. aureus* MRSA. The liquid microfermentations using Duetz systems revealed lower inhibitions of which only 3.03% were active against *B. subtilis*, 0.38% against *S. aureus* MRSA and 0.38% against both. A new screening assay involving bioluminescence visualization was developed against *Alivibrio fisheri* and *Vibrio harveyi*. This screening using the extract from microfermentations revealed 1.14% inhibition against *A. fisheri*, 0.38% against *V. harveyi* and 0.38% against both. In the Janus system, the marine agar media MF, R2A and Starch provided the highest inhibitory numbers while in the Duetz system these were obtained for Marine Broth, MF, marine R2A and marine Starch media. The search of bioactive compounds of heterotrophic bacteria from *Erylus cf. deficiens* revealed biotechnological potential which may be of interest to pharmaceutical companies.

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# Testing the effect of parasites on the immune system of two lizard species from Morocco

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Blood parasites are present worldwide, having devastating effects in some animals, such as malaria on humans, or having imperceptible effects on others. Some intracellular organisms may even present a relationship of mutualism with the host. Recent findings place parasites in a central role for studying populations' ecology and for understanding their dynamics.

The phylum Apicomplexa is a very interesting and diverse group of obligate unicellular parasites, with a vast distribution; it has been suggested that all animals host at least one species of apicomplexan parasites [1]. Their study has become progressively more important as studies reveal that emergent diseases caused by Apicomplexa pose serious health risks both for animals and humans [2]. Studies conducted in lizards have particularly focused on the analysis of parasite prevalence and intensity, using Haemogregarines as a model since it is the most common blood apicomplexan parasites in lacertids [3]. However information about the impact of these parasites on host fitness and life-history is still scarce.

Since the condition of the immune system is a vital component of every animal's life, this study aimed to analyse the effect of blood parasites on the lizard's immune system. For this, we used the simplified phytohemagglutinin skin-testing (PHA) technique in the field, which is a method that, despite of its limitations, has been commonly used as a tool for estimating host immune response [4]. The PHA compound induces a swelling on the injection site (the hind foot pad) with different intensity depending on the host immune condition. This response is related with parasite intensity and circulating blood cells (erythrocytes, thrombocytes and leukocytes), after examining the lizard's blood under the microscope. Identity of parasites was confirmed using 18S rRNA sequences.

Since they are known to have high parasite loads from previous expeditions, we used two sympatric lizard species (*Podarcis vaucheri* and *Scelarcis perspicillata*) from three Moroccan sites. Results were analysed accounting for variation of parasite load between sexes, species and sites, and the apparent impact on the host immune system.

Since no correlations were found between the PHA response, parasitemia levels or any other factor, we discuss it in the light of different possible scenarios, particularly that these parasites have limited or no effect on the immune system of both species.

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# **A comparison of avian haemosporidian parasite communities across the strait of Gibraltar**

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One of the major concerns of ongoing environmental global changes is the ability of parasites to shift distributions, hosts, and to increase in virulence. In order to understand the structure of host-parasite communities across the Mediterranean Sea we used forest avian haemosporidian parasite communities across the strait of Gibraltar as a model system. We sampled 459 birds of 36 species from Portugal and 324 birds of 46 species from Morocco and tested them for the presence of infections using molecular tools (PCR). Overall parasite prevalence and diversity was higher in North Africa than in Iberia. Parasite specificity did not change between both study areas, but was different between parasite genera. Although the composition of haemosporidian communities differed between Maghreb and Iberia, the high proportion of shared lineages and the lack of spatial structure in their phylogeny suggest that haemosporidian parasites are easily able to cross between Iberia and North Africa.

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# Assessment of diversity of apicomplexan parasites in selected snake species

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Parasites are a highly diverse group of organisms with essential roles in the ecosystems they inhabit. Cataloguing their biodiversity has not been an easy task, mostly due to the difficulties in simply detecting and identifying many of these organisms. The impairment is even greater for intracellular parasites, such as the members of the phylum Apicomplexa. These include some of the most notorious parasites of anthropogenic interest. Regardless of this, they remain one of the most poorly studied groups of organisms, especially in wild hosts [1].

Snakes are hosts to a wide variety of apicomplexan parasites, with members of the hemogregarine genus *Hepatozoon* being one of the most common [2]. In this work, parasite detection was performed through molecular screening by PCR of tissue samples from several wild snakes, using hemogregarine-specific primers for the 18S rRNA gene.

In our first study, samples from four snake genera of the Mediterranean area with different dietary regimens were analysed. The saurophagous snakes presented infections and the retrieved *Hepatozoon* sequences were closely related to those previously found in lizards of the same region. This suggests that these parasites might exploit trophic associations for their transmission [3]. For our second study, samples of snakes of the genus *Psammophis*, also saurophagous, were analysed. As these occupy a more desertic habitat, the inherent hypothesis was to test if ecological differences reflect differences in infecting *Hepatozoon* lineages. However, our results show that these were similar to lineages previously found in Mediterranean snake species. Other apicomplexan parasites were also found in these studies. For instance, infections of *Sarcocystis* spp. were detected and their phylogeny did not reflect host taxonomy, in contrast to what had been suggested in other studies.

Our results show that wild snakes can be parasitized by several apicomplexan groups, and that molecular tools are an efficient way of detecting them, while also providing characters for phylogenetic analyses. From these, it could be concluded that the diversity of these parasites is not distributed in a straight forward way. More sampling of taxa and characters is therefore essential to completely understand their phylogenetic history and relation with the several hosts.

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Oral Sessions ▶ A3

# Engineering IV



# Behaviour of a meshless method in the analysis of the incisor

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This work aims to study the behaviour of a recent developed meshless method in the elastostatic analysis of the maxillary central incisive. The two dimensional model used in the analysis was obtained based on the literature [1]. The numerical method used in the analysis is the Natural Neighbour Radial Point Interpolator Method (NNRPIM), an efficient and flexible meshless method [2], presenting various numerical advantages when compared with other numerical methods such as the Finite Element Method (FEM). The NNRPIM uses the Voronoï concept to force the nodal connectivity and to construct an integration mesh, both completely node-dependent. It is this node-dependency that gives the NNRPIM the advantage comparing with the FEM. The interpolation functions possess the delta Kronecker property, which simplifies the imposition of the boundary conditions. It was verified that the stress field obtained with the NNRPIM is more accurate and smooth when compared with the FEM [2]. The computational model used in this work was obtained with the data available in the literature from a computerized tomography scan performed by Poiatea et al. [1]. A literature review on the maxillary bone mechanical properties was conducted. Five distinct material cases were analysed using the NNRPIM. For each case the stress field was obtained. This work also studied the inclusion of restorative materials for the enamel, with the objective to determine which material lead to the most homogeneous stress field and strain field distributions. The restorative material properties were obtained in the literature [3]. The results obtained with the NNRPIM analysis are compared with the FEM available literature. The results obtained with the NNRPIM shown that meshless methods are a suitable numerical tool to analyse the biomechanical behaviour of dental structures. Permitting in the near future the inclusion of more complex assumptions, such the material inelasticity, the large deformations, and transient analysis.

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# Mandible numerical remodelling due to the insertion of dental implants

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The present work assumes a structural optimization model based on the deformation energy with the purpose of simulating the bone remodeling process induced by the insertion of dental implants.

The bone remodeling model considers the bone to be a biological anisotropic material that continually aims to maximize its structural stiffness. A mathematical law was developed, based on experimental data found in the literature, which allows to correlate the bone apparent density with its own mechanical properties [1]. Thus, the referred mathematical law is able to predict the anisotropic behaviour of bone.

The proposed bone remodeling algorithm requires accurate and smooth stress and strain fields. For this purpose, the numerical method used in this work is the “Natural Neighbour Radial Point Interpolator Method” (NNRPIM), a flexible and efficient meshless interpolator method which shows several advantages in the topological analysis when compared with others numerical methods, such as the Finite Element Method [2]. After the problem discretization in a computational nodal mesh, the NNRPIM uses the concept of Voronoï diagrams to establish the inter-nodal connectivity. Considering the same geometrical concept a background integration mesh is constructed based uniquely on the computational nodal mesh with the aim of numerically integrate the equilibrium equations obtained with variation principles. The NNRPIM shape functions possess the delta Kronecker property allowing the easy imposition of the boundary conditions.

The efficiency of the proposed bone remodeling algorithm was tested with the analysis of classical microstructural problems available in the literature as well as with the analysis of a microstructural section of the implant/bone interface.

The results show trabecular architectures, obtained with the proposed method, very similar to the ones obtained with other numerical approaches.

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## **A meshless method applied on dental implant analysis**

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Many variables are to be considered in the evaluation of the long-term success of a dental implant. This work focuses on the mechanical simulation of the interface bone/implant and the stability of the implant/abutment using a meshless method. The two-dimensional model used in the analysis was based on the available literature [1].

The numerical method used to obtain the interest variable fields is the Natural Neighbour Radial Point Interpolator Method (NNRPIM), an efficient and flexible meshless method [2], presenting various numerical advantages when compared with other numerical methods such as the Finite Element Method (FEM). The NNRPIM uses the Voronoï concept to force the nodal connectivity and to construct an integration mesh, both completely node-dependent. It is this node-dependency that gives the NNRPIM the advantage comparing with the FEM. The interpolation functions possess the delta Kronecker property, which simplifies the imposition of the boundary conditions. Previous NNRPIM works [2] showed that the stress field obtained with the NNRPIM is more accurate and smooth when compared with the FEM [2]. A two-dimensional model was constructed for the analysis, considering the geometry suggested in [1]. A Ø3.5mmx12mm ITI solid-screw implant model and solid abutment, 4 mm in height were assumed. A Cobalt–chromium alloy was considered as crown framework material and for the crown surface was assumed a feldspathic porcelain [1]. After the model validation, other materials were considered for the crown surface and the respective stress fields were obtained. The results obtained with the NNRPIM analysis are compared with the FEM available literature.

The obtained NNRPIM results permit to gauge that meshless methods are a suitable numerical tool to analyse the biomechanical behaviour of dental prostheses. Permitting in the near future the inclusion of more complex assumptions, such the material inelasticity, the large deformations, and transient analysis.

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## Bar-implants with meshless methods: applications

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Bar-implants are fixed prostheses subjected to recurring loads and should transfer efficiently and smoothly the occlusal loads (loads applied during masticatory activity) to the bone tissue. These type of implants are connected by a metal alloy bar are usually prescribed to partial edentulous or total edentulous patients. The occlusal loads induce naturally stress paths on both the prosthetic structures and bone tissue. It is very important to obtain the correct stress paths, in order to correct the existent solutions and develop more efficient prostheses. This work focuses on the mechanical simulation of a bar-implant using a meshless method.

The two-dimensional model used in the analysis was based on a three-dimensional model available in the literature [1]. The numerical method used in the analysis is the Natural Neighbour Radial Point Interpolator Method (NNRPIM), an efficient and flexible meshless method [2], presenting various numerical advantages when compared with other numerical methods such as the Finite Element Method (FEM). The NNRPIM uses the Voronoï concept to force the nodal connectivity and to construct an integration mesh, both completely node-dependent. It is this node-dependency that gives the NNRPIM the advantage comparing with the FEM. The interpolation functions possess the delta Kronecker property, which simplifies the imposition of the boundary conditions. Previous NNRPIM works [2] shown that the stress field obtained with the NNRPIM is more accurate and smooth when compared with the FEM [2].

Since the used two-dimensional model is an adaptation of a three-dimensional model [1] several adjustments were performed. Therefore, the considered essential and the natural conditions reflect the model dimensional adjustment. As suggested in [1] the model has two ITI implants placed in the mandible in the first and second molar sites. The fixtures were connected with a gold alloy fixed bar (crown) ending with a cantilever. The results are compared with FEM results available in literature. Meshless methods are a suitable numerical tool to analyse the biomechanical behaviour of dental prostheses, permitting in the near future the inclusion of more complex assumptions, such the material inelasticity, the large deformations, and transient analysis.

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## A solution for footwear active cooling

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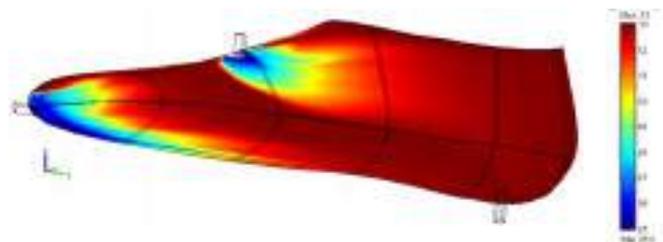
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Footwear products should help the body maintain thermal balance [1, 2]. However, since footwear are relatively closed “systems”, the temperature and humidity of the air trapped inside footwear can escalate over time due to sweat and heat released by the body. In this situation, user comfort is at stake and the conditions can favour the growth of bacteria and fungi. As a possible solution for this problem, a cooling solution based on enhanced convection inside footwear was studied numerically. For this purpose, a FEM-based approach [3] was used to simulate numerically the fluid flow and heat and mass transfer near the skin. This allowed the study of the effect of different parameters over the cooling potential of the envisaged solution, such as the air flow rate and the number and position of air inlets. Furthermore, it allowed the study of the temperature and relative humidity distributions along the footwear skin, as well as the analysis of the systems’ energy requirements and associated weight, as a function of the characteristics of the electronics used to impose air motion and power the system.

Despite evidence of sub-optimal positioning of air inlets (i.e. at the footwear front edge and at the midway between toes and ankle), which resulted in zones with negligible evaporative and dry heat cooling (see Fig. 1), interesting overall cooling potential was obtained. Further research is needed to identify the optimal characteristics of the system, e.g. air inlet positioning, air flow rates and system’s operating point.



**Figure 1** – Temperature distribution (°C) inside footwear, for a solution with 2 air inlets

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## 3D Methodology for Leg Prosthesis Modelling

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(The presenting authors had similar contributions to the development of the work)

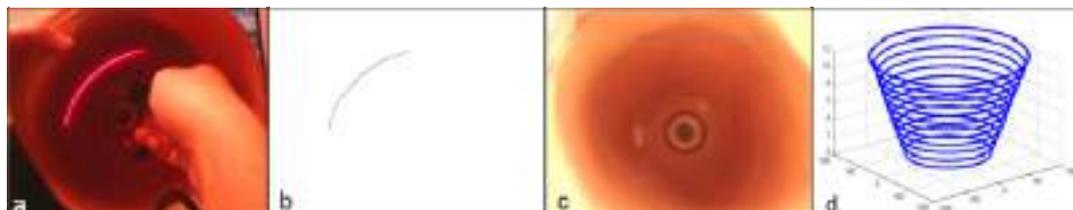
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A system for (three-dimensional) 3D modeling of the leg prosthesis and posterior limb-prosthesis fitting measurement is being developed. The lack of adaptation between the stub and the prosthesis is a well-known identified problem which needs to be solved in order to diminish the associated complications, such as pain, injuries, different pressure distribution along the limb, thus causing discomfort and prosthesis substitution after some time [1,2]. Prosthetic design is mainly manufactured using plaster bandages, therefore the production is imprecise. Its accuracy depends on the used instruments, the operators' skills, on mechanical properties of the plaster at the measurement time and on the status of the patients' limb [2]. Even routine consultations are based on the subjective evaluation of the fitting.

The approach will consist on the projection of a red circumference with a laser on the inner surface of a conventional prosthesis, and its recording with an EyeToy® camera. Image processing techniques can be used to calculate the prosthesis dimensions for the construction of a 3D model. The segmentation of a laser line projected on the inner surface of the prosthesis and the detection of the geometric center of the prosthesis recorded image was tested. Also, a simulated scanning of the prosthesis was performed. The segmentation algorithm was used to detect red artificial circumferences, whose diameter increased with the height of the prosthesis, drawn over a prosthesis recorded image.

The segmented line had 1 pixel width (Figure 1b), with no distortion and the center detection was adequate (Figure 1c). Red artificial circumferences detection and 3D representation was successful (Figure 1d) and had a conic form.



**Figure 1.** Obtained results. In a) the original recorded image is displayed, b) presents the segmented laser line, c) presents the geometric center detection and d) presents the result of the simulated scanning along the prosthesis' height.

This system would fill a gap in the existent technology as it would allow prosthetists and doctors to do a more accurate and faster design of the prosthetic devices. As it will be possible to quantify the adaptation, the comfort to the amputee will be increased.

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Oral Sessions ▶ A4

# Literature & Cultural Studies



# A Cor do Êxtase: A poesia como extensão da ferida

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The resort to image in Manoel de Barro's (1916) poetry, in light of Roland Barthes (1915-1980) photography notes, *La chambre claire*, with special interest in the elimination of the *studium* concept through the overvaluation of the *punctum* concept.

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# ***Maria* de José Craveirinha: uma poética da sublimação do sofrimento**

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With *Maria*, José Craveirinha gives to his readers a whole new manner of expressing a whole new poetical subject. Craveirinha, the major figure of Mozambican literature, is the author of some of the most powerful political and social engaged poems of the 20<sup>th</sup> century. With *Maria* and its 200 poems, the Mozambican warrior turns into a fragile man who can't hide his pain and shares his torment with the universe: an elegiac poem appears, a widower tries to purify his guilt, the literary world acquires one of the most beautiful and meaningful poetry books of all time.

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## ***A Door of Hope is Open: Utopia and Eschatology during the Cromwellian Period (1642-1658)***

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Focusing on the Cromwellian Period, the present paper deals with a context of profound transformation at different levels, which was rich in new ideas and experiments. In this work I try to understand how religion, especially eschatology, determined the mentality of the English people in the mid-seventeenth-century, guiding them through constant political mutations. This study testifies that in many cases the eschatological orientation became intertwined with utopian-oriented projects. Eschatology and utopia united and complemented each other during this short period with the single aim of transforming England not only in a nation well-deserving of transcendental election, but also in a nation where the ordinary individual could fight for freedom of thought and conscience.

By investigating three distinct aspects of this revolutionary period, *Digger Communitarianism*, *Fifth Monarchism* and the *Protectorate of Oliver Cromwell*, I endeavour to prove that the large majority of English people were utopians, millenarians, or both. During the Cromwellian Period, even the ordinary individual had an idea of how society ought to be organised, how religion ought to be professed, how the country ought to be ruled.

## **We built this city: an essay on its representations**

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This research project is still in its initial phase and results from investigations for our dissertation and for the elaboration of several scientific papers. When studying two cities - Espinho and Matosinhos-Leça - with which we interact in a day-to-day basis, we see the continuous construction of an *image of the city*, recalling the concept of Kevin Lynch in his homonymous work.

Art History, with the aid from interdisciplinary studies, provides a range of methodologies to discover the city by exploring numerous sources: written documents, photographs and illustrated postcards, films, paintings, cartography, etc. Each of these sources, thus explored, will produce a new *image of the city*, but can also corroborate or transform an *image* previously established. Like any work of art, it also echoes the context of its production. Our research focuses on the analysis of the sources that we then apply to the object, the city and its components, either still existing or already absent.

Although our investigation is still in progress, some results are already noticeable. We present two examples. For Espinho we have a repository of illustrated postcards allowing us to monitor the evolution of the town and its representations. Since the chronology of many of the views is unknown, we need to use a dating process, by analogy with the evolution of the urban network. Until about the 40s it was not yet possible to detect a predominant criterion in the choice of views, and we deal with kaleidoscopic *images* of the city amidst which there are certain *leitmotifs*, including what we call “reception and recreation areas”. Is it only with the construction of the *Piscina-Solário Atlântico*, a boldly modernist architecture, that the representations are consolidated around the leisure and recreational activities, reflecting an *image* of a modern and cosmopolitan town, assumed and defined as such, with the equivalent architectural stage. About Matosinhos there are two reference documentaries: one from 1928, produced by the Army, which illustrates several "aspects" of Matosinhos and surrounding areas and a second one, from 1948, commissioned by the municipality to promote the town as a tourism destination. In addition to the views we need to analyze the comments, written or narrated, and the construction of one or various narratives. Thus, in the first case we are dealing with the fascination, still romantic, for natural and built landscapes, monuments and historical aspects, but also by the progress and the industry, expressed by several segments without unity. In the second, the film clearly passes the impression of a well-defined town, with remote origins, but transformed and modernized, that has chosen its path and progresses confidently towards the future.

The above examples are not surprising in view of the aesthetic changes between different eras. However, analyzing the various *images* created (apprehending what is represented, how and for whom) may help to appreciate and understand Portugal's difficult relationship with modernity as well as inquire about its immediate impact on urban transformations. The information gathered by crossing the built city with the built *images* will also contribute to an improved knowledge about it, and to better understand, protect and value its heritage amongst the inhabitant and the tourist.

# Redefining City's Image: Guimarães, European Cultural Capital 2012

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In the last three decades, competition between cities for foreign investment, tourism revenues and new residents has been strongly increasing [1]. In this sense and as a way to differentiate themselves from their competitors, cities must identify the changes taking place within their market segment and understand the needs of their target audiences [2]. It is in this context that marketing techniques find their foundations. It is a process by which the city can achieve its goals by developing a brand as a mean to achieve a competitive advantage, strengthen its position in the market and enhance the identification of its target audiences [3]. Therefore, the creation of a city's brand must be founded on three fundamental pillars: brand identity, brand object and brand market [4]. The city brand and its management are related to the concept of city's identity, which it is intended to be distinctive [5]. To this end, some cities have appealed to culture and cultural events, such as European Cultural Capital (ECC), as a way to improve their image, strengthen their identity, stimulate urban development and attract new audiences [6].

The aim of this study is to understand how a medium-sized city with an industrial background can redefine its image through culture. To this end, the study analyzes the Guimarães ECC 2012 brand in terms of its identity, object and market. It is intended to answer to three research questions: Q1: What is the identity of the Guimarães ECC 2012 brand?; Q2: How was the marketing-mix of the Guimarães ECC 2012 brand developed?; and Q3: What is the target audiences reaction to Guimarães ECC 2012 brand? The empirical analysis was conducted using a mixed approach based on both desk research and intensive application of questionnaires to the brand's target audiences (n = 938).

By creating Guimarães ECC 2012 brand, the city intended to enlarge the symbolic value of Guimarães, focusing on an identity that reflects the cultural diversity and the historical legacy of the city. With the goal set on urban, economic and social regeneration, the city privileged a marketing-mix based on urban design (consolidation of the territory), infrastructures (mainly technological), basic services (namely education) and attractions. In general, the target audiences have a positive image of Guimarães. The "heritage" and "beauty" are the best rated dimensions and "international visibility" has a less positive evaluation by the respondents.

The ECC 2012 event enabled to develop a strong, unique and distinctive identity and renew the pride of its citizens. Simultaneously, the event permitted to add a new life to the city, which incited a visit of "old" and "new" visitors and create the bases in terms of infrastructures and knowledge to strengthen and sustain cultural city positioning.

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# Hume's *Of the Standard of taste* and the quest for objectivity

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The *Social Responsibility* project arises from the conviction that philosophy and literature may contribute to a better understanding of the specific content of UNICER's social responsibility. Along with one rather promising suggestion a fairly unexplored way in which a company like UNICER could fulfill its obligation towards society would be by promoting the diffusion of what the philosopher R. Scruton once called the "culture of symposium", namely the system of human norms, manners and rituals that should accompany our drinks if our human condition is to be elevated and ameliorated, and not degraded, by the consumption of intoxicants. Key to the achievement of this goal is to uncover the aesthetic dimension that is constitutive of the truly human relation we establish with intoxicants when we experience their gustative properties and we try to describe them, thereby trying to put the ineffable traits of our own subjective experience into public and sharable words that other persons could understand. In the present study I shall try to contribute to a better understanding of this topic by presenting the answer(s) defended by the Scottish philosopher David Hume to the question about whether we could as much as develop shared and universal standards of taste concerning alcoholic substances. To summarize the conclusion I shall try to defend, in *Of the standard of taste* Hume seems to defend an affirmative answer. In particular, Hume's answer seems to imply that, at least to some extent, our judgments of taste are answerable to the specific objective traits of the substances they are about, so that they are capable of being correct, when they convey appropriate responses to these traits, and incorrect otherwise. How far does Hume's answer reach is a question I shall touch upon in my presentation. In particular I shall address the question about whether Hume's proposal justifies the more ambitious plan of developing universal standards in light of which the *aesthetic worth* of an intoxicant should be assessed, and ranked accordingly, and the related question of how, from Hume's own point of view, objective knowledge of the aesthetic worth of an intoxicant could ever be as much as achieved.

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# IJUP Social Responsibility Research Project: Drinking as an Aesthetic Phenomenon

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The art of writing and the act of drinking have long since shared a peculiar bond that few have ever attempted or succeeded in representing. Combining different areas of investigation, from social sciences to philosophy and literature, the project entitled ‘Drinking as an Aesthetic Phenomenon’ aimed at analysing the link between the creative process and the regular or occasional ingestion of alcoholic drinks. Due to the participation of several researchers, each with its area of expertise, it was established that the project would be divided into two different yet intersecting parts: one regarding the philosophy of mind, and the other consisting in a reflection and study of the relation between aesthetics, literature and drinking, first by exploring the notions of experience, desire and gratification and secondly by justifying them with examples from well-known literary works. It was equally established that the chronology of the research would comprise literary texts written and published between the 19th and 20th Centuries from Baudelaire, Algernon Swinburne and Oscar Wilde to F. Scott Fitzgerald. In *The Beautiful and Damned* (1922) Fitzgerald provides us with an insight into the mind of young men and women who absorb and personify the beliefs of an age of glamour and excess, even describing one of his character’s views on the act of drinking as that of someone who had ‘taught himself to drink as he would have taught himself Greek—like Greek it would be the gateway to a wealth of new sensations, new psychic states, new reaction in joy or misery.’<sup>[1]</sup> In addition, an open lecture series dedicated to literature and the aesthetics of drinking was held at the Faculty of Letters of the University of Porto on 9 November 2012 with the attendance of three main speakers: Filipa S.Paiva (University of Porto), Teresa Louro (University of Porto) and Sara Ramos (Quinta and Vineyard Bottlers- Vinhos S.A: Caves Croft, Fonseca e Taylor, Port Wine House) each addressing the peculiar nature and individual identity of alcoholic drinks. The bibliography used in these presentations and research in general was quite extensive, ranging from specialized books on alcohol to literary quotations taken from specific novels and short-stories including Hemingway’s *A Moveable Feast*<sup>[2]</sup> and Fitzgerald’s *On Booze*.<sup>[3]</sup> Furthermore, meetings were prepared to discuss the organization of future conferences and seminars in order to promote both Unicer and the University of Porto. The full report to be presented in February will elaborate further on these issues and the work developed by the research project.

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[2] Hemingway, Ernest (2009), *A Moveable Feast*, New York: Simon and Schuster.

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Oral Sessions ▶ A5

# Astronomy



# Probing Unification Scenarios with Atomic Clocks

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The observational evidence for the accelerating Universe shows that current theories of physics are incomplete, if not incorrect. The LHC evidence for the Higgs particle strongly suggests that fundamental scalar fields are among nature's building blocks. These have been invoked to explain several paradigms (such as dynamical dark energy), but the most direct way to infer their presence is (arguably) to search for spacetime variations of nature's fundamental constants. It is known that fundamental couplings run with energy, and many particle physics and cosmology models suggest that they should also roll with time.

Recent astrophysical measurements have led to claims for and against variations of the fine structure constant and the proton-to-electron mass ratio at redshifts  $z \sim 1-3$ . Moreover any Grand Unified Theory predicts a specific relation between variations of the fine-structure constant and the proton-to-electron mass ratio, and therefore simultaneous measurements of both provide key consistency tests.

Atomic clock comparisons provide one such consistency test. The operation of these devices is based on specific atomic transitions, whose frequencies depend on several combinations of fundamental constants. The frequencies of these transitions are very stable (hence their usage as clocks) and experimental tests of their drifts allow us to obtain direct constraints on the phenomenological parameters which characterize unification scenarios.

Our work has used the latest atomic clock measurements to improve the constraints on the variation of fundamental couplings [1]. We also carried out a first analysis of the impact of atomic clock measurements on the phenomenological parameters describing the class of varying fundamental coupling scenarios under consideration (specifically  $R$ , related to QCD physics, and  $S$ , related to electroweak/Higgs physics). These measurements are only sensitive to a particular combination of these parameters, and our results are in agreement with theoretical expectations on unification scenarios.

This work was done in the context of the project PTDC/FIS/111725/2009 from FCT (Portugal), with additional support from grant PP-IJUP2011-212 (funded by U. Porto and Santander-Totta). The work of CJM is supported by a Ciência2007 Research Contract, funded by FCT/MCTES (Portugal) and POPH/FSE (EC).

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[1] M. C. Ferreira, M. D. Julião, C. J. A. P. Martins and A.M. R. V. L. Monteiro, *Phys. Rev. D* **86**, 125025 (2012).

# Probing Unification Scenarios with Quasar Absorption Lines

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Fundamental couplings are dimensionless quantities that are used to compare magnitudes of interactions. Current experimental data provides evidence for the existence of four fundamental interactions in Nature. Fundamental means that all phenomena are ultimately described in terms of these interactions. So, if it is assumed that these couplings are constants in time and space, it is expected that physical laws are the same everywhere at every time. But, what if these couplings vary? What if one interaction has a greater magnitude in one place than in the other? Being true, variation of couplings would be a seminal finding.

Recently there has been some evidence for the variation of one of these couplings based on absorption spectra from quasars[1]. However, the consequences of these facts have to be digested and treated in a way theorists can compare with their results.

There are many theories of unification that encompass variation of fundamental couplings. The best way to probe them is to compare their predictions with experimental data. In a wide class of unification theories it's possible to squeeze into two coefficients, R and S, their predictions on variation of couplings. Different theories produce different coefficients. Translating experimental data into these coefficients allows one to sort out theories for which the coefficients don't agree with the ones found with the data.

The absorption spectra lines of quasars occur as a result of gas clouds at cosmological distances and so they can be used as a sensitive probe of physics at the time of the absorption of light. What we'll present is a translation of quasar absorption spectra data obtained with Keck and VLT into the R and S coefficients. Combining these results with our previous work[2] it will be possible to constrain even more the freedom theoretical physicists have when conceiving a new unification theory.

This work was done in the context of the project PTDC/FIS/111725/2009 from FCT (Portugal), with additional support from grant PP-IJUP2011-212 (funded by U. Porto and Santander-Totta). The work of CJM is supported by a Ciência2007 Research Contract, funded by FCT/MCTES (Portugal) and POPH/FSE (EC).

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# Linkages between NEO's and the birth environment of the Solar System

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The comparison bidirectional reflectance spectra of asteroids and meteorites, allows the establishment of possible genetic linkages between them. Asteroids can be seen as “fossils”, which carry information of the late solar nebula and early Solar System. Since meteorites are asteroid fragments, their analysis can provide mineralogical data that can be essential to reveal the primordial chemical elements of the late solar nebula, as well as a better understanding of the conditions and processes that prevailed on it [1].

This work gathers spectral data from asteroids, mainly NEO's (Near Earth Objects) taken from MIT-UH-IRTF Joint Campaign for NEO Reconnaissance, and meteorites and minerals searched in RELAB (Keck/NASA Reflectance Experiment Laboratory at Brown University). Some meteorite-asteroid genetic linkages were checked through spectrum comparison and curve matching, to infer possible element abundances in the early stages of the Solar System [2]. Processes of asteroids surface alteration and their implications in reflectance spectra were also discussed [3]. In particular, spectral similarities were observed between basaltic achondrites and V-type asteroids and between ordinary chondrites and S-type asteroids, which are the most abundant in the inner main belt [4-5].

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# Mineralogy of V-type near-earth objects: constraints in early Solar System environment

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Asteroids represent a potentially rich source of a variety of materials of the early Solar System. According to dynamical studies, most of the meteorite types were originated from the Main Asteroid Belt and not only from near-Earth asteroids. Visible and near-infrared spectral observations of asteroids and meteorites allow us to infer about their mineralogies and particle grain sizes [1]. From similarities of the absorption features of reflectance spectra of meteorites and asteroids, several genetic linkages between meteorites and asteroids are pointed out [1]: HED meteorites with 4 Vesta and Vestoids; ordinary chondrites with S-type asteroids; iron meteorites and enstatite chondrites with M-type asteroids; CM and CI chondrites with C-type asteroids.

In this study, the genetic relationship between HED meteorites and 4 Vesta and Vestoids was tested. For this purpose, reflectance spectra of a set of 30 HED meteorites were obtained from the RELAB database [2] and of 8 V-type asteroids from the publically available MIT-UH-IRTF Joint Campaign for NEO Reconnaissance [3]. These spectra were compared to identify the meteoritic analogs to each selected V-type asteroid. Reflectance spectra were simulated using a Microsoft Excel implementation of Hapke radiative transfer model [4]. The mineralogical composition of each selected asteroid was determined according to Hapke mixing model [5], through a linear least-squares spectral deconvolution implemented with the Solver add-in [6].

Our results strengthened the genetic linkage between HED meteorites, 4 Vesta and Vestoids and provide hints about some processes which took place in the early Solar System.

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# Varying Constants and Dark Energy: an observational strategy for ESPRESSO

**P. O. J. Pedrosa<sup>1,2</sup>, A. C. O. Leite <sup>1,2</sup> and C. J. A. P. Martins<sup>2</sup>**

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Understanding what constitutes dark energy is a key problem of modern cosmology. In particular, we would like to find out if it is a cosmological constant (as introduced by Einstein), since there are many possible alternatives. These alternative often involve scalar fields, an example of which is the Higgs field which the LHC recently discovered.

The main difference between the cosmological constant and the models involving scalar fields (which are often collectively called dynamical dark energy models) is that in the first case the density of dark energy is always constant (it does not get diluted by the expansion of the universe) while in the second one the dark energy density does change. One way to distinguish the two possibilities is to find ways to measure the dark energy density at several epochs in the universe.

Astrophysical measurements of nature's dimensionless fundamental coupling constants can be used to study the properties of dark energy, either by themselves or in combination with other cosmological datasets (such as Type Ia supernovas and the cosmic microwave background).

In recent work [1] we discussed methods based on Principal Component Analysis to constrain the dark energy equation of state using a combination of Type Ia supernovae at low redshift and spectroscopic measurements of varying fundamental couplings at higher redshifts. Specifically, we discussed the performance of this method when future better-quality datasets are available, focusing on two forthcoming ESO spectrographs (ESPRESSO for the VLT and CODEX for the E-ELT), of direct interest to CAUP and which include these measurements as a key part of their science cases. It was shown that these can realize the prospect of a detailed characterization of dark energy properties almost all the way up to redshift 4.

In this talk we will discuss how the results of the above theoretical analysis can be applied in practice, in order to define an optimal observational strategy for future spectrograph.

In a related talk (by A.C.O. Leite) the results our analysis will be compared with the performance of a current spectrograph (UVES, at the VLT).

This work was done in the context of the project PTDC/FIS/111725/2009 from FCT (Portugal), with additional support from grant PP-IJUP2011-212 (funded by U. Porto and Santander-Totta).

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[1] Amendola, L. Leite, A.C.O. Martins, C.J.A.P. Nunes, N.J. Pedrosa, P.O.J. Seganti, A. Variation of fundamental parameters and dark energy. A principal component approach Phys.Rev. 063515 D86 (2012)

# Varying Constants and Dark Energy: from HIRES and UVES to ESPRESSO and beyond

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Future high-resolution spectrographs include, among their key scientific goals, precision tests of the stability of nature's fundamental couplings. Apart from the fundamental nature of such tests (confirmed evidence for spacetime-varying couplings automatically implies that gravity isn't a purely geometric phenomenon, as postulated by Einstein, and that there's a fifth force in nature) these measurements are also crucial to shed light on the enigma of dark energy.

In recent work [1] we quantified, through methods based on Principal Component Analysis, how varying constants measurements can constrain dark energy (specifically, its equation of state) either on their own or in combination with Type Ia supernova measurements. This analysis can be used to define specific observational strategies, and described in a related contribution (by P.O.J. Pedrosa),

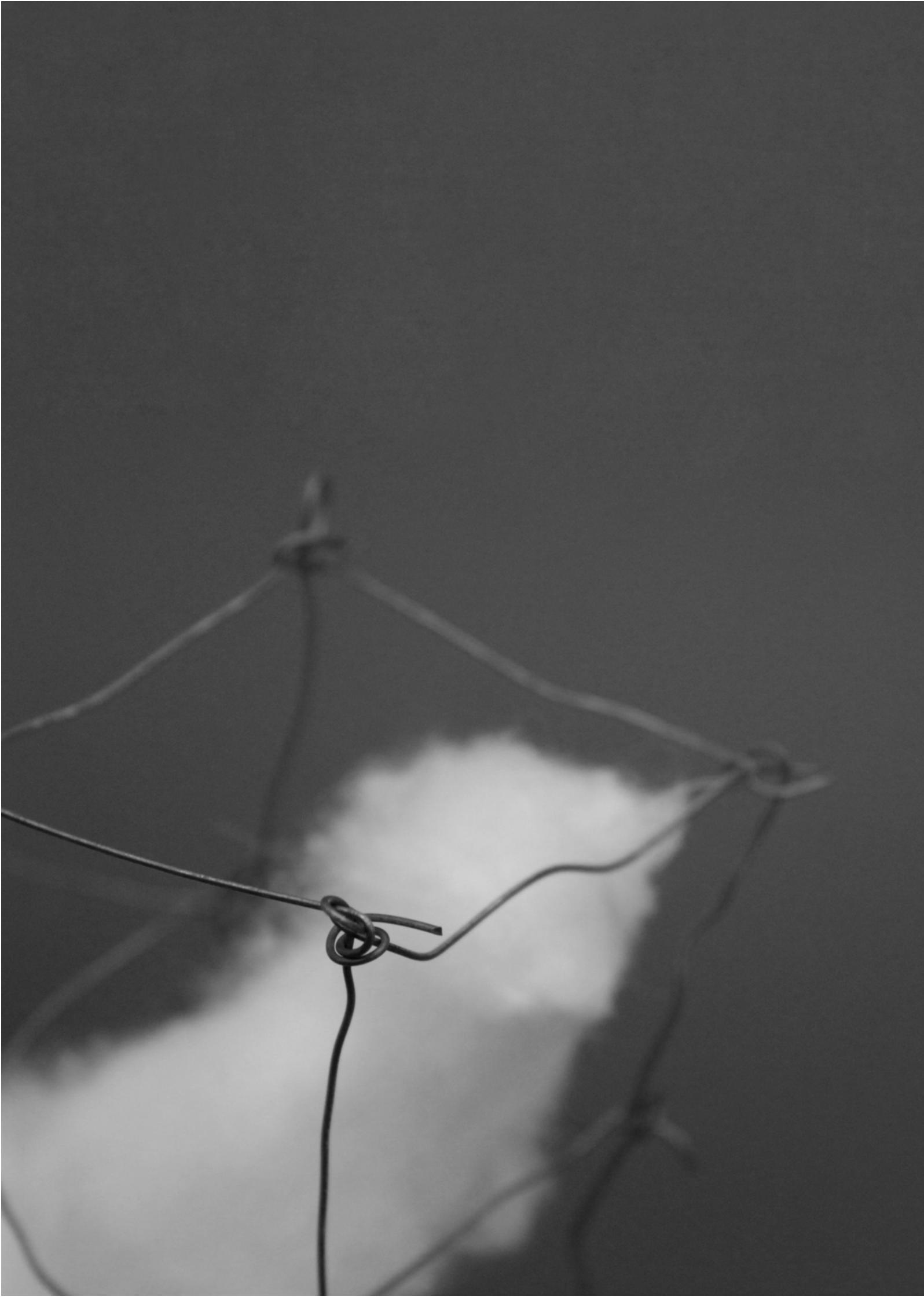
In this talk these theoretical tools will be calibrated by applying them to an existing dataset of about 330 measurements of the fine-structure constant, obtained with the VLT's UVES (Ultraviolet and Visual Echelle Spectrograph). We will describe the main characteristics of this dataset, and then proceed to extrapolate our analysis to future (higher-resolution) spectrographs of direct interest to CAUP, namely ESPRESSO for VLT (under construction) and CODEX (planned for the E-ELT).

This work was done in the context of the project PTDC/FIS/111725/2009 from FCT (Portugal), with additional support from grant PP-IJUP2011-212 (funded by U. Porto and Santander-Totta).

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**ORAL SESSIONS**

**VI**



Oral Sessions ▶ A1

# Biomedicine V



## Anti-inflammatory eicosanoids in chronic heart failure: relationship to disease severity and modulation by low-dose acetylsalicylic acid

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**Introduction and aims:** Lipoxins are anti-inflammatory eicosanoids that promote the resolution of inflammation and tissue regeneration. It has been hypothesized that cardiac dysfunction may be secondary to a deficiency in lipoxin A<sub>4</sub> (LXA<sub>4</sub>) formation and consequent enhancement of leucocyte activation, inflammation and fibrosis. Acetylsalicylic acid (ASA) was recently shown to induce the synthesis of 15-epi-lipoxin A<sub>4</sub> (15-epi-LXA<sub>4</sub>) which shares the potent anti-inflammatory actions of lipoxins and may contribute to cardiovascular protection. However, despite the protective effects of LXA<sub>4</sub> and 15-epi-LXA<sub>4</sub>, little is known about their actions in the pathogenesis and progression of chronic heart failure (CHF). Therefore, this study aimed at evaluating the bioavailability of lipoxins in CHF patients and at investigating the correlation of these lipid mediators with markers of cardiac dysfunction and injury, inflammation and oxidative stress. Furthermore, the effect of low-dose ASA on lipoxins production and CHF biomarkers was also investigated.!!

**Methods:** Thirty-four CHF patients were selected from the Heart Failure Clinic of Hospital São João. Fifteen were on low-dose ASA (100-150 mg daily) treatment. B-type natriuretic peptide (BNP), troponin, C-reactive protein (CRP) and uric acid (UA) were evaluated in the Clinical Pathology Laboratory. Myeloperoxidase (MPO), lipoxin A<sub>4</sub> (LXA<sub>4</sub>) and 15-epi-lipoxin A<sub>4</sub> (15-epi-LXA<sub>4</sub>) concentration was evaluated using commercial kits, while MPO activity was measured by a spectrophotometric assay.

**Results:** Patients were stratified into mild-to-moderate CHF (New York Heart Association, NYHA, classes I, I/II and II) and severe CHF (NYHA classes II/III, III and III/IV). Severe patients had lower plasma LXA<sub>4</sub> (0.262±0.034 vs. 0.362±0.039 ng/ml, p<0.05) and decreased urinary 15-epi-LXA<sub>4</sub> (2.28±0.44 vs 4.88±1.03 µg/day, p<0.05) besides exhibiting an increase in plasma BNP (1464±442 vs 555±162 pg/ml, p<0.05) and MPO activity (45.15±11.56 vs 15.90±2.80 µmol/min/mg protein, p<0.05) compared to mild-to-moderate CHF patients. Plasma LXA<sub>4</sub> was inversely correlated with BNP, troponin, CRP and UA. Treatment with ASA was associated with higher urinary 15-epi-LXA<sub>4</sub> (7.70±1.48 vs 2.06±0.30 µg/day, p<0.05) and attenuated BNP levels (372±155 vs 782±304 pg/ml) in mild-to-moderate CHF, but no differences were observed in severe patients.

**Conclusions:** Worsening of CHF is associated with reduced systemic LXA<sub>4</sub>, decreased urinary 15-epi-LXA<sub>4</sub> and increased plasma MPO activity. The inverse correlation of LXA<sub>4</sub> with HF biomarkers suggests its involvement in CHF pathophysiology. The AAS-induced increase in urinary 15-epi-LXA<sub>4</sub> suggests enhanced renal synthesis of this eicosanoid and may be related to the improvement in cardiac function.

## Efficient transplacental transport of hepatitis E antibodies

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Seroprevalence studies show that a third of the world's population has been infected with hepatitis E virus (HEV) [1]. Predictably, many child-bearing age women and their newborn infants will be positive for anti-HEV immunoglobulin G (IgG) because of the transplacental transfer of maternal IgG [2]. An active placental transport of specific IgG with a higher concentration in the foetal side has been observed in different viral infections [3]. Nevertheless, other studies reported lower levels in cord than in maternal sera [4].

The aim of the present study was to investigate how efficiently are IgG anti-HEV transported through placenta to newborns.

For that, sera samples from 12 mothers and their corresponding newborn were evaluated for the presence of IgG anti-HEV using a commercial ELISA diagnostic kit (Wantai Biological Pharmacy Co ®, Beijing, China). The cutoff value was determined according to the manufacturers' instructions.

Four of the 12 mothers were positive for IgG anti-HEV confirming an exposure to the virus. The corresponding 4 newborns were also positive and all presenting OD values superior to the paired maternal serum. This proves not only an efficient transplacental transport of anti-HEV IgG but also a concentration of the viral specific antibodies in the foetal side.

In conclusion, the present study suggests that infants born from anti-HEV positive mothers are more likely protected to HEV infection in the early life than newborn from non-immune mothers.

Acknowledgements: Wantai Biological Pharmacy Co., Beijing, China, for providing the anti-HEV IgG ELISA diagnostic kits.

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## Evaluation of physicochemical properties of synthetic prenylated xanthone derivatives

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Xanthone derivatives, namely prenylated xanthenes (PX) have been found to exhibit interesting activities such as anti-inflammatory, antibacterial, antioxidant and antitumor [1,2]. The presence of prenyl groups becomes an important structural factor taking into account the influence of the physicochemical properties, including lipophilicity, which may create additional interactions with biological targets [1,2]. Therefore, PX can represent excellent models for the development of new and more effective bioactive compounds, being the introduction of prenyl groups in the scaffold of a “hit” compound one of the strategies used in the CEQUIMED-UP [3].

In this work a small library of PX derivatives (1,3-dihydroxy-2-methylxanthone, 1-hydroxy-2-methyl-4-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone and 1-hydroxy-2-methyl-3-(3-methylbut-2-enyloxy)xanthone) was obtained. The physicochemical parameters, Log P and human serum albumin binding of the compounds synthesized were also evaluated. The lipophilicity was assessed by derivative spectrophotometry and drug-protein binding was evaluated using a fluorimetric assay.

These parameters are determinant to predict pharmacokinetic and pharmacodynamic profile of the obtained compounds. Lipophilicity is a major indicator of interaction with membranes and constitutes a primordial aspect on understanding the interactions of the compounds in the organism. Drug-protein interaction has a great significance in pharmacology as it can affect the biological activity and toxicity of drugs. This study is one of the primary steps to predict the *in vivo* behavior of the referred compounds.

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# Hippocampal neurodegeneration in kainate-treated rats with and without recurrent motor seizures

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Temporal lobe epilepsy (TLE) is often associated with hippocampal sclerosis and other structural changes in the medial temporal lobe region. Generalized motor seizures are present in severe cases of TLE. As TLE is progressive these seizures can become intractable. It has also been reported that TLE patients with hippocampal sclerosis may show memory deficits, anxiety, depression and mood disorders. Thus, it is possible that these behavioral changes are secondary to the TLE-related hippocampal neuron loss.

Animal models of TLE replicate the majority of the neuropathological aspects of TLE. Kainic acid (KA) is a specific agonist for the ionotropic glutamate receptor. When injected to rats, KA induces *status epilepticus* (SE). However, a large percentage of the animals do not develop spontaneous motor seizures (SMS) and are usually excluded from analyses. The purpose of this study was to directly compare the neuroanatomical outcomes of KA treatment in rats that developed SMS to those that did not. To address this issue, we estimated, using stereological methods, the neuron numbers in the dentate hilus and pyramidal layers of the hippocampus proper in 2 groups (SMS and no-SMS).

Male Wistar rats were hourly injected with 5 mg/kg of KA until the onset of SE (n=28). Rats in the control group were injected with saline (n=8). Across the 5-month recovery period the behavior of rats was video recorded. If a rat showed two or more SMS of stage 4 or 5 on the Racine scale, it was assigned to the SMS group. Remaining animals were assigned to the no-SMS group. From the 28 rats treated with KA, 8 rats have died and 9 rats developed SMS. No motor seizures were detected in the 11 remaining KA-treated rats (no-SMS group). After perfusion of the animals, their brains were sectioned in the coronal plane, stained with Giemsa and used for histological analysis.

The total number of neurons was estimated applying the optical fractionator method. The boundaries of the hilus of the dentate gyrus and the CA3 and CA1 hippocampal fields were consistently defined at all levels along the rostrocaudal axis of the brain based on cell morphology and cytoarchitectonic criteria. Estimations were carried out using the C.A.S.T.-Grid System (Olympus, Denmark).

It was found that the patterns of KA-induced neuron loss in the hippocampal formation were considerably different in rats with or without seizures. In particular, in animals from the SMS group there was a significant neuron loss in the dentate hilus and in the hippocampal CA3 and CA1 fields. In sharp contrast, in rats from the no-SMS group there was a significant neuron loss only in the dentate hilus, but not in the hippocampal pyramidal fields. These data suggests that the loss of hippocampal pyramidal neurons is particularly important for the epileptogenesis.

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# **Prrxl1 transcription factor is differentially recruited to chromatin of developing dorsal root ganglia and dorsal spinal cord possibly by a mechanism involving distinct co-regulators**

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The accurate perception of external sensory information by the brain requires well-organized synaptic connectivity between peripheral sensory neurons and their central targets. Transcription factors have a pivotal role in the functional differentiation of sensory neurons. Prrxl1 is a homeodomain (HD) transcription factor (TF) expressed in dorsal root ganglia (DRG) nociceptors and in their putative central synaptic targets in the dorsal spinal cord (DSC), strongly suggesting a role for Prrxl1 in neuronal connectivity. Indeed, analysis of *Prrxl1* null mouse embryos revealed spatio-temporal anomalies in the initial projection of sensory afferent fibers into the developing DSC followed by abnormal migration and increased cell death leading to a diminished response to noxious stimuli in adult mutant mice [1]. Given the multiple abnormalities affecting nociceptive neuronal differentiation, Prrxl1 is thought to be implicated in the orchestration of a complex genetic program that is almost completely unknown.

To unveil the transcriptional network downstream of Prrxl1, we performed a chromatin immunoprecipitation followed by massive sequencing (ChIP-seq) analysis to determine the repertoire of Prrxl1-binding sites in developing DRG and DSC. Unexpectedly, only 10% of the binding events are common in the two tissues, indicating a tissue-specific transcriptional program. *In silico* search for enriched DNA motifs revealed the consensus HD binding motif to be associated with Prrxl1 binding events in both tissues, as expected. Strikingly, when searching for Prrxl1 co-regulators by masking the HD binding motif, we found the Runx-binding motif to be enriched only in the DRG data set. In addition, Runx1 was previously shown to be required for proper central projection establishment of DRG axons [2], reminiscent of *Prrxl1* null mice phenotype. Therefore, it is tempting to hypothesize that Runx1 might work as a co-regulator of Prrxl1. Co-expression studies showed that virtually all Runx1<sup>+</sup> neurons co-localize with Prrxl1 in DRG at E14,5, although in DSC only Prrxl1 was expressed. This result further supports the hypothesis that part of the Prrxl1 transcriptional program is modulated by Runx1 in nociceptors development, suggesting that Prrxl1 has tissue specific co-regulators. Further studies will follow in order to validate the interaction of Runx1 and Prrxl1 to common genomic regions and to assess their cooperation on transcriptional regulation of common target genes.

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# Thymocyte selection regulates the homeostasis of IL7-expressing thymic cortical epithelial cells *in vivo*.

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Thymic epithelial cells (TECs) provide essential instructive signals for thymopoiesis. The differentiation of functionally distinct cortical (cTECs) and medullary (mTECs) subsets relies on bidirectional interactions with developing thymocytes. While the molecular interactions that control mTEC maturation have been partially elucidated, the underlying signals that regulate cTEC differentiation remain elusive, due in part to the lack of detailed intermediate stages. Interleukin 7 (IL-7) is an essential thymopoietic cytokine predominantly produced by TECs. Using IL-7 reporter mice, in which YFP expression identifies TECs that co-express high levels of IL-7 (referred as  $Il7^{hi}$ TECs), we previously showed that  $Il7^{hi}$ TECs gradually decay with age in a thymocyte-dependent manner and are conversely sustained in  $Rag2^{-/-}Il2rg^{-/-}$  mice displaying a profound and early block in T cell development. These findings indicate that thymocyte-TEC interactions regulate the size of the thymic IL-7 niche.

Here, analyzing TEC developmental kinetics, we show that  $Il7^{hi}$ TECs define a  $CD205^{+}BP1^{+}CD40^{lo}$  subset that declines during thymic development, co-expresses *Dll4*, *Ccl25*, *Il7*, *Psmb11*, *Prss16* and segregates from  $CD80^{+}CD40^{hi}$  mTECs expressing *Tnfrsf11a*, *Ctts* and *Aire*. Using thymic organotypic cultures, we demonstrate that the thymocyte-induced reduction in  $Il7^{hi}$ TECs is independent from the RANK- and LTbeta receptor-mediated maturation of mTECs. These observations suggest that  $Il7^{hi}$ TECs are not a direct progeny of mTECs, but instead specify a particular cTEC lineage. To examine the nature of the thymocyte-derived signals that modulate  $Il7^{hi}$ TECs, we employ *in vivo* models of absent, positive, and negative thymocyte selection.  $Il7^{hi}$ TECs are sustained in fetal and postnatal  $Rag2^{-/-}$  thymi. Analysis of anti-CD3-treated  $Rag2^{-/-}$  mice shows that TCR-independent interactions between DP thymocytes and TECs moderately reduce  $Il7^{hi}$ TECs. Interestingly, in Marilyn- $Rag2^{-/-}$  TCR transgenic mice, expressing an I-A<sup>b</sup>-restricted TCR recognizing the male-specific HY antigen, positive selection of thymocytes reduces the frequency of  $Il7^{hi}$ TECs in female mice. Strikingly, in male mice, negative selection provokes a conspicuous more severe degeneration in  $Il7^{hi}$ TECs.

Our results provide evidence that the strength of MHC/peptide-TCR interactions between TECs and thymocytes during thymocyte selection constitute a novel rheostatic checkpoint governing the maintenance of the IL-7-expressing cortical epithelial niche.

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# Physics



# Resistive switching and activity-dependent modifications in Ni-doped graphene oxide thin films

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Traditional memory technologies are rapidly approaching miniaturization limits. An alternative is resistive random access memory relying on the resistive switching (RS) effect occurring in metal-insulator-metal (MIM) cells [1,2]. A link between MIM structures and biological synapses was also recently reported, due to the equivalent dynamical adaptation in response to inputs [3], therefore being investigated towards artificial neural networks.

We studied the RS of two Ni-doped graphene oxide (GO)-based-devices grown on Si/SiO<sub>2</sub> substrates prepared using different methods and electrodes (W, Cu). GO was synthesized by the modified Hummers method and the I–V cycles were obtained by sweeping the voltage in the  $0 \rightarrow V_{\max} \rightarrow 0 \rightarrow -V_{\max} \rightarrow 0$  sequence (where  $V_{\max}$  is the maximum applied voltage). No forming process was necessary to activate the resistive switching effect [4]. We further measured the endurance by applying positive/negative bias voltages large enough to induce RS and also conducted a preliminary study on the activity-dependent modification abilities of graphene-based devices, with consecutive positive/negative voltage sweeps.

The two series reproducibly showed opposite switching polarities, indicating that two processes can be responsible for RS depending on the fabrication method of the GO sheet and on the electrode material. Although some sample-to-sample variations were observed, the switching type and polarity remained the same. We showed that GO-based structures possess activity-dependent modification capabilities in common with long-term synaptic plasticity in neuromorphic systems, emphasized by the increase/decrease of device conductance after consecutive voltage sweeps of opposite polarity.

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# Ab-Initio Calculations in Magnetocaloric Materials

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There has been a great deal of interest in recent years in the energetic potentialities of magnetocaloric materials. A thorough understanding of their physical properties is necessary for the development of refrigeration technologies that take advantage of the Giant Magnetocaloric Effect (GMCE). The  $R_5(\text{Si}_x\text{Ge}_{1-x})_4$  (with  $R=\text{Tb}$  or  $R=\text{Gd}$ ) compounds exhibit such effect [1] and are suitable candidates for economic exploration.

$\text{Gd}_5\text{Ge}_4$  is one of such compounds with higher interest, since it presents a quite exotic effect in the condensed matter (materials science) field: a magneto-structural transition induced by applied magnetic field or external pressure, but absent when only the temperature is varied.

This anomalous effect enhances the interest in studying the other end members of the  $R_5(\text{Si}_x\text{Ge}_{1-x})_4$  composition, such as the  $\text{Tb}_5\text{Ge}_4$  compound. It is conjectured the appearance of a GMCE if a magneto-structural change between an O(II) phase and an hypothetical O(I) phase and a change from AFM to FM is verified (see [2], for a similar discussion on  $R_5\text{Si}_2\text{Ge}_2$  ( $R=\text{Tb},\text{Gd}$ ) materials). To clarify these points, in this work we have performed ab-initio calculations on  $\text{Tb}_5\text{Ge}_4$  considering both structural phases, in order to obtain the internal energy arising from the atomic lattice at  $T=0\text{K}$ , using WIEN2k software [3]. This is a user-friendly program that uses density-functional theory to calculate a variety of properties of materials, like their structural energy, or their density of states.

We will present and discuss the results obtained through numerical simulations, namely the temperature and applied magnetic field dependence of the Helmholtz free energy of these two phases. These studies will allow us to understand the origin of the GMCE on the  $\text{Tb}_5\text{Ge}_4$  compound.

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# A tolerance factor mediated lattice and phonon universal behavior in rare-earth-manganites

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The physical properties of the orthorhombic rare-earth manganites (RMnO<sub>3</sub>) are known to be strongly dependent on the ionic radius of the rare-earth ion ( $r_R$ ), which leads to changes of the tilt of the MnO<sub>6</sub> octahedra, defined as the difference to the ideal cubic perovskite bond angle Mn-O-Mn, connecting Mn<sup>3+</sup> and octahedral apical oxygen ions [1]. Several types of exchanges have been considered associated with Mn<sup>3+</sup> spins: nearest-neighbor ferromagnetic (FM) exchanges  $J_{ab}$ , along  $x$  and  $y$ , next nearest-neighbor antiferromagnetic (AFM) exchanges  $J_a$  and  $J_b$ , along  $a$  and  $b$ , and AFM exchanges  $J_c$  along  $c$  [2]. From the three exchanges,  $J_b$  plays an important role as driving force for the emergence of new phases, crucial for the understanding of the corresponding phase diagrams. Interestingly,  $J_b$  can be continuously changed by modifying the tilt angle [1]. So, the competition between FM and AFM interactions can be unbalanced by changing the size of the rare-earth ion. The balance between these competitive interactions could lead to modulated magnetic structure and, through the Dzyaloshinskii-Moriya interaction, ferroelectricity arises.

The solid solutions, R<sub>1-x</sub>X<sub>x</sub>MnO<sub>3</sub> with R = Gd<sup>3+</sup> or Eu<sup>3+</sup> and X= Y<sup>3+</sup>, Ho<sup>3+</sup> or Lu<sup>3+</sup> ions, are very interesting compounds to be addressed to. Since by modifying the effective A-site ionic radius, through substitution, the tilt angle is altered accordingly, the balance between competing FM and AFM exchanges varies, and thus new physical properties are expected to emerge.

This work is aimed at studying the effect of the effective A-site ionic radius on the orthorhombic distorted perovskite: Gd<sub>1-x</sub>Y<sub>x</sub>MnO<sub>3</sub>, Eu<sub>1-x</sub>Y<sub>x</sub>MnO<sub>3</sub>, Eu<sub>1-x</sub>Lu<sub>x</sub>MnO<sub>3</sub> and Eu<sub>1-x</sub>Ho<sub>x</sub>MnO<sub>3</sub> at room temperature. The  $x$ -dependence of the lattice parameters and the tilt Raman active mode frequency is analyzed and discussed. A universal behavior of both lattice parameters and Raman frequency can be achieved if the Goldschmidt tolerance factor is used instead the doping concentration  $x$ . This outcome evidences that the tolerance factor is in fact a more reliable representative parameter of the lattice deformation induced by substitution, and thus, a universal parameter to figure out the balance between competitive magnetic interactions.

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# AC magnetic susceptometer: a low-cost and versatile system for the analysis of magnetic nanomaterials

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The study of magnetic nanostructures is of particular interest due to their various applications in Spintronics, Catalysis and Biomedicine (e.g., therapeutical hyperthermia). An AC magnetic susceptometer allows the characterization of the magnetic properties of these materials, in particular, their magnetic susceptibility.

In this system, the samples are introduced into one member of a pair of pick-up coils positioned within a pair of Helmholtz coils. The coils are supplied with an AC current by a Kepco source connected to an ultra low distortion function generator, creating an AC magnetic field. Due to flux changes and according to the Faraday law, a voltage signal is induced in the pick-up coils. Since the identical pick-up coils are connected in series opposition, the voltage signal is compensated in the absence of an external sample placed in the pick-up coil. A lock-in amplifier is used to detect and measure the signals' amplitude and phase in relation to the one supplied to the Helmholtz coils [1].

Both the lock-in amplifier and the function generator are connected to a computer and controlled by a LabView based program. It allows the easy configuration of different parameters: frequency and amplitude of the generated function, lock-in sensitivity, etc. The data acquired by the lock-in is represented and saved in real-time. This set-up has been used to characterize commercial magnetic samples with different magnetic susceptibilities (Fig. 1), magnetic nanowires and different amounts of commercial powders of magnetic nanoparticles (MNPs). Currently, the work is in progress on real-time monitoring of the synthesis of Fe<sub>3</sub>O<sub>4</sub> MNPs by aqueous coprecipitation [2].

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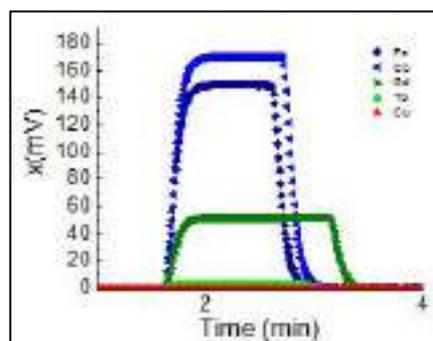


Fig. 1: Voltage signal of Fe, Co, Gd, Tb and Cu obtained by the AC susceptometer.

# Fine-structure “constant” variation in the Symmetron Model

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The variation in time and/or space of some quantities widely held as constants, such as the fine-structure constant ( $\alpha$ ), was first considered by Dirac.

Until recently, there was no experimental evidence that suggested that these constants really changed. However, several astronomical observations in the last decade claim to have detected a variation of  $\alpha$  (the “constant” we focused in this work) over a cosmological time scale [1].

A variety of theoretical models have been proposed in an attempt to explain these astronomical observations (and still be consistent with the constraints that experiments on Earth introduce). These models include the Symmetron Model, the model we examined in this work.

In order to test whether the Symmetron Model could explain the experimental data N-Body simulations were used as described in [2].

It was found that, given appropriate parameters, this model exhibits a variation of  $\alpha$  compatible with the experimental evidence, i.e. it conforms with local constraints and still exhibits a non-zero variation of  $\alpha$  in different environments.

In conclusion, we found that the Symmetron Model is a viable model for explaining the behavior of the fine-structure constant,  $\alpha$ .

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# A Stellar Test Of The Physics Of Unification

**J. P. P. Vieira**<sup>1,2</sup>, **C. J. A. P. Martins**<sup>1</sup>, and **M. J. P. F. G. Monteiro**<sup>1,2</sup>

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There is a group of physical constants whose values are intimately related to the relevant scales for an array of important physical phenomena. In fact, these quantities are so ubiquitous in all branches of Physics that they are commonly referred to as the “fundamental constants” of Nature.

In the last century, the fact that they are actually not constant but instead vary with energy was realized by particle physicists looking into the mechanisms of the fundamental forces of Nature. Nowadays, whether they also vary in time and space is one of the most controversial open questions of modern Cosmology [1,2].

In our work we discuss how the impact of the variation of these quantities on the internal structure and evolution of computational models of our Sun can be used to constrain the variations of said “constants” – in this case, the electromagnetic fine-structure constant ( $\alpha$ ) and the gravitational fine-structure constant ( $\alpha_p$ ), whose variations are related via two phenomenological parameters usually called R and S. In a first approach, the analysis is limited to a simple polytropic model [3], but the study of a more realistic evolving case, made possible by the already existing stellar evolution code MESA [4], is currently in progress.

From the study of the impact of these variations on the central temperature of a polytropic Sun [3], the following approximate inequality is found:

$$|4R + S| < 10^{-144} \left| \frac{\Delta\alpha}{\alpha} \right|^{-1} \quad \text{Eq. (1)}$$

constraining a relative variation of  $\alpha$  to  $10^{-4}$  for a “canonical” choice of R and S.

Although this bound is rather conservative, it clearly confirms that we are justified to look for evidence of variations of fundamental constants in solar-type stars. In particular, the case of an evolving Sun we are currently working on is of considerable relevance [3].

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# An Econophysics Approach to the Theory of Migrations

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We present a new approach to the modeling of migrations, understood as the demographic flows from one region to another. Inspired by results from fundamental Physics, including Thermodynamics and Statistical Physics, the model discussed may be applied to study migratory phenomena within such varied fields as Biology, Anthropology or Economics.

A great part of the models proposed to study migrations [1] shares close analogies with models used in Physics, being the most notable the so-called gravitation model of migration, an adaptation of the Newton's law of universal gravitation. However, empirical data to support such models is scarce and the forecasting ability of many of these is highly questionable, meaning that consensus is still to be achieved.

We pursue a different perspective, based mainly on concepts of Statistical Physics and Game Theory. It is possible to generalize the concept of temperature to non-thermal systems [2]. Indeed, an analogy between economical parameters and thermodynamic variables was already proposed [3] and fundamental ideas of Statistical Physics were already applied to study economical phenomena such as the distribution of money [4].

Following these ideas we face migrations in a similar manner and propose a model to study the distribution of individuals within a given region with the tools of Statistical Mechanics and Thermodynamics. We propose a plastic model, suitable for most of migration phenomena, in analogy with the ideal gas equation, enlightening in particular its close relation to Neoclassical Economics and Game Theory.

Finally, we discuss the range and limitations of this model and compare its results with empirical data from interregional migration in Portugal.

As part of the emerging branches of Econophysics and Social Physics the study of migrations within the frame of Statistical Mechanics allows an interdisciplinary and mathematically resourceful point of view, as shown by this model.

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Oral Sessions ▶ A3

# **Sport Sciences I**



# Effects of Rally Practice in Pedal Dexterity

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The present study aimed to understand whether the practice of Rally affects or not affects the skill on the level of the pedal Preferred Foot, Non Preferred Foot and Functional Asymmetry.

The sample consisted of 12 male subjects, aged between 30 and 36 years, divided into two groups - Rally racers and non-racers.

To evaluate the skill test, it was applied a Tapping Pedal battery of tests described in the FACDEX (1990).

Statistical procedures included descriptive statistics (mean and standard deviation), and the test for independent variables, as the preferred foot, the non-preferred foot and functional asymmetry. The level of significance was set at  $p \leq 0.05$ .

The main conclusions obtained in this study were the following: i) at the level of the Preferred Foot, there are statistically significant differences between the two groups - racers and non-racers; in addition, racers had a better performance than non-racers on pedal dexterity; ii) in what concerns the non-Preferred Foot, there are also significant differences between the two groups - racers and non-racers of Rally.

Thus, it can be concluded that there is a tendency for racers to have better results than non-racers at the level of dexterity pedal; iii) at the level of functional asymmetry, there are significant differences between the two groups - Rally racers and non-racers. For this reason, racers showed lower levels of functional asymmetry compared to non-racers.

**KEYWORDS:** RACERS AND NON-RACERS OF RALLY, DEXTERITY FOOT, FUNCTIONAL ASYMMETRY

# Effect of gender in simple and two-choice reaction time. Study with university students

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The present study aims to know the effect of gender on simple reaction time (SRT) and two-choice reaction time (TRT) in university students and check the levels of correlations between these two abilities. Our sample comprises 11 males and 12 female university students of the first degree in Sport Sciences, with right foot preference and with no sports practice at least on year.

Coren (1993) [1] Foot Preference Questionnaire was applied to identify foot preference. The Foot Reaction Test [2] and the Two-choice-Response-Movement Test [2] were used to evaluate the SRT and TRT, respectively. Statistical analysis was conducted using PASW Statistics 20.0 and procedures included descriptive statistics followed by the Mann-Whitney U test and Spearman test with a 5% level of significance.

This study allowed us to conclude that: i) concerning the preferred foot, the non-preferred foot and the functional motor asymmetry, differences between genders were not statistically significant with respect to the SRT; ii) in TRT there were differences statistically significant between genders in all dimensions assessed, being male more proficient than female; iii) the correlations were negatives between the two abilities; iv) inside TRT dimensions we observe strong and positive correlations between TRT total and CRT for left and right sides; v) the correlations were negatives concerning males and positives in female, but not statistically significant; (vi) there were weak and non significant correlations between SRT and TRT.

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# Effects of head-out aquatic program on body composition, cardio respiratory endurance, muscular strength and flexibility of adolescents and young adults

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Head-out aquatic exercise is a fitness activity performed by non-athletes of several ages. The main goal of this kind of activity is to improve the physical fitness in order to get positive effects on well-being and mainly in health. Being the head-out aquatic exercise a sport with practitioners of different ages, it is imperative to know the effects of the programs on the practitioner's physical condition. However, assessing the effectiveness of head-out aquatic exercise programs is still needed. The studies already performed in this area have used mainly old-aged subjects, especially women, and almost no findings were observed with reference to adolescents and young adults. The present study aimed to determine the effects of a program of head-out aquatic exercise on weight, body composition, cardio respiratory endurance, muscular strength of upper limbs/trunk, muscular strength of lower limbs, muscular strength of abdominal and flexibility of adolescents and young adults. Twenty-two subjects were separated in two groups, an experimental with 12 subjects (19.0±1.41 years, 62.38±4.88 kg, 157.0±0.03 cm, 25.50±2.35 kg.cm<sup>2</sup> and 31.05±2.49% body fat) and a control group, with 10 subjects (18.8±0.50 years, 60.08±11.16 kg, 157.0±0.06 cm, 24.44±4.66 kg.com<sup>2</sup> and 28.05±8.92% body fat). The subjects fulfil a head-out aquatic exercise program that lasts for 9 weeks, in a total of 31 sessions of 50 min long, developed three times a week. Each session was divided in 4 parts: (1) 5 min warm-up with an intensity correspondent to the levels 13 to 14 of Borg scale; (2) 20 min aerobic segment composed by exercises with moderate to severe intensity (levels 14 to 18 of Borg scale); (3) 20 min strength segment composed by sequences of movements for the legs, abdominal and arms/body muscles; (4) 5 min stretching and relaxation of the muscles exercised. The movements used were performed with a musical cadence of 135 bpm. Subjects were assessed prior and after the implementation of the program. Five tests for physical assessment were applied: yo-yo intermittent endurance test, sit up's test, arms extensions test, squats test, and sit and reach test. The percentage of presences of the subjects in the exercise program was 72.31±16.76%. The experimental group abdominal strength increased significantly (p<0.05) from pre (24.25±10.01) to post-test (44.50±21.69), as well as the flexibility (30.63±7.36 vs. 32.00±5.94). A huge variability of the responses to the several tests was observed. It was concluded that the head-out aquatic program induced an increase of abdominal strength and flexibility of the hamstring muscles.

**KEYWORDS:** HEAD-OUT AQUATIC EXERCISE PROGRAM, ENDURANCE, STRENGTH, FLEXIBILITY

## **A look at blindness: Case study of two blind children included in Orientation and Mobility Program**

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This study emerged in order to analyze a specific set of characteristics belonging to two visually impaired children, in order to know the specific problems about this issue and subsequently try to understand how these same peculiarities influence the normal development of a child with visual impairment.

We could count on the participation of Mariana and Soraia, two blind children aged 7 and 13 years and both belonging to the Orientation and Mobility Program of the School Rodrigues de Freitas.

After performing the various tests we concluded that in fact the child's development is greatly affected and exists a number of factors which will highlight along its development. We decided to observe the various indicators of poor posture on two students (scoliosis, hyperlordosis, kyphosis, etc...), the notable lack of balance both in static balance and dynamic as in the different gait patterns comparing the two legs of each student. The test propriocetividade, however, was the test had better results.

After analyzing the results, we could understand the reason for the existence of several specific characteristics (physical and motor) that are observed in this general population and allowed us to carry these same results for a better understanding of the fundamentals of Orientation and Mobility Program.

# SPACES OF PHYSICAL EDUCATION PHYSICAL: ANALYSIS OF MUNICIPAL SCHOOLS CASTANHAL-PA/BRASIL

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The physical space has its importance in the teacher pupil in the school. These sites should be inviting for teachers, as well may represent relationships of intimacy and affection, which can manifest themselves through visual or aesthetic appreciation and senses from the coexistence (Matos, 2007).

This study aimed to perform a job fostering a discussion about the teaching of physical education in public schools in the town of Castanhal -PA/Brasil in order to contribute to the analysis of physical infrastructure and how this allows (or not) in the design Teacher of Physical Education for the proper conduct of curricular activities and physical conditions in which they are held. This research had the tool, a questionnaire of questions and answers created exclusively for this study. Municipal schools were mapped and their specific physical spaces for physical education classes. Made up of 11 randomly selected schools in a total of 27 existing in the urban area being interviewed their teachers, representing a sample of 40.7% of institutions.

As Medeiros (2009), to treat the physical space under the shed of school physical education, are common in Brazil Physical Education classes happen in places without the least appropriate structure where the teacher can get to provide their own materials and / or develop their classes solely with alternative material, putting into practice the theory of romantic creativity. Of all the schools surveyed, 54% have a sports field, and in 27% of institutions do not exist own physical spaces for classes, in this case, the teachers assumed the use of neighborhood sports courts, as also stated there is no other physical spaces in school, as alternative spaces (video room, computer lab, auditorium etc.).

It is noteworthy that in one of these schools (9%), an indoor sports court was under construction. As the site of action, more than 50% of teachers classified as good / very good spaces in which they work, while in other 3 schools (27%), such spaces are classified as satisfactory and only 1 teacher (9%) ranks as insufficient. When asked about the possible interference in the teaching of physical education in the workplace, 18% indicated not suffer any interference, while over 80% indicated a lack of space, lack of materials, such as the factors that influence negatively developing their work plans. We conclude from this study that despite the presence of blocks in most schools, spaces and materials for teachers are essential to achieve the goals proposed by the Physical Education, since the lack of these create some professional motivation.

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# Perfect body and its connection with the waiver of exercises

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The study of the body in a biological perspective has been well studied and is widely used in various fields of studies, however, the body senses beyond the biological also brings many psychological senses.

One of the earliest ways of human communication was the gestural expression. Today these sets of signs are widely used to make communication more understandable or no verbal [1].

The importance of studying these signs are necessary because today this nonverbal language became a mass instrument for the marketing. Understanding the evolution of this language makes it clear why certain body type has the meaning checked today [2].

Since its beginnings that primitive man uses his survival instinct of nature to collect their food. For this purpose uses physical components such as running, jumping, crawling, throwing and rolling, these are some of the components that characterize the hunter man body [3]. These elements are expressed in his body and may be perceived by those which are able to translate these signs.

In order to survive longer the Human Being does everything to be healthy and it may explain why he seeks a model of body leaner and stronger, however, does not justify all cargo that media are selling to people. The advertisers often offer body ideals that are not the right for the Human being needs.

What would be the ideal Healthy body? This is a very important question to be asked, because nowadays the ideal body is seen as the perfect body sold by the Media. The practice of physical exercise remains sabotaged by false idea that will never reach the level that is expected, generating demotivation and subsequently waiver of any workout plan while the society has as reference the inadequate nonfunctional body sold by marketing.

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# Effect of manual preference and sport practiced on manual dexterity of female handball and volleyball players

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The adaptation represents the need that living beings have to develop structural and functional characteristics. Following the acquisition of bipedalism we became increasingly skilled with hands, reflecting on our manual dexterity, which is present in many sports. This study aims to determine the effect of manual preference and the effect of the sport practiced in manual dexterity and in functional motor asymmetry of volleyball and handball players. The sample comprises 18 female athletes: 12 handball players (6 right-handers and 6 left-handers) and 6 volleyball players (all right-handers), between 16 and 18 years old. Manual preference was assessed with Van Strien Questionnaire (2002). Athletes practicing for at least four years and training three times a week for two hours. *Soda Pop Test* (Osness et al., 1996) was used to assess manual dexterity. Statistical procedures included descriptive statistics and the *Mann-Whitney* test to compare both sports and handedness groups. The results of the study show that there are no significant differences in manual dexterity between modalities or between right-handers and left-handers. Also demonstrate that there are no significant differences that allow us to conclude that left-handers are better with their non-preferred hand than right-handers. Regarding the functional motor asymmetry, we did not find significant effects of sport practiced and manual preference.

**Keywords:** Manual dexterity, Volleyball/Handball, Manual preference, Functional motor asymmetry

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Oral Sessions ▶ A4

# **Sociology & Communication Sciences**



# Human Resource Management and social entrepreneurship profiles in Third Sector Organizations

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In the context of Multidisciplinary Projects IJUP-2011 on "Social Entrepreneurship in Portugal: contributions to the analysis of its emergence in educational and organizational areas" and another on "Social Entrepreneurship in Portugal" funded by the FCT, which 2-year research career, it was considered important to analyze the relationship between social entrepreneurship profiles and the Human Resources (HR) management in 7 organizations of the third sector (OTS).

Currently the social entrepreneurship is focus of attention worldwide. An issue that attracts several sectors of society, amongst other reasons, for being regarded as a form of innovation aiming to overcome the challenges of the crisis of the Welfare State. The term *social entrepreneurship* emerged in the 90s (twentieth century), combining a diversity of conceptual approaches which come from the Anglo-Saxon, European and Latino-American approaches.

We propose answering to the following survey question: what relationship is there between the HR management and social entrepreneurship profile in OTS? To this end, the social entrepreneurship profiles were defined from an extensive analysis of the information gathered on 89 OTS, wherein one of the analytical vectors was HR management. Through a combined multivariate analysis (with the use of hierarchical cluster analysis and of the simple correspondence analysis as exploratory techniques) three profiles of social entrepreneurship were established: low, moderate and strong guidance.

In this communication, we focused the analysis on 7 cases of OTS with moderate and strong entrepreneurship so as to deepen the characteristics of HR management. The approach was made through indicators available in secondary source: the Single Report of 2011. The set of variables that characterize the HR which we tried to relate to the social entrepreneurship profiles were the personnel evolution, sex, age, educational and professional qualification, antique, absenteeism, leaves, training, promotions, contracts and salaries.

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# Comissões Unitárias de Mulheres do Porto: Recovering the Memories of a social Movement

**J. Correia<sup>1</sup>, C. Lopes<sup>1</sup>, A. Andrade<sup>2</sup>, S. Araújo<sup>1</sup>, J. Caramelo<sup>1</sup> and T. Medina<sup>1</sup>**

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Looking forward to rebuild and contribute to the preservation of social, cultural and political memory of the Oporto city in the late 20<sup>th</sup> century [1], the research on the *Comissões Unitárias de Mulheres do Porto* (Unitary Commissions of Women from Oporto) aims to reconstruct the trajectory of this social movement (forms of organization, communication, mobilization and socio-educative meanings) and its relations with a specific historical context filled with struggles and social interventions. This research is integrated in the Young Researchers Program of Oporto's University, supported by the Santander Bank, which promotes research projects that include undergraduate students.

Emerging after the 25th of April's revolution and lasting until the early 90's, the Unitary Commissions of Women from Oporto represent a movement of social and political action claiming a new role for women in the public sphere. Their actions may be defined as a proactive and participatory struggle around social issues directly related with their living conditions. With a well-structured organization, these commissions acted by raising awareness of the population on the value of active participation and mobilizing them for actions of manifestation. Their targets were governmental institutions and their claim was for equitable living conditions for the population.

With a team involving researchers and students from fields such as Education Sciences, Fine Arts, History and Computer Sciences, this research began with the collection of public documents on this movement (posters, flyers, and newspaper reports) that lead us to some of the activists of these commissions and created the possibility of constructing a memory of the Unitary Commissions of Women from Oporto. Since one of our goals, on top of preserving the collective memory, was the divulgation of this materials, we were led to the classification of the documents within the portfolio, which was organized following consistent criteria, and allowed us to draw the history it contained by creating a chronological table and a website where we share the documents (<http://memorias.dcc.fc.up.pt/cum/>). The methodological path based on documental analysis and oral history [2] permitted us to cross information and testimonies and draft their common memory. We retrieved important moments in history that testify the identity reconstruction of women from Oporto after a period of dictatorship, leading us to the discovery of how this process occurred and its impact on both individual and social issues.

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## ***East of Eden... reconstructions of the immigrant identities around music***

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The construction of the social world is inseparable from the representations of the intervention of a diversity of actors that (re)produce endlessly throughout space-time coordinates. It is in this continuous production of identity representations that we search for the genesis and shaping of our object. We intend to address a Sociology's key subject, the ability to understand multiplicity of relationships that are established between experiences in social structure and the appropriation of music, meaning this, to evaluate the significance of music in the construction of identities [1].

We consider that music is a communicational language of particular interest for it knows no boundaries and, as so, it "talks and belongs to everyone". Concomitantly, music is one of the most pervious manifestations to contagious, and, steadily more, an important marker of boundaries and group identity, expressing ideas and emotions shared within a community, embroiling individuals in the defence and claim of identity. Music is socially significative because it actively encourages means though which people recognize social identities, highlighting differentiations in class, status, ethnicity, age, gender, etc.. Connecting this approach with immigration, it is possible to affirm that social agents are most likely to face dislocation, separation, insecurity and discrimination. The only reason to be an emigrant is therefore, as Sayad [2], states, work, a non-permanent job, in transit. For that reason, to the immigrant it is significative to use music as a identitary framework of reference to "re-establish" himself.

This communication will present the preliminary results of a number immigrant's representations based on semi-directive interviews on musical affiliations, arguing that there is a re-materialization of culture regarding situational and relational practices, as the circumstances of activation of musical practices are privileged instances from which we are able to read the unfolding of attitudes and typologies of interaction [3]. Culture is beyond ideas and values and it is impossible to define the relation between culture and social structure as a pure homology as it is instead a incessant process of (re)construction where different and plural identities meet. Consequently, this is an underlying contribute of Sociology towards the understanding of multiculturalism as it formulates music as the transposition of structural principals of social life, being a framework to shape new subjectivities and specific typologies of migratory integration.

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## The dialogue of public opinion on current censorship

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Since the decade of 1980, the Library of the School of Communication and Arts is responsible for the Miroel Silveira Archives: a collection of 6.137 documents of prior censorship process of theater plays that occurred in Brazil, in the period from 1930 to 1970. That rich amount of documents – which includes censorship requests, plays copies, censors analysis and appraisals and so on – underlies the research work that has been done at the Communication, Freedom of Speech and Censorship Observatory (OBCOM). However, aware of the importance of developing studies that surpass the notion that censorship is just part of an overcame past, typical of dictatorial governments, the researchers started to consider new ways of apprehending and considering the existence of a current censorship. As professor Luiz Francisco Rebello affirms, “the censorship has only one face, but many masks”[1].

When looking at a recent censorship, the public opinion – which is the primary focus of this research - appears as an important way of trying to redefine the concept of censorship, since it allows us to establish contact with the current social values about this subject. In the contemporary panorama, it seems to be impossible not to explore the world of Internet and its possibilities as a dialogical communication vehicle: “far from encouraging the irresponsibility linked to the anonymity, the virtual community explores new forms of public opinion”[2].

Among the main activities developed by the research, it is important to highlight the creation of a virtual newspaper library called Hemeroteca Digital Miroel Silveira, which recovers the recent materials that has been published on the media about censorship. Started on April 2012, the collection already has more than 960 cataloged documents, and is available to all interested users.

A significant part of the material that presents virtually expressed public opinions about censorship is the comment platform that many virtual addresses have, as newspapers sites and personal blogs. Allowing readers to express themselves freely about recent facts, it can unveil ideological positions, discover relevant researches topics and raise discussions about polemic questions that concerns to our research.

Analyzing an amount of comments published on news that are linked to the censorship subject, and comparing these recent public opinion manifestations with the censorship requests that came from the society and are archived among Miroel Silveira’s documents, some supportive censorship arguments seems to have been gone, as relevant new topics appeared, such as the freedom of speech discussion and the thin limit between censorship and regulation in democratic countries.

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# Som à Letra

## A Civic Digital Media

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Journalism lives times of crisis. The RTP is in the process of being privatized, Lusa will suffer huge cuts in state support, and journalists from Publico will see their salary reduced dramatically. Unemployment for journalists (both generations) is a reality.

Lusa, for instance, lives difficult times. A group of workers from different organizations representing employees of the agency delivered at the office of Pedro Passos Coelho, Sao Bento, Lisbon, with a framework document explaining the agency's current situation and the dangers of a reduction in state funding, in particular *weakening of Democracy* that this represents, the lower the resulting news coverage"[1]

In what matters for RTP, the concept of public service is questioned, accompanied by a possibility of firing the professionals. "Channel presents new grille and new image for this year of decisions for the future of the company. Advertising investment fell 30% in 2012" [2]

For young people is idyllic or impossible to get jobs in the area, having no alternative but to become entrepreneurs. The difficulties begin, however, self sustaining the respective media

"Som à Letra" was born amid this background of uncertainty on September 18<sup>th</sup>, 2009, a media that not only informs, but also teaches, entertains and helps readers through it's projects: "Som Fm, Som TV, Som Civico".

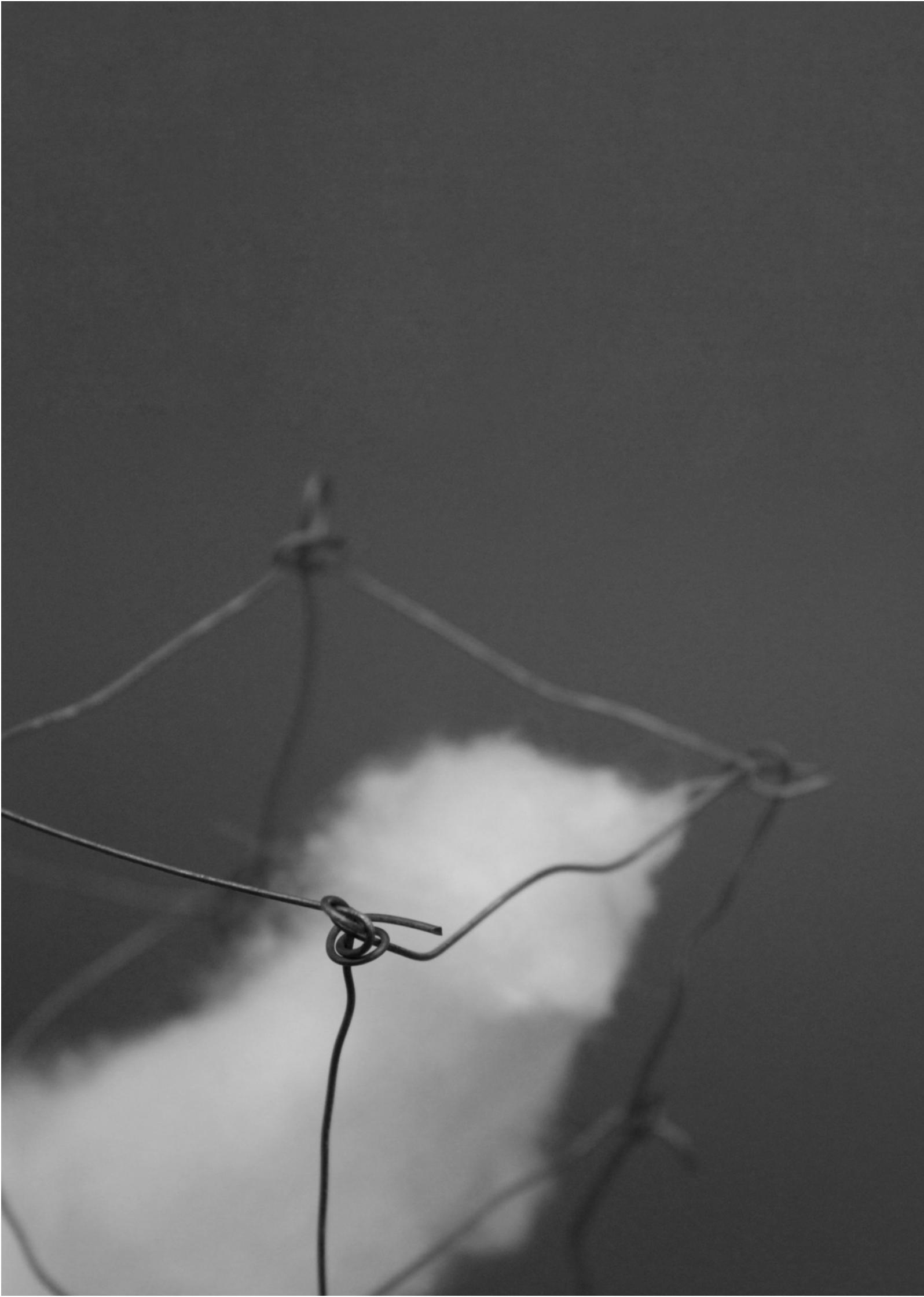
Civic journalism "at its heart is a belief that journalism has an obligation to public life - an obligation that goes beyond just telling the news or unloading lots of facts. The way we do our journalism affects the way public life goes. Journalism can help empower a community or it can help disable it. [3]

Som Civico [4] appeared with the main objective of helping communities and associations, without any fait divers or dependence from the Government. Many activities are already being prepared, by Som à Letra's team.

We hope in this article relate the history of this medium, both in terms of journalism, both in terms of coordination (business model and social responsibility).

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**ORAL SESSIONS**

**VIII**



Oral Sessions ▶ A1

**Public  
Health &  
Epidemiology  
II**



# Compliance of lunch portions for 3 to 10 years old children

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Menus planning and portions compliance with recommendations are essential for an adequate nutrient supply of food services [1, 2].

The evaluation and monitoring of school meals either in qualitative and quantitative aspects is important to improve food service quality, cost control, as well as promoting health and well-being of children[3].

The objective of this work was to compare portions served and consumed with recommended quantities, according to the nutritional needs of children.

The study was conducted in a Social Institution, in Águeda municipality. 48 children aged from 3 to 10 years old were evaluated. Lunch portions served and consumed, of 10 selected menus were registered.

Portion were much higher than recommendations concerning protein and carbohydrates suppliers' (except potato). On the contrary vegetables portion served and consumed was below the recommended value. We have found significant differences between the average portion served and consumed in relation to recommendations, to all components, which determines overfeeding.

The oversizing of servings contributes to high and excessive energy consumption, increasing risk of overweight [4].

No associations were found between Body Mass Index classes and portions served or consumed.

This study demonstrates the need to calculate lunch portions and compliance to needs of different age groups as well as employees training on quantities to be served, pondering the purchase of tools that facilitate compliance with the recommended amounts.

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# Influence of satisfaction with school meals on food waste in fourth-grade children

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**Introduction:** School meals play an important role in children's food habits development [1, 2]. In spite of schools meals usually being nutritional balanced, these are frequently rejected [3], so that benefits are no longer assured [4]. Food sensory characteristics influence children acceptance concerning school meals [5], being an important determinant of food waste [6].

**Objectives:** To evaluate children's satisfaction with school lunch sensory characteristics and its impact on plate waste.

**Population and methods:** There were included all fourth-grade children attending 6 schools from Oporto. Data collection was performed in two sequential days: on the first day, plate waste was evaluated by the physical measurement; on the second day, it was collected socio demographic and anthropometric data and satisfaction of children with school lunch in relation to sensory characteristics was evaluated.

**Results:** Plate waste was 20,5% for soup and 32,4% for the main dish. The waste obtained by protein and carbohydrates-rich foods was 13,5% and 31,3%, respectively. The most dissatisfaction was observed for appearance and smell of meals served. It was found that as satisfaction with sensory characteristics increased, food waste reduced, with significant results for soup taste and smell and the main dish taste.

**Conclusion:** Children's satisfaction with school lunch had influence on plate waste. In order to reduce food waste, besides improvement of taste and appearance of meals, interventions for school community should be planned, to increase satisfaction and acceptability of school lunch.

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# **Adherence to mediterranean dietary pattern and nutritional status association in primary school children from Póvoa de Lanhoso**

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**Introduction:** Portugal is moving away from the traditional mediterranean dietary pattern (MDP). Since the 70's, coincidentally or not, the prevalence of obesity in school-age children has hugely increased.

**Objective:** To assess adherence to MDP, its determinants and association with nutritional status.

**Methodology:** Children from the 3rd and 4th years, of two primary schools from Póvoa de Lanhoso, participated in this cross-sectional study. During the months of May and June 2012, personal data were collected (sex, age, household, etc.) as well as anthropometric data (height and weight) and the application of the KidMed questionnaire.

**Results:** Of the 166 children studied, aged between 8 and 10 years old, 56% were female and 44% male. It was observed that 66.9% presented appropriate weight and 33.1% were at risk for being overweight or obese. 61.4% had good adherence to MDP and 38.6% had a moderate to weak adherence. Female children showed a greater adherence to MDP ( $p=0.041$ ). No statistically significant differences were found between adherence to MDP and the type of household with whom the child lived ( $p=0.402$ ). Children with overweight or obesity showed less adherence to MBP ( $p = 0.013$ ).

**Conclusion:** In this study it was found that more than half of the population had good adherence to MDP, more specifically, the female children. There was no association between the adherence to this dietary pattern to the type of household with whom the child lived. Children with better adherence to the MDP had a lower prevalence of overweight and obesity.

# Assessment of Food Insecurity in households of a social neighbourhood

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The assessment of food insecurity enables not only to characterize the food security of a population, but also to draw conclusions about the food security of a regional population or local groups. [1] This study evaluates the security of households living in a social neighborhood. A scale of perception of food insecurity was applied and the relationship with socio-cultural factors was evaluated.

The sample included 99 households and the scale used was adapted from the Brazilian Food Insecurity Scale. [2, 3] This scale includes 14 questions focused on the perception of food insecurity, ranging from concern about the possible lack of food, to a severe food restriction due to economic difficulties.

It was found that about 85% of households were living with food insecurity at different levels: mild, moderate, or severe, corresponding to 38.4%, 26.3% and 20.2%, respectively. Higher levels of food insecurity appear to be associated with lower education levels, lower family income, inexistence of children, changes in consumption of essential foods, decreased number of visits to doctor and purchase of medicines and if a woman represents the household.

In Portugal, more studies are needed in order to assess the prevalence of food insecurity, given the increasing difficulties that an increasing number of families are facing. It is important that local authorities develop regional studies to better assist the population in terms of nutritional education and social support, aiming to minimize the magnitude of the problem and its consequences.

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# **Anthropometric evaluation of primary school children from Póvoa de Lanhoso: comparison of two methods**

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**Introduction:** It is now recognized the importance of the accumulation of abdominal fat in the development of cardiometabolic diseases. It is extremely important to develop screening tools that are reliable and low cost. The ratio between waist circumference and height (WHRt) has been suggested as an important tool in the assessment of cardiometabolic risk.

**Objectives:** To investigate whether the WHRt is dependent on the sex and age, to describe and compare the prevalence of risk associated with the values of WHRt and BMI.

**Methodology:** The sample consisted of students attending the 3rd and 4th years of two schools from Póvoa de Lanhoso. Data were collected during the months of May and June 2012. The children were measured (weight, height and waist circumference) according to standard procedures. The WHRt was calculated and the children categorized as "at-risk ( $\geq 0.5$ )" and "out of risk ( $<0.5$ )." BMI was calculated and the classification made by the respective percentiles according to the CDC criteria. The agreement between the WHRt and BMI was calculated using Cohen's kappa.

**Results:** The study comprised 166 children, 56% were female and 44% male, aged between 8 and 10 years. There were no statistically significant differences between the WHRt and sex ( $p=0.404$ ) or age ( $p=0.661$ ). The proportion of children considered at risk was lower when measured by the WHRt (20.5%) than according to the percentiles of BMI (33.1%). The agreement between these two methods was moderate ( $k=0.594$ ,  $p<0.001$ ), and there have been a greater level of agreement among females ( $k=0.740$ ,  $p <0.001$ ) than in males ( $k=0.405$ ,  $p <0.001$ ).

**Conclusions:** In this study, the WHRt was independent of sex and age, indicating that this may be an easy tool to use in children. There was a strong correlation between BMI and RPCA and there was a moderate agreement between these two methods.



Oral Sessions ▶ A2

# Math



# Dynamics of Human Decisions

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**Abstract:** We study a dichotomous decision model, where individuals can make the decision yes or no and can influence the decisions of others. We characterize all decisions that form Nash equilibria. Taking into account the way individuals influence the decisions of others, we construct the decision tilings where the axes reflect the personal preferences of the individuals for making the decision yes or no. These tilings characterize geometrically all the pure and mixed Nash equilibria. We show, in these tilings, that Nash equilibria form degenerated hystereses with respect to the replicator dynamics, with the property that the pure Nash equilibria are asymptotically stable and the strict mixed equilibria are unstable. These hystereses can help to explain the sudden appearance of social, political and economic crises. We observe the existence of limit cycles for the replicator dynamics associated to situations where the individuals keep changing their decisions along time, but exhibiting a periodic repetition in their decisions.

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# Influence of tourist relations in resort prices

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When a group of consumers faces a choice between several products, we may consider their decision is based not only upon the prices and characteristics of the products, but also on the decisions made by others, as the decision model in [1]. Identical consumers may then have different strategies in equilibrium due to the way they relate to the decision of others. A natural question arises: how are prices affected by consumer relations?

We approach this problem as a resorts-tourist game inspired by [2]. Here we consider a two stage game, where a group of tourists has to choose between two resorts. In the first stage of the game prices are set by the resorts and in the second stage tourists make their choice following the model developed in [1]. Their decision thus depends not only on the resorts characteristics and prices, but also on the influence resulting from how much tourists like or dislike to be with each other in each resort. We observe that a crucial parameter is the average influence tourists have on each other.

The outcome of the game is the pair of prices and the tourists allocation. We characterize game equilibria using the notions of Nash equilibrium and subgame-perfect Nash equilibrium. Depending on the model parameters, the second stage may have multiple equilibria, which is known to pose a problem on finding game equilibria. We allow for two different cases: on one hand tourists reaction to price changes may depend on which resort is changing its price; on the other hand tourists reaction depends only on the price difference between resorts.

In the latter case, if the average influence is positive then the subgame-perfect Nash equilibria are either monopolies or competitive equilibria where resorts have zero profits. When there are negative average influences, non-monopolistic and non-zero profit subgame-perfect Nash equilibria exist. We find the resort prices and tourist preferences for which all these equilibria occur.

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# Modelling the abundance of birds in Porto: a longitudinal count regression model

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Regression models for count data have been widely used in several research fields and they require special methodological frameworks as the response variable only takes non-negative integer values [1,2,4]. The Poisson distribution has probably been the most used distribution to model count data although every time there is overdispersion, the assumption that the average is equal to the variance is not satisfied. In this situation, it is necessary to use the QuasiPoisson distribution, if average and variance are linearly related, or the Negative Binomial distribution, if the variance presents a quadratic relation with the average.

In this work we model the abundance of birds in the green public spaces of Porto between april 2011 and july 2012. The considered data is from the project “Urban Green Structure: Study of the relation between public space morphology and flora and fauna diversity in the city of Porto.” (<http://bio-diver-city.fc.up.pt>) developed by CIBIO – Research Center in Biodiversity and Genetic Resources, University of Porto [3]. In particular, we analyse the effect of time and different environmental variables on the abundance of birds of the *Passeriform* order.

A Poisson mixed regression model with a random effect for the variable *time* and fixed effects for the variables *area covered with phanerophyte* and *presence of water* are chosen. Regarding marginal models, the choice falls on a type-1 autoregressive model having *time* and an *indicator of presence of water* as statistically significant explanatory variables.

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# Modelling and computing carbon footprint of Douro vineyards region

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Carbon plays a key role in the life of a living being. In this presentation, a model [1] of the behavior of this element in the different structures of a vine will be shown. The goal is to understand whether it is possible to reduce consumption and carbon emission of greenhouse gases by the plant producing, thus, wine with neutral carbon footprint.

At a later stage, the models will be calibrated and validated using real data collected in the Douro vineyards region.

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# Comparison of Analytic Models for the Evolution of Semilocal String Networks with Numerical Simulations

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Recent developments in fundamental physics have led to a resurgence of interest in the cosmological properties of topological defects. First, it was realised that the production of cosmic strings is much more generic than previously thought in a wide class of cosmological models. In addition, new exciting developments in string phenomenology led to a generic picture of inflation ending in a phase transition that produces cosmic (super)strings

These objects are much more complex than ordinary field theory strings and their properties depend sensitively on fundamental high-energy physics parameters of the underlying theory. Moreover, a broad range of different defects may form, depending on specific details of the phase transitions. Thus, not only it was realised that string-like defects are much more generic objects that could play a subdominant role in structure formation, but, also, their potential observability could open a window into physics at the highest energy scales.

Here we concentrate on a particularly interesting example of such a defect network, known as semilocal strings. These can be envisaged as a network of local (gauged) string segments ending in a global monopole-antimonopole pair.

We use previously developed analytic models for the study of semilocal string networks [1] and compare them with numerical simulations that we recently made. We focus our comparison in the evolution of individual semilocal segments and more precisely on the phenomenology of segment growth.

We find that our models are generally agreeing with the numerical simulations.

This work was done in the context of the research grant PTDC/FIS/111725/2009 from FCT, Portugal.

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# Quantification of flux and turnover of microtubules of the mitotic spindle in metaphase

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This work addresses the problem of quantifying the spindle microtubules flux and fluorescence decay during metaphase on cells of different organisms. The present approach took advantage of the Induced Speckle Imaging technique developed by researcher António Pereira [1].

An algorithm for automatic flux quantification based on a strategy derived from Block Matching [2] was created. Two operators for spatial displacement detection were evaluated in perfectly controlled test situations: a correlation function generalized to  $n$  signals (Eq. (1)) and a function measuring absolute differences from the average, ADA (Eq. (2)).

$$GCorr(f_1, \dots, f_n) = h(v) = \sum_{x=1}^L \prod_{i=1}^n f_i(x - v \cdot \Delta t_i) \rightarrow Max(h) \rightarrow \vartheta \quad (1)$$

$$ADA(f_1, \dots, f_n) = h(v) = \sum_{x=1}^L \sum_{i=1}^n |f_i(x - v \cdot \Delta t_i) - \langle f \rangle| \rightarrow Min(h) \rightarrow \vartheta \quad (2)$$

Test films were created where the movement of artificial speckles was fully known and controlled. The results obtained in this case, with both the generalized correlation function and the ADA function, were extremely accurate, even in situations where the signal-to-noise ratio was close to 100%. Through testing it was possible to conclude that the absolute differences function is the most efficient and noise robust on the determination of displacement. The results obtained for the original images were produced using the Block Matching strategy with this operator.

For the estimation of fluorescence decay a semi-automatic algorithm was created. The method performs exponential regression using the intensity of microtubules. The robustness of the exponential fits to the fluorescence decay was tested in controlled situations, obtaining very good results.

The programming language used to build the codes for these algorithms was MATLAB.

Flux maps obtained from real data are as homogeneous as expected and the estimated values are within plausible ranges. Several maps can be combined to produce more robust flux estimates. The fluorescence decay determination tool returned results with significant determination coefficients for the regressions.

This work results from collaboration between the Master in Mathematical Engineering and the Chromosome Instability & Dynamics Lab team, from the Institute of Molecular and Cell Biology, led by Dr. Hélder Maiato.

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Oral Sessions ▶ A3

# **Sport Sciences II**



# Association between habitual physical activity and blood pressure of children Pre-school

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**Introduction:** Although the essential hypertension is considered a frequent disease only in the adult population, is now recognized in the literature the increasing prevalence, overcoming cases of secondary hypertension in children and adolescents (Din-Dzietham et al., 2007).

Research suggests that moderate levels of PA are predictors of lower values of systolic blood pressure (SBP). However, few studies addressed this issue in pre-school children.

**Purpose:** The purpose of this study was to analyse the associations between compliance of PA recommendations and SBP preschool children.

## **Methods:**

The sample comprised 439 preschool children (48.8% female) with mean age of 3.5 years. Physical activity was assessed during 7 consecutive days by accelerometer (Actigraph GTM1). We analyzed the recommendation of at least one hour daily of moderate-vigorous PA (MVPA) (Tremblay et al., 2012). Blood pressure was measured using the Colin monitor. The measurements were performed with each subject seated and rested for at least five minutes before the test. Percentage of fat (% MG) was calculated using the equation of Westrate and Durenberg. (1990).

**Results:** We found that about 12.2% of the children did not accomplish the recommended daily MVPA. The prevalence of BP above the 90<sup>th</sup> percentile (P90) was 7.8% and 1.3% respectively for SBP and DBP. Children who did not meet the daily recommendations of MVPA were twice as much more likely to have SBP values above the P90 compared to those who meet the daily recommendations (OR: 2.7; CI 95%:1.2-6.0; p<0.05), even after adjustment to fat mass.

**Conclusion:** We found an association between the accomplishment of daily PA recommendations and SBP in pre-school children.

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# Level of physical activity in the elderly above 60 years who participated in a training monitored walk

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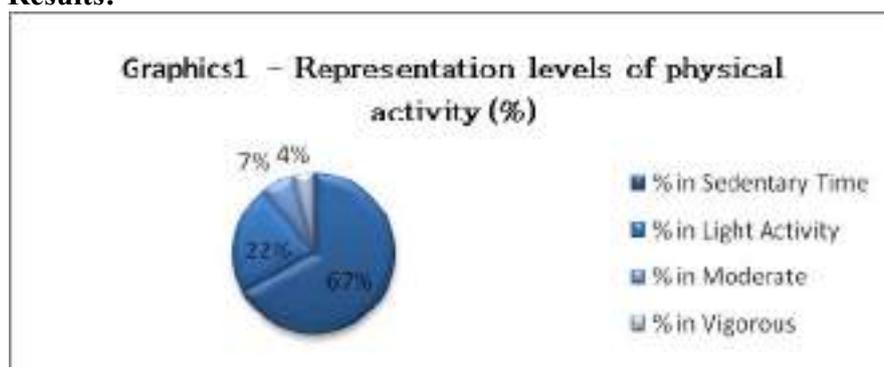
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**Introduction:** According to Nahas (2001), physical activity can be defined as any bodily movement produced by skeletal muscles that results in energy expenditure above resting levels and can be performed by any population group (children, youth, adults and seniors) having or not impairment or motor difficulty, physical and / or sensory.

**Objective:** Analyze the level of physical activity of elderly participants in a training monitored walk.

**Methods:** Twenty-one elderly above 60 years were subjected to the use of an accelerometer for seven days.

## Results:



**Conclusion:** Through data analysis, it was found that 67% of participants had a low physical activity level. The same can be identified due to the fact that individuals have begun the practice of physical activity for a short time and do not perform the same every day. Thus, the program can assist in improving the level of physical activity, but does not prevent the individual ceases to be sedentary.

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**Keywords - Keywords:** elderly, physical activity level, accelerometer, walk.

# Physical Activity, Obesity and Metabolic Syndrome in Portuguese youth

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In the last decades, several worldwide youth lifestyles changes have contributed to a significant decrease in physical activity (PA) levels, and a consequent increase in obesity and cardiometabolic prevalences. The present study has the following purposes: (1) to analyze the prevalence of overweight and obesity, PA levels, as well as metabolic risk factors in Porto youth; (2) to examine the association between PA and body mass index (BMI) with metabolic risk scores.

The sample comprises 212 subjects [(116 boys (B) and 96 girls (G)] from Porto, aged 12 to 16 years. PA was estimated with the Bouchard questionnaire [1] (3 days recall), as well as with the pedometer New Lifestyles NL-1000 (used for 5 days). Metabolic risk factors comprised fasting glucose, triglycerides, HDL-cholesterol, blood pressure and waist circumference; Cook et al [2] cut-offs were used to determine the presence, or not, of metabolic syndrome (MS). Maturational status was indirectly estimated with the maturational offset procedure [3]. A continuous metabolic risk score was computed (zMS) and PA values were divided into tertiles. ANOVA and multiple regression models were used with SPSS 18.0, and the significance level was set in 5%.

Main results were: (1) a high prevalence of overweight and obesity in both genders (40.5% B; 38.6% G) and they expend most of their time in sedentary activities (1250.42±89.04 min.d<sup>-1</sup> B; 1251.85±103.61 min.d<sup>-1</sup> G). (2) In the association of PA and zMS there was a positive trend in B and G, i.e., more active youngsters tend to have lower zMS; (3) when relating BMI and zMS, obese adolescents have higher zMS when compared to normal weighted (p <0.001).

In conclusion, the present data showed that adolescents with higher levels of PA have lower BMI and cardiometabolic risk. This finding suggests that increased levels of PA associated with a balanced diet have a positive role against the development of MS.

Acknowledgments: The authors acknowledge the financial support of U.Porto/Santander Totta/Projetos Pluridisciplinares 2010

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# The association between physical fitness, cardiorespiratory fitness and metabolic syndrome in Portuguese youth

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Modern youth current lifestyles are also characterized by the presence of some risk behaviors, such as lower levels of physical activity and physical fitness (PF), which contribute to an increased risk of metabolic indicators. The purpose of this study is to analyze the association between metabolic risk and PF levels of the great Porto youth.

The sample comprises 212 subjects, 96 girls (G) and 116 boys (B), aged between 12 and 16 years. PF was assessed with the Fitnessgram™ test battery. Metabolic indicators comprised the clustering of triglycerides (TG), cholesterol (HDL-C), fasting glucose (FG), waist circumference (WC), and systolic blood pressure (SBP), so-called metabolic syndrome (MS). MS prevalence used cut-off values suggested by Cook [1]. A continuous metabolic risk score (zMS) was computed from the sum of the metabolic indicators, after being transformed into a z score. Adolescents were also classified into fit and non-fit based on the cut-off points from the test battery. SPSS 18 software was used and the significance level was set in 5%.

In the *push up* test, B presented higher success rates than G (66% e 56.1%, respectively); *curl up* test showed high success rates in both gender (61.7% B; 62.4% G); in *aerobic fitness*, B were better than G (76.7% e 63.6%, respectively), while in *trunk lift* both groups were highly fit. The prevalence risk for each of the MS indicators was: TAS, 77.6% B, and 79.2% G; HDL-C, 28.4% B, and 16.7% G; in TG, G group showed higher risk (15.6%) than B (6.9%). A lower prevalence for WC (5.2% B; 6.3% G) and the absence of subjects with FG risk were found. zRM and PF presented an inverse relationship, but only for G group ( $p=0.003$ ), where girls with higher PF levels had lower metabolic risk.

Taken together, these results suggest that the Portuguese youth sampled in this study present some risk behaviors that may induce to cardiometabolic risk. The inverse association between PF and metabolic risk was also present in other studies [2], emphasizing the need to promote healthier and more active lifestyles in youth, as well as strategies for morbidities prevention linked to cardiovascular diseases.

Acknowledgments:

The authors acknowledge the financial support of U. Porto/ Santander Totta/ Projectos pluridisciplinares 2010.

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## **After effects of a single session of resistance or walking exercises on the blood pressure and heart rate of patients with intermittent claudication.**

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**Background:** Although exercise training have been recommended for treatment of intermittent claudication (IC) patients, their effects on cardiovascular function remains unclear.

**Aim:** To investigate the effects of a single session of resistance or walking exercise on blood pressure and heart rate of IC patients.

**Methods:** Nine patients of both gender with peripheral arterial ‘disease and symptoms of IC participated of study. All subjects were submitted to three experimental sessions in random order: resistance exercise (RE), walking (WE) and control (C). In the RE patients performed 8 exercises for upper and lower limbs with two sets of 10 repetitions, with intensity corresponding to 5-7 on OMNI-RES scale with two minutes of rest between sets and exercises. In the WE subjects performed 10 cycles of two minutes cycles on a treadmill with an intensity corresponding to the onset of symptoms of CI. A two-minute interval was given between each cycle. Before and after the experimental sessions systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were collected.

**Statistical analysis:** Normality of data was confirmed by Shapiro-Wilk test. The one way ANOVA was employed to compare the groups at baseline. The responses of the cardiovascular variables were compared using two-way ANOVA (session x time) for repeated measures. When significance was obtained, a Newman-Keuls post-hoc test was used to identify the differences. For all analysis, the significance level was  $P < 0.05$ .

**Results:** In comparison with the pre-intervention values, SBP did not change and DBP increased in all sessions ( $p < 0.04$ ). In comparison with the pre-intervention values, HR also did not change after all sessions. No differences were observed between sessions for all variables.

**Conclusion:** A single session of resistance or walking exercises did not affect blood pressure and heart rate in subjects with intermittent claudication.

# Cardiovascular Risk in Elderly participants of a Walk Program

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**Introduction:** Older adults have becoming the least physically active age group. This sedentary lifestyle has been described as determinant risk factor for several chronic conditions, namely cardiovascular diseases. . In fact, cardiovascular risk stratification seems to be of special importance in the exercise programs for older adults. However, besides the potential benefits, this procedure is not normally performed at the beginning of exercise programs for elderly.

**Objective:** To describe the risk of cardiovascular seniors interested in physical activity.

**Methods:** Twenty-one elderly over age 60 years, underwent evaluations initially comprised: anamnesis of ERC, the body DEXA evaluation and measurement of arterial tension.

**Results:**

**Table 1.** *Prevalence of cardiovascular risk in elderly*

Risk	Absoluty Frequency (n)	Relative Frequency (%)
<b>MODERATE</b>	<b>10</b>	<b>50</b>
<b>HIGH</b>	<b>11</b>	<b>60</b>

**Conclusions:** Part of seniors interested in significant physical activity is at high cardiovascular risk, suggesting that they require more comprehensive cardiovascular evaluation. Thus, the appropriate risk stratification provides a basis for recommending additional testing, medical evaluation or surgical interventions prior to participation in the exercise. (MCARDLE; KATCH, 2008). So, it is suggested that the ERC is always performed by the elderly and that program always performs this type of action.

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**KEYWORDS:** CARDIOVASCULAR, PHYSICAL ACTIVITY, RISK FACTORS, BUTCH.

# Effects of qigong on performance related anxiety and physiological stress functions in transverse flute music schoolchildren

## A feasibility study

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**Background:** Performance-related anxiety is a frequent phenomenon in musicians impairing musical performance and the overall development of quality musicians in music schools. It may furthermore be seen as a model of anxiety generally associated with presentation in public and may be regarded as a specific kind of challenge that may be faced in many professions. The nowadays almost constant obligation to present in public, on meetings, in conferences etc. may considerably contribute to elevated stress and burn out levels. It may also individually present severe obstacles in career development which may be independent of personal qualifications. Qigong has been used for ages to promote both physical and mental health. One obstacle for the distribution and integration of Qigong may be the need of time and space for exercises. A special kind of Qigong, the white ball exercises, takes 2x 5 min per day only and can be done without special requirements of extra space.

**Objective:** To evaluate if and how Qigong-related effects may be further objectified by physical measurable parameters and psychological scores.

**Methods:** Prospective controlled interventional study with waiting list design. In the Qigong group 8 children were included and they received specific Qigong lessons of the so-called white ball Qigong over 7 weeks, twice a week, for 30 minutes. They also were instructed to do the exercises at home daily. In the control group 8 children were included and they didn't receive any intervention (waiting list design). Subjective perception of anxiety (measured by the Portuguese version of Depression Anxiety and Stress Scale adapted for children), salivary cortisol, heart rate variability, blood pressure, surface electromyography of the trapezius muscle and time reaction were measured in the beginning and in the end of the study.

**Results:** Qigong reduced subjective perception of anxiety (mean of 4.5), salivary cortisol level (mean of 0.198 µg/dl), heart rate (mean of 11 beats per minute) and blood pressure (6.54 mmHg). However, only heart rate decrease showed a significant change for an  $\alpha=0.05$  (p value=0.005). There is no evidence that Qigong is effective in the reduction of muscular tension of trapezius and in the reduction of time reaction.

**Conclusion:** Although data is from a small sample size, the results obtained are consistent with the hypothesis that Qigong may positively influence anxiety levels of transverse flute schoolchildren, aged 10 to 12, before the auditions. The study confirms that Qigong related effects may be conventionally objectified by psychological scores and physiological parameters including cortisol measurements, however further studies with a proper control, randomization and blinding procedures should be carried out.



Oral Sessions ▶ A4

# **History & Cultural Studies**



# **The Relations Between Municipalities and Military Orders in Portugal on Middle Ages (XII-XIV centuries)**

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This presentation aims to exhibit the first results of the dissertation in medieval history under the guidance of Professor Paula Pinto Costa. This work consists on the intersection of municipal and military orders historiography in the twelfth to fourteenth centuries, and revisits the municipal charters (called "cartas de foral") to understand the relations between the municipalities and the military orders, focusing on the relationships of dependence and confrontation. This approach allows us to determine the strategies of settlement and defence by the military orders and understand the importance of municipalities in these same strategies on the period of Christian reconquest. Understanding how the municipalities of military orders were structured based on municipal charters at economic and social level and such as the point of view of justice and administration, are central items on our dissertation. The charters are fundamental to understand the role of the military orders in Portuguese reconquest. These lines are the main goal of this work.

The military orders granted the lands of their landlords, at least, 24 charters. Knights Templar, since 1156, granted 11 charters, especially in the central region of Portugal, which played a leading role in defending the line of the Tagus river against the moors, building castles and establishing commanderies to administer the territory. Since 1194 the Order of the Hospital began to have a greater role in the Portuguese reconquest, collaborating in the defence line of the Tagus river, granted four charters after this time and managing castles and commanderies to. This fact has been caused by the incursions of the Almohads in the late twelfth century. In addition to the Order of the Hospital, the order of Santiago and the Order of Avis were in aid of the defence of the frontier line but settling in areas further south of the Tagus river. The Order of Santiago was occupying the Alentejo coast to the Algarve granting 5 charters, managing castles and establishing many commanderies. Finally, the Order of Avis occupied the lands south of the Tagus river, but especially for the innermost zone settling in high and low Alentejo, where granted four charters, managed and created castles and commanderies. After the reconquest period, begin to emerge a record of various disputes between the military orders and municipalities, which shows a difficult coexistence between these institutions of power.

Through this small contextualization and of the presented methodology, we intend to show, that the military orders were key institutions in the consolidation of the kingdom of Portugal, having an important role in the process of Portuguese civilizing space, using the municipalities as key tools in this process.

## Notaries public in Porto from the XIII-XIV centuries

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<sup>2</sup> Investigator at CITCEM – Centro Interdisciplinar Cultura Espaço e Memória.

This abstract presents a very short version of a two year investigation in which the author studied the notaries public of Porto between 1242 and 1383. The analysis of this particular group of men, in a town of high political and economic importance in the Portuguese kingdom in Middle Ages, aims to identify notaries public and establish a relation between their activity and the history of the city, establishing a chronology of their notarial career, and analyse the development of organization, articulation, hierarchy and succession of the notaries public of Porto, as well as to uncover their socio-economic position. Although very briefly, we also payed attention to the existence and the work of episcopal notaries and scribes. Furthermore, the exercise of the notarial activity in monasteries is also studied and might indicate a wider client network.

Listing these people was very difficult due to homonym, similarity of periods during which they worked and unclear references between those who write, appear as witnesses or are only mentioned in the acts. The information extracted from the type of documents and commercial references, accompanied by their mobility, helped us to understand a little more about this particular group of individuals.

We compared the frequency of these notaries' working timelines as well as the number of officials per year with the history of the city. The result was a shifting number which can be related with the city's political and economic life: in fact, the king tried, by all means possible, to gradually enlarge his influence in the city, process in which the notaries public played an important role. We believe that the importance of these references is shown through a superior validation of the written act, which bestows a stronger safety, authority and solemnity to the document. The vast majority of these documents were drawn up in the city of Porto. Nevertheless, there is an undoubtful mobility of the notaries public: until the first half of the XIV<sup>th</sup> century the documents are written mainly in side or near the See. Thereafter, the notarial acts are drawn up primarily in the city council.

The organization, hierarchy and succession of these notaries is observed in the "oficinas" to which they seemed to belong. There are several examples that testify to a relationship of generational succession ("master / disciple") embodied in the use of the same *signum tabellioni*. The bonds that we observed between these individuals, mainly through the study of references and testimony subscription, allowed us to demonstrate possible notarial networks.

We took note on the participation of notaries in the commercial and economic life of Porto, as we were able to pick some examples that illustrate their social position: they were involved with chartering of ships from Flanders and Normandy, trading (wine, olive oil, bread) as well as being involved in building construction and debt collection. All this allowed us to verify the privileged relations with political and economic key people of the city, and meddling in the affairs of the "concelho".

# **The excavations in Muge shellmiddens during the 1930's by Mendes Corrêa: new contribution to the study of archaeological investigation**

**Ana Abrunhosa**<sup>1</sup>

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The archaeological excavations in Muge shellmiddens (Tagus Valey – Portugal) during the 1930s by University of Porto Professor Mendes Corrêa (1888-1959) and his team were the subject of research leading to a Master Dissertation in Archaeology at the Faculty of Arts of the University of Porto.

This year is celebrated the 150<sup>th</sup> anniversary of its discovery by geologist Carlos Ribeiro (1813-1882). Since then, research on these sites and its diverse materials has been carried out by various teams. The Mesolithic shellmiddens of Muge turned out to be one of the major Portuguese and European Prehistoric archaeological sites.

A new and abundant collection of documents concerning the most diverse subjects underlying the archaeological excavations and investigation carried out in Muge shellmiddens by Mendes Corrêa was found in the 1980s and saved by A. Huet B. Gonçalves, then curator of the university museum founded by Professor Mendes Corrêa in the early XX century. This collection contains notes, correspondence, reports, photographs, drawings and sketches authored by Mendes Corrêa and his team, namely Rui de Serpa Pinto, Santos Júnior, Alfredo Athayde from the Anthropology Institute of the same University, also founded by Mendes Corrêa. It fills an important gap in the already known significant documentation about the development of archaeological investigation on this site especially because the beginnings of Mendes Corrêa intervention, aimed at (but not limited to) obtaining new anthropologic specimens for the study of a human population he called *Homo afer taganus*, are now put in evidence.

This presentation shows the results obtained from the study of the new documentation regarding the history of archaeological research there and its integration in the development of ideas and archaeological practice. It revealed the motivations, strategies, programming, development work and field work techniques of the time. This area is usually poorly described, even though considered important for the knowledge of the development of archaeological practice in Portugal and Europe.

## Some considerations about the architect João Antunes

Rúben Ribeiro

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The text presented here is intended to summarize the journey of the greatest portuguese architect between the second half of the XVII century and the first decade of the XVIII century. It is during this time that in Portugal prevails a economic crisis, as well as peace with Spain in 1668. This will imply a financial effort in the development of military architecture, aiming to ensure the Portuguese restoration. Furthermore, there is still the issue of governmental succession, since D. Pedro II will replace D. Afonso VI in 1667.

Entering in the life of architect João Antunes, he was born in 1643 and there are records of his baptism on 30 September in Lisbon. During his lifetime, he will assume various positions, such as the *Apprentice of Civil Architecture*, *Architect of King D. Pedro II* and *Architect of the Military Orders*.

Enumerating some of the projects in order to understand the magnificence of his *métier*, his first drawing has been to Saint Engracia's Church (1681), through the «*Irmandade dos Cem Escravos*». Referred to as his greatest work, the architecture has a distinct game of light and dark, integrating concave and convex plans, having only been completed in the 60s of the XX Century. His impressive performance will be recognized in the early 80s, when he will fill one of three vacancies for *Apprentice of Civil Architecture* at the «*Aula do Paço da Ribeira*» [2].

Among projects of his own, as well as works that have been attributed to João Antunes, I would highlight the project for the Louriçal Convent, in Vila do Louriçal (Pombal), and the College of Saint Antão-o-Novo, Lisbon, in particular the sketch and execution of the sacristy and the marbled portal that gives access to the same vestry, and a room lined with marble. Note also that this architect participated in the drawing of the Tomb for Princess Saint Joana (Convent of Jesus, Aveiro). I conclude this brief presentation with the work made in the sacristy of the Cathedral of Braga, as well as the sketch of the Senhor Bom Jesus da Cruz Church, Barcelos, order issued by D. João de Sousa (who has been Bishop of Porto, Archbishop of Braga and Lisbon). A year before his death, he has yet outlined the Menino Deus Church, Lisbon (1711).

Finally, it is important to mention the knowledge João Antunes had of the roman baroque, the manuals architectural (Serlio and Sagredo), as well as the circulation of prints and engravings. He seems to have had contact with Spanish architecture that was produced on the order side of the border. In conclusion, we can call the architect João Antunes global, since produced religious architecture but also noble houses, yet having sketched marble altars and carving baptismal fonts and other works.

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[1] The dissertation in progress and that will be defended during this academic year corresponds to the life and work of architect João Antunes (1643-1712).

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# THETHYS WOOS: GEOPOLITICAL CONSIDERATIONS ON PORTUGAL IN THE EARLY XXI CENTURY

H. Pinto Abreu<sup>1</sup>

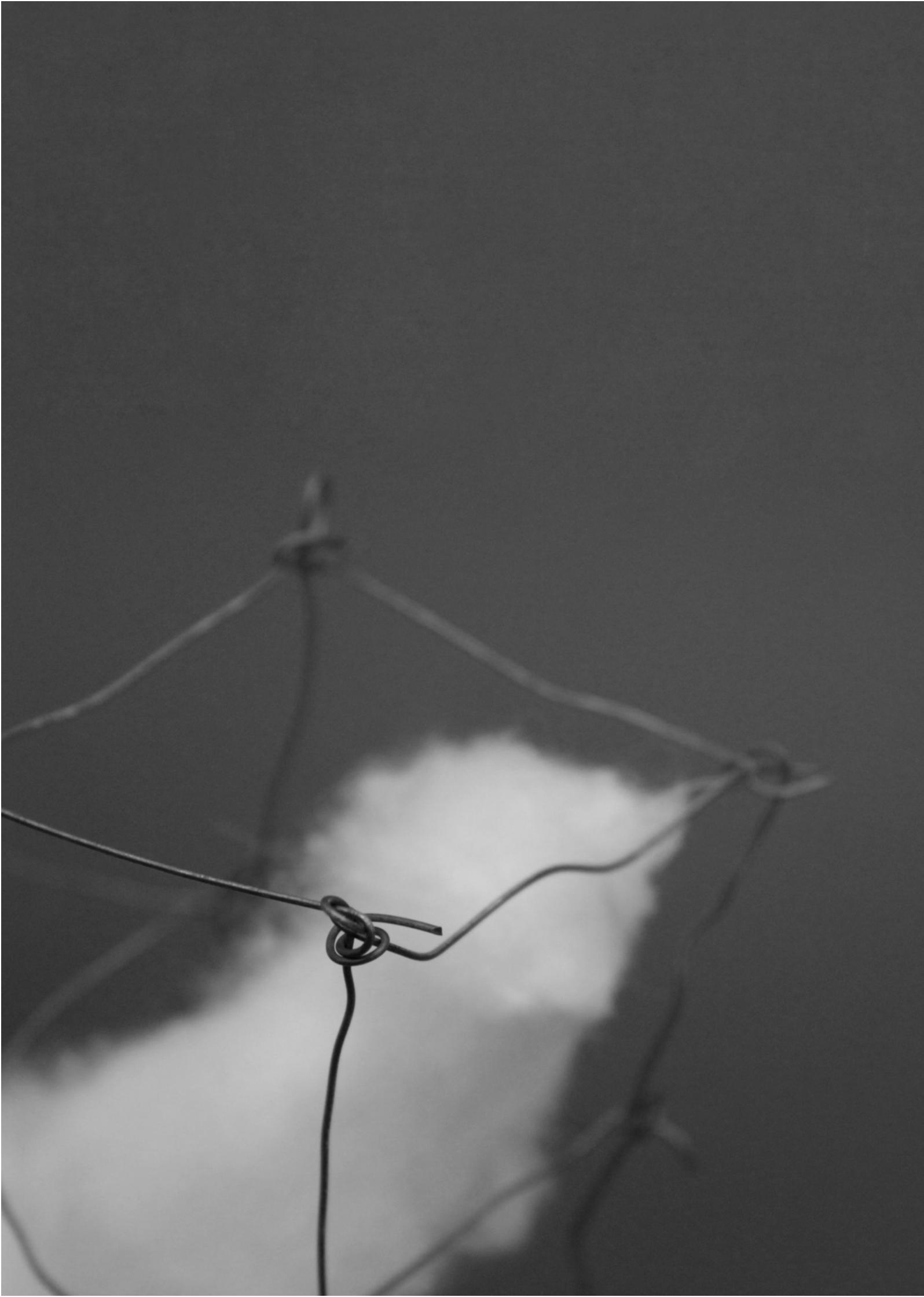
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Starting from an overview of the current political situation in Portugal and in Europe, with a special emphasis on the impacts of the 2007-2008 financial crisis and the ensuing global recession and sovereign-debt crisis, focusing not only on the economic and financial spheres, but also on political, social and demographic issues, amongst others, this essay closely examines the unfolding of recent events in Iceland and Greece, looking for geopolitical insights that may provide the key to a better understanding of the future paths and potentialities for the Portuguese nation, also analyzing the influence and the ramifications of the rise of a multipolar world, with the loss of influence of the United States of America and of the North Atlantic Treaty Organization (NATO), and the growing relevance of Russia and the Popular Republic of China, amongst others.

In order to apply and to give form these geopolitical insights, a brief historical review is conducted, with two goals in mind, namely: to gain a better understanding, through historical events, of how the current Portuguese situation has come into being; and to discern the specificities of the Portuguese nation, which must be taken into account when applying the geopolitical insights we gained.

By processing and analyzing all these inputs, we deduct that there are basically two paths – which need not be, but will probably be mutually exclusive - for Portugal: the first, which is being followed at least since the 1970s, is European integration (or, alternatively, Iberian integration), a path that is threatened by the current crisis, which is of a civilizational nature; the second is a much closer cooperation with Portuguese-speaking countries, especially with the Republic of Angola, a country with a fast-growing economy and vast natural resources. We emphasize the importance of this second path, namely of establishing a close political and strategic cooperation – a cooperation of equals, and not one of a neocolonial type - with Angola and other Portuguese-speaking countries, with a special emphasis on the economic and educational fields.

We emphasize the need to destigmatize Angolan investment activities in Portugal, also demonstrating they pose no threat whatsoever to the democratic system or to the freedom of speech in Portugal, and instead are highly beneficial for the reinforcement of Portuguese autonomy, and indeed to the accomplishment of the deepest traits of the Portuguese nationality, that is, the promotion of cooperation, tolerance and friendship among peoples.



**ORAL SESSIONS**

**VIII**



Oral Sessions ▶ A1

# Biomedicine

## VI



## **Bridging the gap between nature and antioxidant therapy: development of mitochondria-targeted antioxidants based on benzoic scaffold**

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Mitochondria are cellular organelles fundamental for the survival of the cells, being their dysfunction associated with some diseases, named mitochondrial diseases that encloses, for example, mitochondrial myopathies, diabetes, cancer, neurodegenerative diseases, such as Parkinson and Alzheimer diseases, and amyotrophic lateral sclerosis.

Besides providing cellular energy (ATP), mitochondria are involved in other biological processes, such as signaling, differentiation, cell growth and death, also in the production of reactive oxygen species (ROS). In fact, it was estimated a daily production of  $10^{11}$  ROS in a typical aerobic cell.

The neurodegenerative diseases constitute a public health problem affecting millions of people worldwide. The overall process in the genesis of neurodegenerative diseases may be considered antagonistic to the one that mediates cancer since in cancer occurs uncontrolled proliferation of the cells and in neurodegeneration the final result is cell death. In both cases, mechanisms of necrosis and/or apoptosis as well as mitochondrial dysfunction are intrinsically involved.

Therefore, it is urgent to find an approach to reduce or delay the progression of neurodegenerative processes and, consequently, the associated ageing processes. In this context, mitochondria are currently considered an important target and the selective inhibition/mimimization of the mitochondria oxidative damage is considered a promising therapeutic solution for this type of disorders.

Accordingly, the present project encompasses the rational design and synthesis of new hydroxybenzoic acid derivatives, presenting antioxidant activity, with positive charges at physiological pH, thus displaying the ability of accumulation inside mitochondria.

In order to achieve this goal, structural changes were performed in natural phenolic antioxidants present in human diet (protocatechuic and gallic acids) by inserting an aliphatic carbon chain spacer linked to a triphenylphosphonium cation (TPP<sup>+</sup>). The synthesized compounds (6-(3,4-dihydroxybenzamido)hexyltriphenylphosphonium bromide and 6-(3,4,5-trihydroxybenzamido)hexyltriphenylphosphonium bromide) present antioxidant activity, measured by using ABTS, DPPH, and GO methods and also redox potential determinations, and their performance in mitochondrial and neuronal systems is being attained. There is the hope that, in a near future, this new therapeutic approach can improve the lifestyle of people who suffer from diseases related to oxidative *stress*, namely of neurodegenerative nature.

The synthesized compounds, methods and applications are in patenting process.

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# Effects of superparamagnetic iron oxide nanoparticles on differentiated human SH-SY5Y neuroblastoma cells under hyperthermia

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Superparamagnetic iron oxide nanoparticles (SPIONPs) have earned the interest of the biomedical community and are promising in a variety of applications such as magnetic resonance imaging, drug and gene delivery, and destruction of tumor tissues through hyperthermia [1,2]. In the event of their biomedical applications, SPIONPs can reach CNS [3], but knowledge about their putative neurological effects is scarce.

The present study aimed at investigating whether elevation of temperature could dictate a different susceptibility of the neuronal cells to SPIONPs. Therefore, we have investigated SPIONPs-induced effects under normothermic (37°C) and hyperthermic (40°C) conditions, on differentiated human neuronal SH-SY5Y cells.

Two types of ~10.5 nm magnetite (Fe<sub>3</sub>O<sub>4</sub>) SPIONPs were tested: uncoated vs polyacrylic acid (PAA)-capped. SH-SY5Y cells were cultured in DMEM with 10% FBS, 100 U/mL penicillin, 100 µg/mL streptomycin, 0.25 µg/mL amphotericin B and 0.1 mM nonessential amino acids. To induce differentiation, cells were grown for 7 days in DMEM with 3% FBS and 10 µM retinoic acid. Cells were exposed for 4 or 24h to increasing concentrations of the SPIONPs (6.25 to 100 µg/mL) and thirty minutes before completing the incubation period exposed to 40°C for 30 min. Cytotoxicity was evaluated by assessing esterase activity and membrane integrity using the calcein-AM and propidium iodide (PI) assays, respectively. Production of reactive oxygen species (ROS) was determined by the DCFH-DA assay.

After 4h of exposure to uncoated SPIONPs, a decrease of the esterase activity was observed at 37°C and 40°C. However, for the lowest (6.25 µg/mL) and highest (100 µg/mL) tested concentrations a more accentuated decrease in esterase activity was detected at 40°C comparing with 37°C ( $p < 0.01$  and  $p < 0.0001$ , respectively). After 24h of incubation, a clear concentration-dependent decrease of esterase activity induced by the uncoated SPIONPs was visible at both temperatures. A significantly marked decrease of esterase activity was detected in cells incubated with 12.5 µg/mL at 40°C comparing to 37°C ( $p < 0.05$ ). For PAA-SPIONPs exposed cells, a concentration-dependent decrease of esterase activity was observed at 4h and 24h. No differences were observed between the magnitude of the PAA-SPIONPs effects between 37°C and 40°C. A decrease in PI fluorescence levels was detected in cells exposed either to uncoated or PAA-SPIONPs, at both temperatures, indicating an interference of the NPs in the assay. In addition, at both time-points, the ROS levels were not elevated in response to uncoated or PAA-SPIONPs comparing with untreated SH-SY5Y cells.

In conclusion, hyperthermia slightly exacerbated the effects on esterase activity induced by uncoated but not by PAA-SPIONPs, under our experimental conditions. Cytotoxicity induced by both SPIONPs is not mediated by ROS. Furthermore, it was shown that SPIONPs interfered with the PI assay, drawing attention for the importance to assess multiple toxicity endpoints for an accurate toxicological assessment of the SPIONPs.

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# **Lipid nanoparticles for topical and transdermal application for alopecia treatment: development, physicochemical characterization and *in vitro* release and penetration studies**

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Alopecia is a dermatological disorder, commonly known as hair loss, which affects up to half of the Caucasian male population by middle age, and almost all Caucasian men by old age (95%). Considering that alopecia affects many people and that no scientifically proven treatment for alopecia, with few side effects, is already available, is urgent to develop new drug delivery systems able to improve alopecia therapy. With this purpose, the present study aimed to develop lipid nanoparticles (nanostructured lipid carriers) with the ability to incorporate and deliver anti-alopecia active compounds (minoxidil and finasteride), into the dermis and hair follicles. Lipid nanoparticles, prepared by ultrasonication method, showed mean particle sizes around 200 nm, as desired to achieve the dermis and the hair follicles, and zeta potential values around -30 mV, which indicates a good physical stability. Over 28 days of storage little variations in these parameters were observed, which indicates that all nanoformulations are stable in storage over that period. Cryo-SEM measurements showed that all the lipid nanoparticles exhibit a spherical shape and a smooth surface independently of their composition. Differential scanning calorimetry studies allowed the determination of phase transition temperatures. A high loading efficiency was achieved for finasteride (between 70 and 90%), while only nearly 30% was achieved for minoxidil nanoparticles, over 28 days. Controlled release assays in physiological conditions demonstrated that nanoparticles loaded with minoxidil yield a prolonged release, as desired. Penetration assays through pig ear skin demonstrated that nanoparticles loaded with minoxidil and finasteride have low levels of penetration. These results suggest that the proposed novel formulation presents several good characteristics which indicate that can be suitable for dermal anti-alopecia active compounds' delivery.

# Mitochondriotropics derivatives based on the natural polyphenol caffeic acid as an way out for oxidative stress related diseases

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The mitochondrion is an important organelle for the genesis of ATP in which occurs the intracellular formation of the reactive oxygen species (ROS) and for that reason is particularly vulnerable to oxidative damage. Thus, one way to prevent or retard the progression of oxidative damage is the regulation of ROS production in mitochondria. In this context, it is believed that the modulation of mitochondrial dysfunctions throughout the antioxidant therapy can correspond to an appropriate strategy to prevent or retard the deleterious oxidative effects present in aging and degenerative diseases, such as Alzheimer's disease. Mitochondriotropic antioxidants can be a successful approach as they are able to penetrate in the phospholipid bilayer of mitochondria's membrane and have the capacity of accumulation in the negatively charged compartments of the mitochondrial matrix.

Hydroxycinnamic acids, and in particular caffeic acid, are phenolic compounds present in the diet that have been used as a model for the design and development of new antioxidants. However, despite exhibiting an interesting *in vitro* antioxidant activity their application in therapy was not successful. Failure in the antioxidant therapy of compounds of natural origin is often associated with setbacks related with their physicochemical characteristics, particularly its low lipophilicity, which are not suitable for their biodistribution and penetration into the target site.

In this context the aim of the present project is the rational design of new mitochondriotropic antioxidants based on caffeic acid, namely ((*E*)-2-(3-(3,4-dihydroxyphenyl)prop-2-enamido)ethyltriphenylphosphonium methanesulfonate (*D<sub>i</sub>D<sub>1</sub>*), (*E*)-6-(3-(3,4-dihydroxyphenyl)prop-2-enamido)hexyltriphenylphosphonium methanesulfonate (*D<sub>i</sub>D<sub>2</sub>*) and (*E*)-6-(3-(3,4,5-trihydroxyphenyl)prop-2-enamido)hexyltriphenylphosphonium methanesulfonate (*Tr<sub>i</sub>D<sub>2</sub>*). The structural modifications performed, such as modification of the length of the aliphatic spacer and the number of hydroxyl groups will allow establishing the structure-activity relationship and the optimization of the lead compound. The compounds were characterized by nuclear magnetic resonance spectroscopy (<sup>1</sup>H, <sup>13</sup>C and DEPT) and electronic impact mass spectroscopy (MS/IE) and the antioxidant activity was evaluated by DPPH<sup>•</sup> and ABTS<sup>•+</sup> methods. In addition, the redox potentials of the synthesized compounds were evaluated by the techniques of differential pulse and cyclic voltammetry. According to the results it was possible to assign a hierarchy on the antioxidant efficacy and redox data of the lipophilic antioxidants according to the order: *Tr<sub>i</sub>D<sub>2</sub>* > *D<sub>i</sub>D<sub>2</sub>* > *D<sub>i</sub>D<sub>1</sub>*.

The compounds, processes and application have been patented- patent 20121000003813.

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## Survivin Role in Pulmonary Arterial Hypertension

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Survivin exhibits pro-proliferative and anti-apoptotic properties being of a pivotal role in the vascular of pulmonary arterial hypertension (PAH) and the left ventricular remodeling of heart failure [1,2,3]. Smac/DIABLO is an inhibitor of survivin, thus promoting apoptosis. This study aims to investigate right ventricular (RV) expression of survivin and smac/DIABLO in monocrotaline (MCT)-induced PAH and to characterize the effects of terameprocol, a pharmacological suppressor of survivin gene expression, in pulmonary arterial smooth muscle cells (PASMC) of adult male Wistar rats [4].

Hemodynamic and morphometric data were collected at different time points (1-21 days after injection). Survivin and smac/DIABLO expression in the RV was determined by immunohistochemistry and western blotting. A primary culture of PASMC isolated from sham and MCT-treated rats (day 21) was established and the effects of terameprocol in cell proliferation and apoptosis were evaluated by BrdU and TUNEL assays, respectively.

Immunohistochemistry and western blotting demonstrated a significant increase in RV survivin expression in the MCT groups since day 7, when compared with the SHAM groups. Smac/DIABLO followed an inverse expression pattern. This time-point also corresponded to the first histological evidence of RV hypertrophy in MCT-treated rats. Survivin overexpression preceded hemodynamic manifestations of the disease, which only started at D14. Terameprocol significantly inhibited proliferation and induced apoptosis of PASMC from sham and pulmonary hypertensive rats in a dose-dependent manner. The pattern of proliferation and apoptosis did not differ significantly between SHAM and MCT groups.

These findings suggest that targeting survivin in PAH could have dual beneficial effects by reversing pulmonary vascular remodeling and myocardial hypertrophy.

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# The use of lubricants in the preparation of solid dosage forms

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Lubricants have a vital importance in the production of solid dosage forms, namely tablets and capsules. To ensure the consistency of dosage units it is necessary to obtain a uniform flow of the powder mixtures before tableting or filling the hard capsules. Several studies have stated weight, content uniformity, hardness, disintegration/dissolution as being affected by the formulation flow. [1]

There is a wide range of lubricants available for the preparation of solid dosage forms namely anti-adherents like magnesium stearate or glidants like talc or silicon dioxide (aerosil). These excipients and their use have been described for many years; hence the purpose of this work is to understand how different parameters may modify the flow. The parameters studied were concentration of the lubricants, particle size of talc[2], moisture and particle size of granules.

To perform this study lactose (95 %) and povidone (5%) were wet granulated. The granules obtained were separated by a nest of sieves. Angle of repose and flow time (using Erweka GT apparatus) were studied according to Portuguese Pharmacopoeia 9. The moisture of the granules was also estimated by an infrared moisture balance (AD- 4713).

The results show that fractions containing granules between 180  $\mu\text{m}$  and 355  $\mu\text{m}$  have better flow than the fractions with particles of higher size. It is also shown that fractions containing 2 to 3% of moisture have optimal results when blended with 1% magnesium stearate. Different particle sizes of talc (2.2, 8 and 12  $\mu\text{m}$ ) do not produce relevant changes in the measured flow properties. Magnesium stearate, a lubricant with anti-adherent properties, has shown the best flowing results.

Still, other tests should be carried out, to assess the compressibility index or the ejection force. To understand flowability it would also be relevant to study different composition of granules like microcrystalline cellulose and methylcellulose or calcium phosphate and sodium alginate.

A special thanks to the Mondo Minerals, for kindly supplying samples of talc.

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Oral Sessions ▶ A2

# Chemistry



## Virtual screening of a focused library of cell growth inhibitors to identify potential inhibitors of p53:MDM2 interaction

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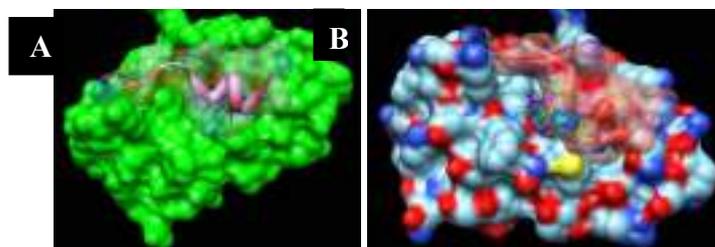
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The p53 tumour suppressor protein is a major regulator of cell proliferation and death. In tumours that retain a wild-type (wt) p53, the activity of this protein can be inhibited by the endogenous negative regulator MDM2. Inhibitors of p53:MDM2 interaction have been considered promising drugs for cancer therapy [1].

The virtual screening of a library of xanthone derivatives inhibitors of tumor cell growth led us to the identification of potential novel MDM2 ligands. Using this approach, the pyranoxanthone **LEM1** was identified as a putative small-molecule inhibitor of p53:MDM2 interaction. By computational docking studies, it was predicted that, like nutlin-3a, a known small-molecule inhibitor of p53:MDM2 interaction, **LEM1** binds to the p53-binding site of MDM2 (**Fig. 1A**).

$\alpha$ -Mangostin and gambogic acid are natural products with potent antitumor activity against several human tumor cell lines. However, the molecular mechanism of action of these xanthenes remains largely controversial. In this work, the computational docking studies supported that these xanthenes have the potential of acting as putative inhibitors of MDM2 (**Fig. 1B**).



**Fig. 1.** **A.** LEM1 in the binding site of MDM2 (1YCR); **B.**  $\alpha$ -Mangostin (yellow), gambogic acid (purple) and nutlin-3a (green) in the binding site of MDM2.

Docking studies with MDM2 protein (1YCR) confirmed a high binding affinity for **LEM1** and showed that this inhibitor may block the interaction between MDM2 and p53 protein. Biological assays, in order to evaluate their efficiency as inhibitors of p53:MDM2 interaction, confirmed the computational results.

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# The catalytic mechanism of human renin protease – a quantum mechanics/molecular mechanics study

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The aim of the present work is to describe the catalytic mechanism of human renin protease with atomistic detail. This enzyme belongs to Renin-Angiotensin-Aldosterone System (RAAS) which represents the most important regulating system of the arterial blood pressure. Hypertension promotes the occurrence of cardiovascular diseases and this is the reason why these studies are so important.

Renin is an aspartic protease and its exclusive activity and determinant velocity on the RAAS cascade makes this enzyme an ideal target for new antihypertensive drugs [1,2].

In this work we started with a crystallographic model of human renin complexed with its substrate, the angiotensinogen. This model was divided in two layers that were studied at different theoretical levels (Density functional theory - DFT - and Molecular Mechanics). The geometries were optimized at the ONIOM (B3LYP/6-31G(d):Amber) level, with which we have obtained the structures of the reactants, transition states, reaction intermediates and products. The energies were recalculated with single-point calculations using different density functionals (B1B95 and MPWB1K) and a progressive increase in the number of atoms in the DFT layer.

Our results suggest that the renin reaction (hydrolysis of the angiotensinogen) occurs through three elementary steps. It begins with the formation of a stable gem-diol intermediate, followed by the protonation of peptidic bond nitrogen between Leu10 and Val11 and it ends with the completely cleavage of this peptidic bond. The first step is rate limiting, with a barrier of 23.5 kcal.mol<sup>-1</sup>, while the others have activation energies of 16.3 kcal.mol<sup>-1</sup> and 14.7 kcal.mol<sup>-1</sup>, respectively. We also conclude that the residues around the active center greatly influence the catalytic mechanism of this enzyme.

The present data were compared with the results obtained recently by our group to mouse submandibular renin [3]. In the future this study will improve the rational design of antihypertensive drugs.

## Acknowledgements

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## Aminoacid-based surfactants as potencial drug or DNA carriers

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Despite the recognized advantages of delivering drugs or DNA into cells using liposomes or viruses, these carriers lack on long-term stability and efficiency, and are usually highly toxic to cells. Therefore, it is essential to find biocompatible, less toxic new molecules able to survive cell metabolism and to deliver their content successfully.

Ionic amino acid derived surfactants seem to be a good alternative to the existing drug delivery and transfecting agents since, in addition to the desired performance, they often present low toxicity levels and enhanced biocompatibility and biodegradability.

This presentation is an overview of a multidisciplinary project concerning the synthesis and the assessment of the physicochemical and toxicological properties of different serine derived monomeric and gemini surfactants (Fig.1) [1-3]. The main goal has been to synthesize various series of different families of compounds in order to perform a systematic study on the influence of chain length and nature of spacer linkage on aggregation properties, toxicological profile and potential for drug encapsulation of these surfactants.

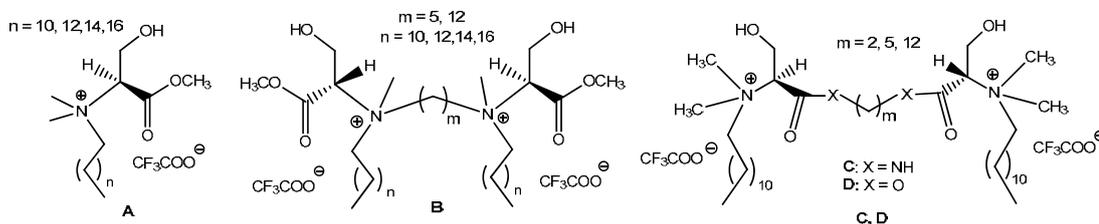


Figure 1: General structure of some of the surfactants studied. **A:** Monomeric Series; **B:** Amine Series; **C:** Amide Series; **D:** Ester Series

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# Bisnaphthalimidopropyl polyamine as promising electroactive specie for perchlorate-selective electrodes

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Potentiometry with ion selective electrodes is a well-established technique with intensive uses in many laboratories either carrying out routine analyses or in research. Its attractive features include the simplicity of use, enabling of fast and accurate results, economy of implementation, non-destructive and environmentally competitive with other traditional methods [1]. Useful electrodes arise from a careful combination of a hydrophobic charged ion exchanger that selectively pulls counter signal ions and excludes similar charged ions through the membrane/sample interface barrier (permselective properties of the membrane) and from a selective hydrophobic complexing agent (called an ionophore) which selectively buffers the activity of target ion in bulk membrane [2]. In this context, a new compound of the family of bisnaphthalimidopropyl polyamine (BNIP) [3], called bisnaphthalimidopropyl-4,4'-diaminodiphenylmethane (BNIP Dapm), has been exploited as ionophore candidate for potentiometric membranes preparation. Several solid contact electrodes, incorporating a plasticized PVC membrane with BNIP Dapm as ionophore and different mediator solvents in the presence or absence of additives (cationic or anionic) were prepared and evaluated against some common inorganic and organic ions. The membrane showing better characteristics consisted of 68% (w/w) of 2-nitrodiphenyl ether, 31% (w/w) PVC and 1% (w/w) BNIPDapm which responds to salicylate, thiocyanate, perchlorate, citrate, periodate, iodide and chlorate anions. However, the anion with the better response was perchlorate as shown by the practical limit of detection of  $1 \times 10^{-7} \text{ molL}^{-1}$ , the slope of  $-52.8 \text{ mVdec}^{-1}$  and the lower analysis time (less than 15s), when tests were performed in solutions of ionic strength adjusted to  $0.01 \text{ molL}^{-1}$  with  $\text{Na}_2\text{SO}_4$ . The response of the potentiometric sensor was not influenced by pH variation in the range of 3.5 to 11.0. The selectivity of the membrane was assessed against fluoride, sulfate, hydrogenphosphate, acetate, chloride, nitrate, bromide, chlorate, iodide, thiocyanate, periodate and carbonate, for which reduced values of potentiometric selectivity coefficients were attained. Due to the good characteristics of the electrode an analytical method for the determination of perchlorate in soils and waters, a priority emergent contaminant according to the Environmental Protection Agency's regulation of United States (EPA 505-F-10-002), is now under development.

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# Development of ibuprofen selective electrode based on molecularly imprinted polymer

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Ibuprofen (IBU) is a well-known non-steroidal anti-inflammatory drug with analgesic and antipyretic properties that is mainly used in the treatment of mild to moderate pain and in the management of spondylitis, osteo-arthritis, rheumatoid arthritis, and soft tissue disorders [1]. In urine, the percentages of free and conjugated IBU are approximately 1% and 14% respectively, the remaining is eliminated in faeces [2]. The availability and widespread use of over-the-counter IBU has raised concerns about environmental contamination. Nowadays, it can be determined by optical and electroanalytical methods or resorting to separation techniques. A simple, sensitive, low cost and potentially highly selective analytical method based on potentiometric detection is being developed which can be further applied to the analysis of river waters.

Recently organic compounds known as plastic antibodies have been used as ionophores due to the high selectivity to bind to the analyte. This is possible thanks to a specifically design that allow stereochemical interactions [3-5]. In this work a molecularly imprinted polymer (MIP) specifically designed for ibuprofen was successful incorporated in a polymeric membrane as a recognizing molecule for development of a analytical detector. Several potentiometric PVC membranes incorporating 1% (w/w) of MIP as ionophore and different mediator solvents with cationic lipophylic additive were prepared and evaluated. A blank membrane without additive was also prepared in order to check the potentiometric properties of the ionophore.

In order to optimize the membrane characteristics three different mediator solvents were tested at three different pH values ( 5.5, 7.4 and 10 ). A greater sensitivity was obtained at pH 5.5. When 2-fluorophenyl 2-nitrophenyl ether is used an analytical linear range of  $1.06 \times 10^{-5} - 1.00 \times 10^{-2}$  mol L<sup>-1</sup> and a slope of -92.8mV/dec was obtained whereas for 2-nitrophenyl octyl ether values of  $1.07 \times 10^{-5} - 1.00 \times 10^{-2}$  mol L<sup>-1</sup> and -103.22mV/dec were obtained. Results of  $7.93 \times 10^{-6} - 1.00 \times 10^{-2}$  mol L<sup>-1</sup>, and -87.98mV/dec for membranes incorporating dibutylphtalate as solvent were obtained.

Since a greater selectivity is expected regarding the ionophore characteristics, once it is a molecule with a specific chemical recognition to ibuprofen, studies of interferences are being carried out for a number of inorganic and organic anions.

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# Magnetic hyperthermia response of MnFe<sub>2</sub>O<sub>4</sub> nanoparticles synthesized at different reaction temperatures

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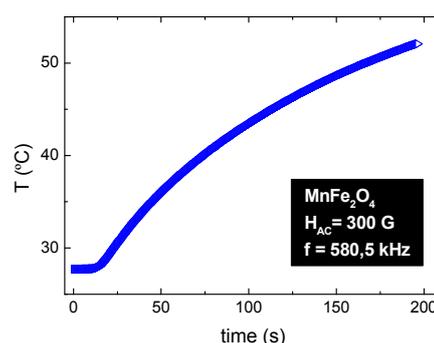
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Tumor-targeted magnetic hyperthermia with magnetic nanoparticles (MNPs) has recently attracted much attention in the context of clinical multimodal therapies for cancer treatment [1]. From the large spectrum of MNPs for biomedical purposes, those based on 3d transition metals are widely employed due to their low toxicity and biocompatibility. The insights on the power released by these MNPs depend strongly on their physicochemical properties: shape, size, colloidal stability and saturation magnetization [1]. Therefore, the fine control and development of new strategies that allow engineering effective “nanoheaters” is continuously being reviewed.

In this work, MnFe<sub>2</sub>O<sub>4</sub> MNPs were synthesized by a new aqueous coprecipitation method using an alkanolamine as precipitating agent [2]. The reaction temperature was modified (between 80 and 100 °C) in order to study its influence on the MNPs size, size distribution and aggregation. After centrifugation and/or magnetic separation processes, the colloidal stability, particle size, crystal structure and magnetic properties of the NPs were studied by dynamic light scattering, powder X-ray diffraction and, SQUID, respectively. Finally, the heating capabilities of the synthesized MnFe<sub>2</sub>O<sub>4</sub> MNPs were studied under  $H_{AC} = 300$  G and  $f = 580$  kHz (see Fig. 1).



**Fig. 1** Temperature versus time under an ac magnetic field.

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# **Degradation of UV filters 4-tert-Butyl-4'-methoxydibenzoylmethane (BMDM) and 2-Ethylhexyl-4- methoxycinnamate (EHMC) in chlorinated water**

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Ultraviolet filters (UV) are one of the key components of the cosmetics industry for sun protection. These compounds have the ability to absorb and/or reflect UV radiation, thus protecting us from its harmful effects. However, it is known that these compounds undergo degradation under light (photo-degradation) and reaction with water disinfectants such as chlorine [1]. From these degradation processes other compounds are formed which exhibit different properties of the parent compounds and which may be more toxic, constituting a risk factor for human health. Thus, there has been growing realization of research in order to identify and quantify the presence of UV filters and their degradation products in the environment, as well as understand what factors may influence this same degradation.

This work is focused on the study of photo-degradation and reaction with free chlorine of two UV filters: 4-tert-Butyl-4'-methoxydibenzoylmethane (BMDM) and 2-Ethylhexyl-4-methoxycinnamate (EHMC). Thus, it was studied the effect of five factors in the degradation of the two UV filters: pH, temperature, concentration of chlorine and dissolved organic matter (DOM) and the time of exposure to artificial solar radiation. It was also studied the effect of chlorine concentration and DOM concentration on degradation of the BDM in two sunscreens commercial brands.

Of the five factors tested in this work the ones that have shown a more significant effect in the BMDM degradation were the chlorine concentration, DOM and pH, and in the case of EHMC it was the temperature and the time of exposure to artificial solar radiation.

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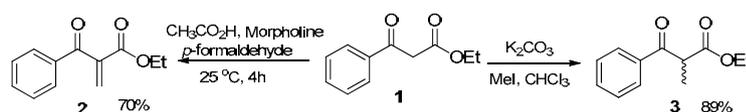
# Biocatalysis approach to the enantioselective synthesis of $\alpha$ -methyl- $\beta$ -hydroxyesters

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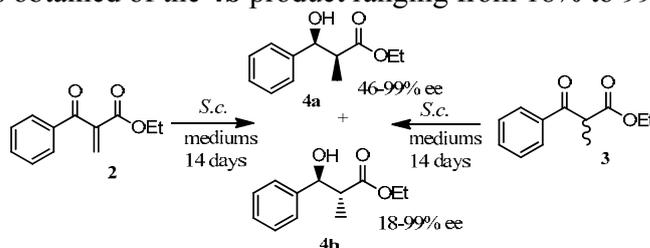
In recent years, the biological activity of substances has been frequently related to their optical purity.<sup>3</sup> Biocatalysis, which is the application of enzymes isolated or contained in microorganisms as catalysts, appears as a very interesting strategy for the enantioselective synthesis of bioactive substances.<sup>4</sup> Thus, the overall goal of this work was to develop a methodology that would permit the enantioselective preparation of  $\alpha$ -methyl- $\beta$ -hydroxyesters, key synthetic intermediates of (+)-discodermolide, a substance that has been used with great success in the cancer treatment.

Substrates were prepared as shown in Scheme 1. While the substrate **2** was obtained by methylenation with paraformaldehyde in acetic acid, the  $\alpha$ -methyl- $\beta$ -ketoester **3** was obtained by direct methylation of the ethyl benzoylacetate with iodomethane in the presence of potassium carbonate.



Scheme 1

The bioreductions were mediated by yeast *Saccharomyces cerevisiae*. Reactions were carried out with free yeast cell grown on YMA,<sup>5</sup> baker's yeast lyophilized Type-II Sigma-Aldrich® and baker's yeast cells immobilized with calcium alginate. The preliminary studies of reduction of the substrates **2** e **3** with *Saccharomyces cerevisiae* were carried out in different bioreduction mediums for 14 days in the presence and absence of glucose, as shown in Scheme 2. The enantiomeric excesses obtained of the **4a** product were ranging from 46% to 99% already the enantiomeric excesses obtained of the **4b** product ranging from 18% to 99%.



Scheme 2

The yeast *Saccharomyces cerevisiae* has catalyzed the enantioselective synthesis of  $\alpha$ -methyl- $\beta$ -hydroxyesters with high enantiomeric excesses. Applications of the methodology in the synthesis of bioactive compounds are ongoing in our laboratories.

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Oral Sessions ▶ A3

# Agro Food III



## Bioactive compounds and reducing ability of medicinal herbal infusions: a regard over their labels

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Consumer interest in a healthy diet has been growing exponentially. Consequently, food industries provide, nowadays, an increasing number of products (including teas and infusions) that label beneficial health and/or well-being effects.

In this work, a preliminary survey of several herbs commonly used in commercial mixtures for infusions (horsetail, lemongrass, green tea, fennel, St. John's wort, senna and tilia) was performed. Infusions of single plants ( $n=13$ ) and mixtures ( $n=18$ ) were analyzed and their labels verified in order to identify possible gaps in their claims.

Total phenolics [1], total flavonoids [2] and tannins [3] contents, as well as the reducing ability (using FRAP assay [4]) of samples, were determined.

In what concerns to the single plant infusions, a green tea sample presented the highest total phenolic content (136 mg EAG/100 ml) and reducing power (931 mg ESF/100 ml). Among the other pure infusions, closer values to the green tea were found in the lemongrass brew (107 mg EAG/100 ml, 667 mg ESF/100 ml). There was a large variability in the values obtained for the infusions of mixtures, even among those prepared with the same plant, which, in part, could be explained by a possible synergistic effect. It was also noted that the information of the labels is hardly enlightening taking into account the possible adverse reactions that these products may originate. Moreover, certain products presented health claims somewhat inconsistent with their constitution.

In conclusion, it was possible to verify the compositional variability of herb preparations for infusions that exist in the market. Healthy consumers can drink them moderately with benefits. However, consumers with some sort of dysfunction should analyze carefully the labeling or any other information related to the infusions content.

Acknowledgments: R. Alves is grateful to FCT for a post-doctoral grant (SFRH/BPD/68883/2010) financed by POPH-QREN-Tipologia 4.1-Formação Avançada, subsidized by FSE and MCTES. This work has been supported by FCT through grant no. PEst-C/EQB/LA0006/2011.

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## Phytochemicals in food industry by-products: The case of coffee silverskin

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Recovery of waste and added value by-products in the food industry is mandatory in a society that aims environmental, social and economic sustainability. It promotes waste reduction and gains instead of expenses.

In the present study, we used coffee silverskin, a by-product (with no added value) of coffee roast industry, as a possible source of phytochemicals with antioxidant activity. Silverskin from three commercial coffee batches (a pure robusta and two blends with arabica and robusta coffees) were provided by a local coffee torrefaction industry.

Extracts were prepared using ethanol:water (1:1) at 40°C, for 60 minutes. Flavonoids [1], tannins [2] and total phenolic contents [3], as well as antioxidant activity, were evaluated by spectrophotometric methods. Antioxidant activity was determined by using both the ferric reducing antioxidant power (FRAP) [4], and 1, 1-diphenyl-2-picrylhydrazyl free radical (DPPH•) assays [1].

No significant differences were found ( $p < 0.05$ ) between silverskin extracts of coffee blends in what concerns to flavonoids (~116 mg ECE/L), tannins (~0.4 mg TAE/L), and total phenolics (~370 mg GAE/L). The silverskin extract from 100% robusta coffee showed significantly lower ( $p < 0.05$ ) contents. Concordantly, higher antioxidant activities ( $p < 0.05$ ) were found for silverskin extracts from blends.

These preliminary results suggest that it could be possible to chemically distinguish silverskin from different coffee species. Furthermore, this by-product appears as an antioxidant-rich product that could be used for incorporation, for instance, in dietetic supplements or foodstuff fortification.

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## 4-methylimidazole levels in balsamic vinegars and processed sauces

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Caramel colours are colouring substances authorised as food additives in the EU, produced by heating carbohydrates under controlled heat and chemical processing conditions. The caramel colours can be grouped into four classes according to the process and reactants used in their manufacture: Class I Plain caramel (E 150a), Class II Caustic sulphite caramel (E 150b), Class III Ammonia caramel (E 150c), and Class IV Sulphite ammonia caramel (E 150d). In caramel corresponding to class III or IV, the use of ammonia or ammonium salt as caramelization promoters involves the formation of undesirable compounds, such as 4-methylimidazole (4-MeI). These kind of caramels are commonly used in a variety of foodstuffs, especially in carbonated beverages, dark beers, condiment sauces such as processed sauces (e.g. soy sauce) and balsamic vinegars, because of their aromatic and colorant characteristics [1,2].

The presence of minor caramel components in most foods and beverages, however, can be hazardous to humans because of their toxicity. 4-MeI is a neurotoxic agent, which has been shown to be a potent convulsing agent that elicits neurological signs in many animals, and some in vitro studies have shown its capability to inhibit the cytochrome P450 isoenzyme which catalyses the oxidation of many known or suspected carcinogens in the human liver. Furthermore, recent toxicological studies showed that 4-MeI can induce alveolar/bronchiolar adenoma and carcinoma in male and female mice [3]. The Codex Alimentarius of the World Health Organization (WHO) and the European Union (EU) have established a maximum of 250 mg/kg for 4-MeI, for caramels class III and IV [2].

The aim of this work was to screen the presence of 4-MeI in balsamic vinegars, soy sauces and other processed sauces containing caramels class III and IV commercialized in Portugal using a previously developed gas chromatography-mass spectrometry (GC-MS) method [1]. This method was validated for this type of sample in terms of: linearity ( $R^2$  always > 0.9936); recovery (78-87% for balsamic vinegars and 86-96% for processed sauces, in 3 levels); and precision (5-16% for both types of matrices, in 3 levels,  $n = 6$ ). The validated method was applied to 33 samples, including 20 balsamic vinegars and 13 processed sauces. 4-MeI was found in all the 20 vinegar samples studied at levels that ranged from 0.630 to 4.150 mg/l, and it was found in 4 of 13 samples of processed sauces at levels ranging from 0.670 to 0.860 mg/l.

**Key Words:** 4- methylimidazole, vinegar, processed sauces, ion-pair extraction, GC-MS

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# High-throughput near-infrared spectroscopy based technology for qualifying grapes based on soil and climate conditions

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Production of high quality wines requires a permanent monitoring of the entire winemaking process. Grapes composition is the result of the “terroir”, which is ultimately influenced by the soil composition and climatic conditions. Grapes composition not only controls the ultimate acid/ethanol balance, but also determines the type and the intensity of further yeast activity. The optimal maturity depends upon the style of wine being made, varietal, interaction of varietal, rootstock and site, seasonal specific factors, viticultural practices and downstream processing events. The major question that this project aims at answering is: is it possible to use efficiently an high-throughput analytical method for estimating the impact of soil quality, tillage and thinning on the grapes quality? The nowadays strategy for analyzing the soil quality is based in wet chemistry methods, which are often laborious, expensive, time-consuming and of limited use during harvesting campaigns. Near infrared spectroscopy (NIRS) proved to be the most efficient tool for direct in-situ analysis of soil and grapes. Despite the proved usage of NIRS for estimating soil chemical and physical parameters, and grapes quality (e.g., to follow the ripeness process), there are no consolidated scientific results for the correlation between soil characteristics and grapes quality using this analytical method. Additionally, Portuguese grapes varieties have distinctive features when compared to other varieties largely used abroad (e.g., Australia, USA, South Africa), for which most results in this area exist. Addressing this technology from the point of view of Portuguese producers constitutes yet a challenge. The major and ultimate deliverable of this project is a cost-effective NIRS based technology adapted for Portuguese winemakers in order to add the valorization of current products, by mapping efficiently the vineyards, not only in terms of soil characterization, but also on soil/climate effect on grapes quality and ripeness process.

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## Meat Quality Traits of Alternative Lines of Broiler

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Researches have been undertaken to evaluate alternative lines of broiler chickens, however, with respect to the carcass and meat quality traits, few studies have been published [1]. The objective was to investigate the effects of strains, sex and rearing system on meat quality of broilers strains.

The experiment was conducted using broilers alternatives of Project "Frango Feliz" of ESALQ: "Caipirão ESALQ" and "7P" - Pinto Preto Pesado de Pasto de Pescoço Pelado de Piracicaba. For that, 240 chicks of each broiler strain were placed in 16 floor pens. Treatments consisted of two strains reared under intensive and semi-intensive system (with free access to the pasture area after 21 d of age). At 84 d of age, a sample consisting of three males and three females from each pen was selected and slaughtered. pH and color (L\*) were evaluated on the pectoralis major muscle, 24 hours postmortem. The results were analyzed using the SAS (Statistical Analysis System, version 9.2.).

Only the strain and sex had significant effect on the parameter pH<sub>24</sub> (Table 1). The mean pH<sub>24</sub> value measured in the meat of alternative lines of broiler was below the border value for PSE meat (5.7) [2]. No sex or strain effects were observed for color measurement. The L\* value was influenced by rearing system. Studies have reported a similar relationship between lower pH values and color, where L\* values (greater than 52) were associated with poor meat quality [3].

The rearing system does not influence the pH<sub>24</sub> values, however, poultry treated under the semi-intensive system has presented a higher L\* value.

Table 1. pH<sub>24</sub> e L\* measured in meat of males (M) and females (F) of broiler strains (7P and Caipirao) rearing in intensive (I) and semi-intensive (SI) systems

pH <sub>24</sub>	7P		Caipirao		Mean	L*	SI		I		Mean
	SI	I	SI	I			7P	Caip	7P	Caip	
M	5,64	5,67	5,64	5,73	5,67A	M	57,37	59,80	59,36	58,54	58,8A
F	5,62	5,67	5,62	5,61	5,63B	F	55,92	56,25	58,38	58,67	57,3A
Mean	5,63b		5,67a				57,34b		58,74a		

Means followed by different small/capital letters within a row/column differ (P<0.05) by Tukey' test.

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## Characterization of Dulce de Leche by texture analysis

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The *dulce de leche* is a typical Latin American product, being produced in large scale in countries like Brazil, Chile and especially Argentina. In Brazil, the cultural differences, changes in technologies and formulations employed make it difficult to obtain uniformity in the dulce de leche found on the market. The aim of this work was to measure textural and physical-chemical parameters of ten commercial brands of this product and also to evaluate the relationship between these characteristics.

The moisture content determination was made by the traditional method – Gravimetric, the water activity by Aqua Lab 4TE equipment, the analysis of texture by CT3 Brookfield Texture Analyzer, the soluble solids by Reichert AR 200 refractometer and the statistical analysis by SAEG software.

The results were: water activity 0,869 ( $\pm 0,032$ ); moisture 32,02% m/m ( $\pm 4,93$ ); soluble solids content 65,65°Brix ( $\pm 4,82$ ); hardness 308,55g ( $\pm 161,22$ ); adhesiveness 21,69mJ ( $\pm 14,77$ ); cohesiveness 0,86 ( $\pm 0,15$ ); stringiness 15,66mm ( $\pm 3,92$ ); gumminess 273,81g ( $\pm 183,27$ ); chewiness 47,90mJ ( $\pm 48,50$ ). It was found significant correlation coefficients ( $p \leq 0.05$ ) between the following variables: water activity and soluble solids content, water activity and cohesiveness, soluble solids content and cohesiveness, hardness and adhesiveness, hardness and chewiness, adhesiveness and cohesiveness, adhesiveness and stringiness, cohesiveness and stringiness, cohesiveness and gumminess, cohesiveness and chewiness, stringiness and hardness, stringiness and gumminess. It was also found that the combination between physical-chemical parameters and rheological attributes is useful for the characterization and the evaluation of the quality of *dulce de leche*.

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Oral Sessions ▶ A4

# **Biological Sciences III**



# ***Spergularia rubra*: A functional beverage to reduce overweight, obesity and diabetes**

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*Spergularia rubra* L. J. Presl & C. Presl is a species distributed all over the world. Its aerial parts are widely used for the preparation of an infusion, consumed for its diuretic properties. In this work, a functional beverage to reduce overweight, obesity and diabetes was tested. Thus, different concentrations of a hydroethanolic extract obtained from *S. rubra* were incorporated in several beverages commercialized by UNICER<sup>®</sup>. Additionally, taking into account that stomach and intestine pH may affect the activity of the functional beverage, an *in vitro* model of digestion was used.

Phenolic compounds were determined by HPLC-DAD-MS. Thirty six flavonoids comprising non-acylated *C*-glycosyl flavones, *C*-glycosyl flavones acylated with aromatic acids, *C*-glycosyl flavones acylated with aliphatic acids and *C*-glycosyl flavones with a mixed acylation were identified.

The capacity of different functional beverages, containing *S. rubra* extract, to inhibit  $\alpha$ -glucosidase and acetylcholinesterase (AChE) was evaluated. All tested mixtures revealed a strong activity against these two enzymes (Fig. 1).

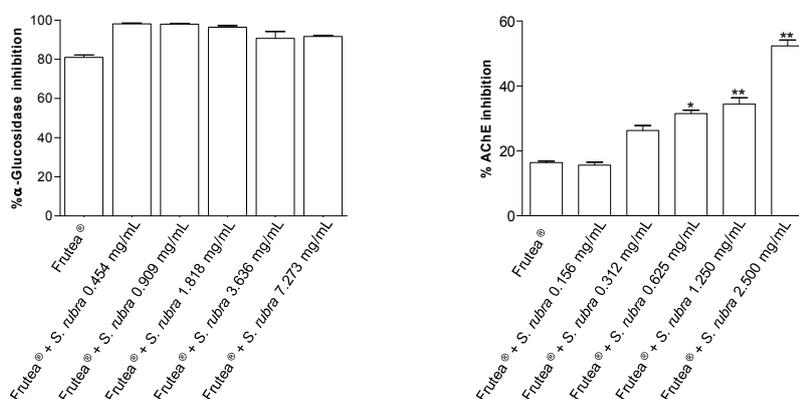


Fig. 1.  $\alpha$ -Glucosidase and AChE inhibitory activities of the functional beverages containing *S. rubra* extract. \* $p < 0.05$  and \*\* $p < 0.01$ , relatively to Frutea<sup>®</sup>.

The results suggest that the ingestion of this functional beverage can have a positive impact in the organism since it can suppress hyperglycaemia. Additionally, due to its capacity to inhibit acetylcholinesterase, it may be useful in the treatment of Alzheimer's disease.

Acknowledgments: The authors are grateful to UNICER<sup>®</sup> and Porto University (PP-IJUP2011-UNICER-84).

## A Role for the Cell Wall in the Regulation of Vacuolar Sorting

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In the past few years, the plant cell wall has been drawing more and more attention from the scientific community, particularly regarding the protein trafficking to and from this compartment. Although the transport of soluble proteins to the extracellular matrix is commonly accepted to occur by bulk flow, new data has emerged regarding the existence of signals and intermediates to control these pathways. In fact, experiments by our group have pointed towards some form of regulation of protein transport by the cell wall. Using cardosins A and B as experimental models, we have shown that the absence of cell wall in *Nicotiana tabacum* protoplasts expressing cardosins, which are typically vacuolar in leaf cells, have originated partial protein secretion. In addition, the importance of cardosins' vacuolar sorting determinants in this regulation was explored and particularly important observations were collected when comparing the divergent results obtained with the Plant Specific Inserts (PSI) and the C-terminal peptides, both fused to the fluorescent protein mCherry. While the C-terminal peptide fusions showed to be partially secreted in the absence of the cell wall, the PSI regions directed mCherry to the vacuole. Based on the data obtained, we consider that the cell wall might have a role on the regulation of protein trafficking to the vacuole, namely on redirecting vesicles from the prevacuolar compartment (PVC) with cargo destined for the vacuole, which have erratically been transported to the cell surface. Following a route that bypasses the PVC, the PSI fusions do not get tangled in the regulation by the cell wall at the PVC level and, consequently, are not secreted to the culture medium. This study enhances the dynamic and active role of the cell wall in the protein sorting events and is the starting point of several studies exploring its participation on the regulation of intracellular sorting and trafficking.

# Foliar application effects of 24-epibrassinolide on biomarkers of oxidative stress in *Solanum nigrum* L. exposed to high levels of Zn

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*Solanum nigrum* L. proliferates in sediments with high levels of metal pollution, and it is known for its ability to hyperaccumulate heavy metals (HM), namely Cd, and to tolerate and concentrate high amounts of other HM (Pb, Zn, Cu, Cr and As) in aerial parts. Zn is an essential micronutrient necessary for plant growth, but at higher levels can be phytotoxic [1]. Brassinosteroids (BRs) are steroidal phytohormones involved in a wide range of physiological processes and are also associated with the plant protection to biotic and abiotic stresses [2]. This work aimed to evaluate the protective role of 24-epibrassinolide (EBR) in *S. nigrum* plants exposed to high levels of Zn. After emergence, *S. nigrum* seedlings were divided in three groups: G1 – control with 2  $\mu\text{M}$  Zn (final concentration of Zn in the Hoagland's solution), G2 - 500  $\mu\text{M}$  Zn and G3 - 1  $\mu\text{M}$  EBR + 500  $\mu\text{M}$  Zn, and all the plants were grown in a substrate of vermiculite:perlite (2:1) watered with Hoagland's solution during 7 days and were maintained in a growth chamber at 23°C, under 16 h/8 h light/dark photoperiod. At this time the G3 plants was sprayed with 1  $\mu\text{M}$  EBR. After 24 h, the two last groups (G2 and G3) were watered for three weeks with Hoagland's solution supplemented with 500  $\mu\text{M}$  Zn, whereas control plants (G1) were only watered with Hoagland's solution. Then, after the three weeks of Zn treatment, at least 5 plants from each group were collected, separated into shoots and roots, and biometric parameters (shoot height, root length and shoot and root fresh weight) and physiological parameters related with oxidative stress, such as photosynthetic pigments, lipid peroxidation and free proline content were evaluated. Regarding shoot and root fresh weight, as well as shoot height, no significant differences were found between the three groups of plants, but the presence of 500  $\mu\text{M}$  Zn in the nutrient solution caused a reduction of 30% in root length compared to the control. Root length of plants pre-treated with EBR was similar to the control. The Zn treatment caused a significant decrease of 25.6% in the chlorophylls, however the pre-treatment with EBR reverted the negative effect of the metal. The level of carotenoids was similar in the three plant groups as well as lipid peroxidation. Proline content, both in shoots and roots, increased in the plants exposed to 500  $\mu\text{M}$  Zn, but proline levels in EBR sprayed plants subsequently treated with Zn were close to that of control plants. These preliminary results reveal that the amelioration of the negative effects of 500  $\mu\text{M}$  Zn treatment, due to the spraying of EBR was reflected in the improvement of root growth and in the increase of chlorophyll and proline content.

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# Unravelling the role of brassinosteroids in response to high Zn levels in *Solanum nigrum* L.

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*Solanum nigrum* L. plants were grown for 30 days in a hydroponic system to analyze the effect of high Zn concentrations and to determine if a brassinosteroid (BR) pre-treatment plays a protective role towards the same high Zn concentration. The high Zn treatment (500  $\mu$ M) induced a reduction of 2.8x in the root's H<sub>2</sub>O<sub>2</sub> levels, but the BR pre-treatment (1  $\mu$ M) increased these levels 2x, similar to those levels of the control situation. The high Zn treatment led to a 1.9x reduction of H<sub>2</sub>O<sub>2</sub> levels determined in leaves, while in the same organs the BR pre-treatment led to a 1.3x increase of this parameter, in relation to the control. In these organs, and compared to the Zn treatment, the BR pre-treatment in leaves led to an increase of 2.5x of the H<sub>2</sub>O<sub>2</sub> levels. The H<sub>2</sub>O<sub>2</sub>-scavenging enzymes, cytosolic ascorbate peroxidase (cAPX) and catalase 1 (CAT1), were studied by RT-PCR. Both cAPX and CAT1 suffered a reduction in mRNA accumulation in response to high Zn levels in roots. However, an increased accumulation of cAPX and CAT1 could be observed in the roots of the BR pre-treated plants, although never reaching the levels detected in the control. Contrary to the roots, Zn led to an increased expression of CAT1 and cAPX genes in shoots. While the BR pre-treatment apparently repressed cAPX expression in shoots, it slightly decreased CAT1 mRNA accumulation. These results suggest that both shoot CAT1 and cAPX act to efficiently decrease H<sub>2</sub>O<sub>2</sub> levels resulting from the abiotic stress of high Zn concentrations, but other detoxifying mechanisms may be acting to decrease the H<sub>2</sub>O<sub>2</sub> levels in roots. Interestingly, BR have a differential effect in the analyzed organs, inducing CAT1 and cAPX expression in roots and inhibiting it in shoots, thus leading to increased H<sub>2</sub>O<sub>2</sub> levels in the shoots in the BR pre-treated plants. Taken together, these results suggest that the BR pre-treatment apparently protected plants against the high Zn levels by inducing an antioxidant response that does not include the participation of CAT1 and cAPX.

# ***Solanum nigrum* L. metallothionein gene family responses to high zinc levels and the effect of brassinosteroids in their expression**

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Although essential to most living beings, some heavy metals (HM) can be deadly in high doses [1]. Anthropogenic activities have resulted in the release and subsequent accumulation of high levels of HM in the environment. Several studies have been done in soil phytoremediation because it is a process more reliable and cheaper than other methods. For its great potential in phytoremediation, *Solanum nigrum* L. has been studied for its capability to extract and store HM from the soil. Several Cys-rich peptides, like metallothioneins (MTs) have been implicated in the detoxification and homeostasis of HM such as Cu and Zn. Other properties include an antioxidant role against reactive oxygen species (ROS) and protection against DNA damage [2]. MTs are encoded by a small multigene family and are based on the arrangement of their Cys residues into four types: MT1s, highly expressed in roots; MT2s, mostly in shoots; MT3s, more specific to ripening fleshy fruits; and MT4s, restricted to developing seeds [1]. Brassinosteroids (BR) are plant growth regulators that have been implicated in the protection of plants to several stresses. To understand the performance of *S. nigrum* when exposed to a high concentration of Zn and the influence of a BR pretreatment regarding the involvement of MTs in Zn homeostasis, two distinct treatments (A,B) plus a control were used. Both A and B treatments consisted on the continuous exposure to 500  $\mu\text{M}$  of Zn diluted in Hoagland solution, but in B a 1  $\mu\text{M}$  BR foliar spray application was performed 24 h prior to high Zn exposure. Plants were grown in a growth chamber under a constant temperature of 23°C and a 16 h light/8 h dark photoperiod and watered when needed. After 30 days shoots and roots from at least 5 plants of each situation were separated and frozen in liquid N<sub>2</sub> and stored at -80°C until used. Total RNA was extracted and used for specific MT-encoding mRNA accumulation analysis by a “two-step, two-tubes” reverse transcriptase-PCR. Both treatments lead to an increased accumulation of MT1-, MT2ab-, MT2cd- and MT2c-related mRNAs in roots and shoots. Interestingly, the BR pretreatment induced a *de novo* accumulation of MT3 mRNAs in roots, where no MT3 expression was expected, but in shoots both treatments increased MT3 mRNA accumulation, being this increased more pronounced with the Zn treatment. These preliminary results suggest that MT2a,b,c,d are Zn-responsive and that BR induced MT3 *de novo* expression in roots, thus protecting the plants from the high Zn cellular levels and/or removing ROS derived by the exposure to the high Zn conditions.

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# Acetophenone-exposed *Solanum nigrum* L. weed plants: stress and toxicity analysis

**J. Cunha, J. T. Moreira, F. Fidalgo & J. Teixeira**

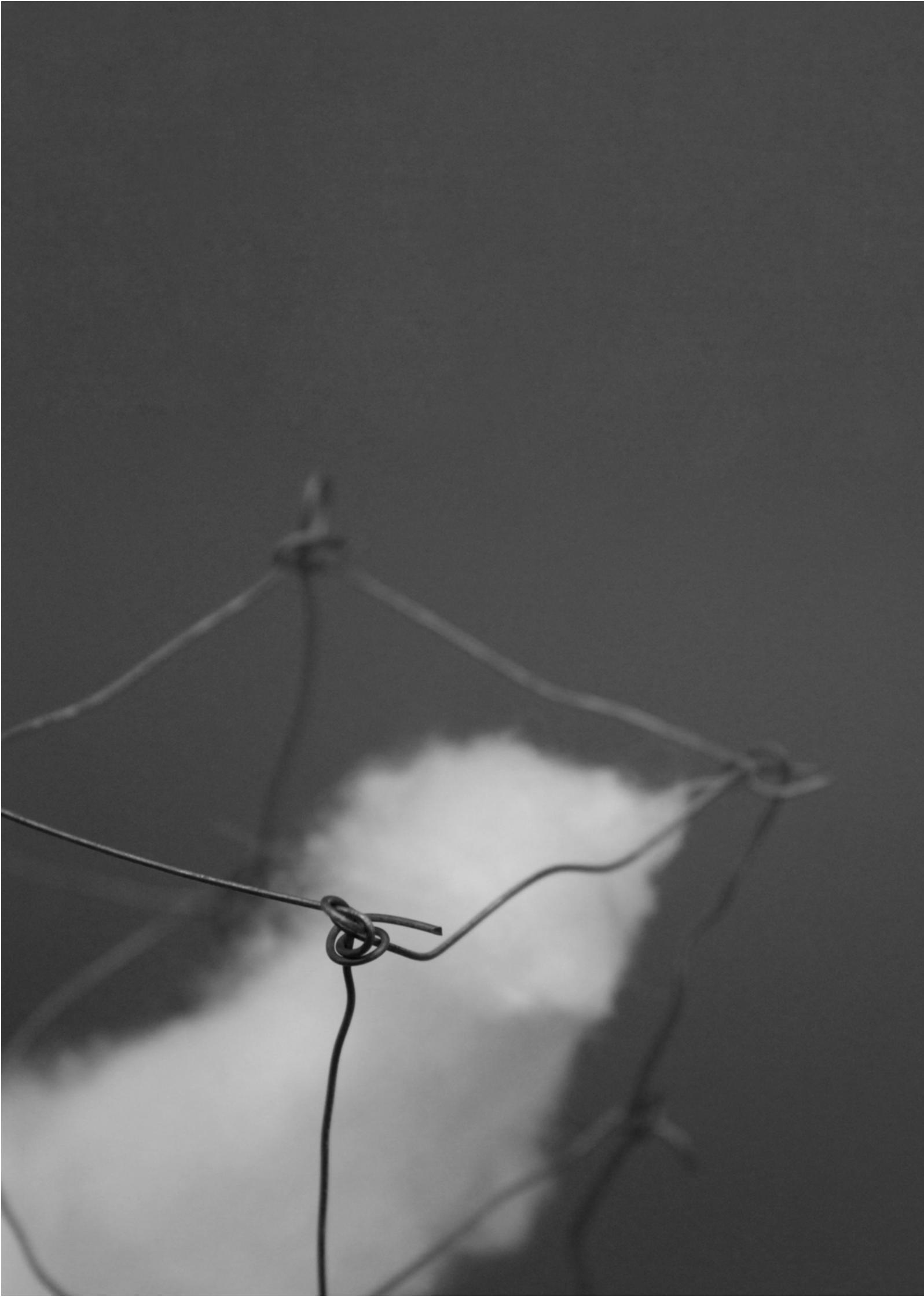
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Pesticide application has become an integral part of agriculture worldwide. Pesticide drift outside the target area is potentially hazardous for nearby non-targeted plants. Phytoremediation is the use of plants and associated mechanisms for environmental cleanup. In previous phytoremediation studies, metalaxyl was chosen as model pesticide because of its common use in vineyards and several horticulture industries, and also due to its physiochemical properties, where it was supplied to *Solanum nigrum* L. plants in the form of a commercial formula (Ridomil®; Syngenta Agro S.A., Portugal) consisting of metalaxyl as active compound (ca. 45% wt.), but also containing acetophenone in its composition. It was showed that this plant species can be used as an excellent metalaxyl phytoremediation tool [1]. In order to determine if acetophenone can be deleterious to, or exert a negative effect on *S. nigrum*, several tests were now performed. Plants grew in the presence of different acetophenone concentrations for one month: The control group (1), without acetophenone; a second group with 0.52 ppm (2); and a third group with 1.04 ppm acetophenone (3). Roots and shoots were analyzed separately to evaluate the effect of acetophenone on plant growth, photosynthetic pigments, lipid peroxidation and free proline levels, and glutathione-S-transferase (GST) activity. The obtained results were statistically analyzed using Fisher and *t*-Student tests. Biometric analysis revealed a significant drop in shoot length for groups (2) (1.18x) and (3) (1.24x), compared to group (1). Root growth was not significantly affected. Carotenoid levels were not affected, but the concentration of chlorophyll A+B showed significant differences only for group (2), dropping 1.14x. Lipid peroxidation significantly increased in roots by 1.40x and 1.52x, in groups (2) and (3), respectively, but only the shoots from group (3) showed a significant increase of 1.25x. The root proline levels showed a significant increase only in group (3) (2.69x). GST activity showed no differences between all groups studied, independently of the substrate used, suggesting that this enzyme does not directly metabolize acetophenone. This work revealed that plants were negatively affected by 0.52 and 1.04 ppm acetophenone and that GST does not play a pivotal role in its detoxification. Despite some oxidative stress, antioxidant defenses such as proline production in roots, and maybe other mechanisms, appear to effectively protect the plant and decrease the acetophenone-related deleterious effects, allowing plants to thrive until the end of their life cycle.

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# POSTER SESSIONS



Posters

**Wednesday, 13<sup>th</sup>**



## **Aerobiology of *Alnus glutinosa* and ozone effects on its pollen fertility, proteins and allergenicity**

**I. Reis<sup>1</sup>, H. Ribeiro<sup>2</sup>, L. Duque<sup>2</sup>, M. Pereira<sup>1,3</sup>, I. Abreu<sup>1,2</sup>**

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The *Alnus glutinosa* tree has been spread out for ornamental purposes and is present in public gardens scattered around Porto. Its pollen is mainly transported by wind, being the cause of pollinosis in the citizens of this city. On the other hand, Ozone is one of the most important atmospheric pollutants. The aim of this work was to study the aerobiology and the effects of in vitro exposure of *Alnus* pollen to Ozone (O<sub>3</sub>).

Airborne pollen monitoring was continuously performed during 2012, using a 7-day Hirst-type volumetric trap set on the roof of the Faculty of Sciences in Porto. Pollen grains were trapped on a Melinex tape coated with silicone oil, which was then cut into daily segments and mounted on slides with a mounting media of glycerol jelly. The daily mean concentration of *Alnus* spp. pollen was estimated using an optical microscope (DMLB, Leica) with x400 magnification along 4 full lengthwise traverses. Pollen counts were expressed as the sum of the number of pollen per cubic meter of air for a 24 -hour period. *Alnus* spp. pollen is mainly present in the atmosphere from January to early March, with the maximum airborne concentration found in February. The annual pollen concentration was of 190.96 pollen grains with a maximum airborne pollen levels attained in a day of 11.408 pollen.m<sup>-3</sup> of air.

For the in vitro assays the *Alnus* pollen was collected directly from the tree and exposed to two O<sub>3</sub> concentrations (3.66ppm and approximately four times this value) during two consecutive days. After exposure pollen viability was determined using the Trypan Blue Dye test. Pollen proteins were quantified and the molecular weight of the polypeptides was determined by SDS-PAGE and the immunoreactivity was assessed by Western blot using patient sera sensitized to this pollen.

The Trypan Blue Dye test showed that, with the tested levels of O<sub>3</sub> pollen viability decreased in comparison to control sample (non-exposed pollen). However, the quantity of protein and the IgE recognition by patient sera was not affected, indicating that the O<sub>3</sub> exposure did not have influence in pollen allergenicity.

This work was supported by FEDER funds through the COMPETE and National funds through FCT (PTDC/AAC-AMB/102796/2008).

## AHP6 inhibits cytokinin signaling to regulate the orientation of pericycle cell division during lateral root initiation

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Lateral root (LR) formation is the major determinant of plant system architecture, occurring postembryonically throughout plant's life. In *Arabidopsis thaliana*, lateral root organogenesis has been divided into eight stages, characterized by specific cell divisions and anatomical changes [1]. In this plant, LRs initiate from xylem-associated pericycle founder cells which undergo the first anticlinal divisions, followed by coordinated cell divisions to organize the lateral root primordia (LRP) [2]. Lateral root development is regulated antagonistically by the phytohormones cytokinin and auxin. In opposite to auxin, cytokinin (CK) has an inhibitory effect on the patterning events occurring during LR formation, disrupting the regular pattern of cell divisions in LRP [3]. However, the molecular players involved in cytokinin repression for a correct pattern of cell divisions are still unknown. In *Arabidopsis thaliana*, AHP6 (ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6) is a repressor of cytokinin signaling during vascular development, allowing protoxylem differentiation [4]. In this research, we aimed to study the AHP6 function(s) during lateral root development in *Arabidopsis thaliana*.

First, we characterized the *AHP6::GUS* expression during lateral root development. Different aspects of LR development in wild-type (WT) Col-0 and *ahp6* mutants were analyzed. To investigate the *AHP6* function as a CK inhibitor, we characterized the phenotype of LR development in WT and *ahp6* plants treated with CK and also the phenotype of *AHP6::CKX2* in *ahp6-1* (plants harbouring a CK catabolic enzyme under the control of the *AHP6* promoter). Finally, we tested the interaction of *AHP6* with PIN1-GFP (an auxin efflux carrier marker) in WT and *ahp6-1*. All these analyses were accomplished under optical, DIC and confocal microscopy.

We found that *AHP6* is expressed in all stages of LR development and that *ahp6* mutants show defects in the orientation of cell divisions in early stages. These defective cell divisions were not observed neither in WT nor in *ahp6-1/AHP6::CKX2*. When treated with CK, *ahp6* showed an increase in the frequency of LRP with abnormal cell divisions. We also demonstrate that *AHP6* influences the localization of the auxin efflux carrier PIN1, which is necessary for patterning the LR primordia.

In conclusion, we reveal that beyond vascular development *AHP6* also functions as a cytokinin repressor during early stages of LR development in *Arabidopsis thaliana*, and is required to establish the correct pattern of early LR primordia.

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## Antibiotic properties of marine cyanobacteria: searching for new antibiotics

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The overuse of antibiotics over the years is leading to an acquired of bacteria to these antibiotics. This can cause a public health problem because bacteria can acquire resistance to antibiotics in the environment. One of the challenges is to find antibiotics that can cure infections caused by multidrug resistant bacteria, such as *E. coli*, *Enterococcus sp.* and *S. aureus*. The main goal of this research is to know if the secondary metabolites of marine cyanobacteria produce any antibacterial product that can act against multidrug resistant bacteria. Cyanobacteria and bacteria were collected from laboratory collections (LEGE, CIIMAR and Food Microbiology, ICBAS). Twelve marine cyanobacteria belonging to the *Oscillatoriales* and *Chroococcales* genera were selected. They grew during one month and the biomass collected and then freeze-dried. With the freeze-dried biomass six organic extractions using dichloromethane and methanol (2:1) were done. Some of the crude extracts were fractionated using three different organic solvents (hexane, ethyl acetate and methanol). According to their polarity all the fractions were collected and the solvent was evaporated being collected 1 mg of each and dissolved in DMSO (dimethyl sulfoxide). Bacteria resistant to the most used antibiotics such as methicilin resistant *S. aureus*, vancomycin resistant *Enterococcus sp.* and ciprofloxacin resistant *E. coli* were used. The method used to test the extracts was the disc- diffusion assay with white discs (Oxoid) impregnated with 20µL of the extracts. The plates were left at 4°C during 1h to let the extract diffuse in the plate. At the end the plates went to incubate at 37°C during 24h. No inhibition was observed in all the plates. The next step is to compare freshwater cyanobacteria with marine cyanobacteria and know if freshwater cyanobacteria produce any type of antibacterial compound.

## Antibiotic resistant bacteria in aquaculture rainbow trouts: a new threat in the food chain?

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**Background.** Aquaculture is currently one of the fastest growing food production sectors, associated with, approximately, 50% of the fish produced worldwide for human consumption. By providing products with standard characteristics, quality control and prices, which are stable and lower than regular fishing products, aquaculture could present advantages for food services. However, food safety threats, such as clinically relevant antibiotic resistance (ABR), are currently a FAO/WHO/EFSA recognized concerns. Our goal was to assess the presence of pathogenic bacteria and *Enterobacteriaceae* carrying clinically relevant ABR genes in aquaculture rainbow trouts (*Oncorhynchus mykiss*) marketed in Portugal.

**Methods.** We analyzed 27 trout aquaculture samples (3 trouts per sample; muscle and viscera), 25 from 8 supermarkets (SM) and 2 from an aquaculture facility (TA) (May-June 2012). We screened for *Salmonella*, *Listeria monocytogenes* and *Escherichia coli* (standard methods) and for ABR Gram negative bacilli using selective media with/without AB (ceftazidime, cefotaxime and ciprofloxacin) after enrichment. Genes conferring resistance to beta-lactams (*bla*<sub>TEM</sub>, *bla*<sub>CTX-M</sub>, *bla*<sub>SHV</sub>), fluoroquinolones [*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *qepA*, *aac(6')-Ib-cr*, *oqxAB*] and other AB were searched by PCR/sequencing. Species were identified by ID32GN/16SPCR and phylogenetic group was determined in *E. coli*. ABR was analyzed by agar diffusion/E-test (CLSI/EUCAST).

**Results.** *Salmonella* and *L. monocytogenes* weren't detected. *E. coli* (n=16; phylogenetic groups A0-5/A1-1/B1-5/B2-1/D-4) was found in 48% of samples (TA/7 SM), with 56% (TA/4 SM) resistant to different AB (nalidixic acid:38%; tetracycline:31%, *tetA/tetB*; streptomycin:31%, *strA/strB*; amoxicillin:25%, *bla*<sub>TEM</sub>; sulfamethoxazole:19%, *sul2*; ciprofloxacin:19%; chloramphenicol:13%, *floR/catA*; trimethoprim:13%). In Gram negative bacteria extended-spectrum beta-lactamases weren't detected (n=0/146) but plasmid mediated quinolone resistance (PMQR) genes were observed in 9% (n=6/68; TA/4 SM) of the isolates, with minimum inhibitory concentration to ciprofloxacin (0.25-0.5 µg/mL) above ECOFF. The *qnrS2* gene was detected in multidrug resistant (MDR) *Hafnia alvei* (n=2; 2 SM; *tetA/floR/strA/strB/sul1*), the *qnrB10/new qnrB* variant in MDR *Citrobacter freundii* complex (n=3; SM/TR; *tetA/floR/sul1/sul2/int1-dfrA12-aadA*) and the recently described *qnrD* in MDR *Proteus vulgaris* (n=1; SM; *tet/sul2*).

**Conclusion.** Trouts for human consumption analyzed in this study seem not to be a source of *Salmonella* and *L. monocytogenes* but are a vehicle of ABR bacteria/genes of relevance for human and animal health. Detection of MDR bacteria and PMQR in fish products is of concern and highlights the need to establish ABR control policies and surveillance in the food chain.

**Acknowledgments:** This research was supported by Fundação para a Ciência e a Tecnologia - PEst-C/EQB/LA0006/2011 and PTDC/AAC-AMB/103386/2008.

# Antioxidant activity of *Colocasia esculenta* (L.) Shott and its possible relation with wound healing

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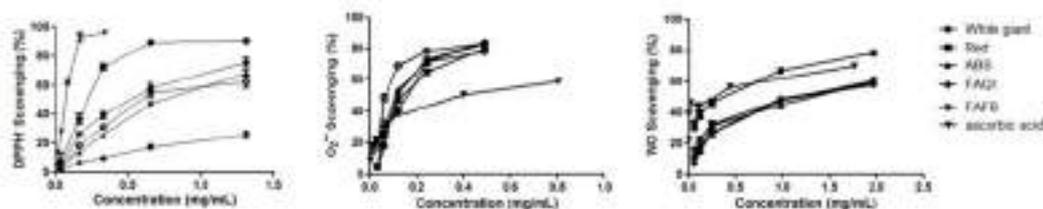
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*Colocasia esculenta* (L.) Shott is an annual herbaceous plant belonging to the Araceae family, commonly known in Portugal as "Inhame". The cultivation of this plant is mainly due to its tuber, an essential food for millions of people. In addition to its nutritional value, taro is known for its medicinal properties and has been traditionally used to treat various diseases. In Azores taro leaves are used by the indigenous to treat dermal wounds. This property may be related to the anti-inflammatory activity of phenolic compounds, which act by sequestering free radicals involved in the inflammatory process that may adversely affect the repair process in protracted situations. Therefore, this work aimed to characterize and compare the phenolic fraction of different varieties and culture conditions of taro from Azores, as well as to relate the phenolic profile with the antioxidant capacity.

The analysis by HPLC-DAD-ESI/MS<sup>n</sup> allowed the identification of 41 phenolic compounds, 34 of which were described for the first time in this species. The application of a reproducible method of quantification by HPLC-DAD demonstrated that there are qualitative and quantitative differences between the different varieties.

The evaluation of the antioxidant activity against the radicals 1,1-diphenyl-2-picryl-hydrazyl (DPPH<sup>•</sup>), superoxide (O<sub>2</sub><sup>•-</sup>) and nitric oxide (•NO) showed that the different *C. esculenta* varieties present great potential, being more effective than ascorbic acid against O<sub>2</sub><sup>•-</sup> (Fig. 1). The phenolic profile of the different varieties is directly related to the scavenging of DPPH<sup>•</sup>. Probably other compounds, not determined by us, are involved in the scavenging of O<sub>2</sub><sup>•-</sup> and •NO.



**Figure 1.** Antiradical activity of *C. esculenta* varieties against DPPH<sup>•</sup>, O<sub>2</sub><sup>•-</sup> and •NO. Values show percentage of mean ± SEM from three experiments performed in triplicate.

The wound repair property attributed to *C. esculenta* may be, at least partially, related to its capacity to scavenge superoxide radical.

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# Assessment of the Ecotourism Potential of Hiking trails in Castro Laboreiro

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Ecotourism can be a very important tool to protect natural areas. Through its educative role, environmental interpretation, local communities' involvement and the generation of revenues, ecotourism can act as a way to raise awareness about the urgency of protecting biodiversity and natural values in such remote areas <sup>[1]</sup> and promote sustainability in the local uses of the territory.

The Peneda-Gerês National Park (PNPG) presents itself as an ideal place for the development of ecotourism projects. Its biodiversity, ecological importance and cultural heritage support the promotion of ecotourism <sup>[2]</sup>.

Most of the scientific research directed to ecotourism tries to achieve the impacts on particular wildlife species and/or on particular sensitive habitat (ecological dimensions); while others want to optimize social-cultural impacts on rural communities (ethical dimensions) <sup>[3]</sup>. The study of ecotourism needs different approaches, with more biological, ecological, landscaping and cultural information linked as one, and studies that address all the four seasons. The main goal of this study is to apply an approach that proves to be useful in assessing ecological impacts of ecotourism projects.

In this study, a spatiotemporal approach has been used so it can be determined which trails offers better ecological, landscaping and cultural values and in which season. A biodiversity survey will be conducted in six predefined trails at the study area of Castro Laboreiro, covering the four seasons. Thereafter, biodiversity indices will be calculated, so we can achieve significant differences, not only between trails (spatial diversity) but also between seasons (temporal diversity). Cultural values will be added in the trails description, as geographical characteristics, topography profiles, and other relevant information.

The knowledge of local and temporal differences in biodiversity of a specific area can offer a new way to determine where and when ecotourism projects should be implemented, so it can function as a conservation tool for protected areas.

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# Authentication of Halal products by a species-specific PCR assay

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In the last few years authenticity of processed meat products has been a common problem. Meat species adulteration is a major problem as a result of fraudulent substitution of higher commercial value meat species by cheaper or lower quality species. Actually, Directive 2002/86/EC says that meat products should declare on their labels the present species and respective quantity. This is particularly important when involves consumers' practices like certain religions. Halal products are specifically made for Muslim people because they should obey to determined practices and should be free of pork meat or derived products. Thus, it is very important to be able to detect this species and verify the veracity of labels.

Different methods have been applied for the identification of meat species based on proteins analysis such as chromatographic, electrophoretic and enzyme-linked immunosorbent assays. Nevertheless, these methods are frequently less sensitive when used in the evaluation of thermally processed foods mainly because of alterations in the protein epitopes. More recently, methods based on DNA analysis have been reported to be more sensitive when thermally processed foods are analyzed [1]. Besides, the ubiquity in every type of cell and the higher stability of DNA when compared to proteins are advantages. Thereby, polymerase chain reaction (PCR) is considered a simple, fast, sensitive and highly specific alternative.

The aim of this work was to develop a highly sensitive species-specific PCR assay adequate for Halal food authentication. For method development and optimization, a set of binary mixtures as references was prepared by adding known amounts of pork meat to cow meat in the range of 0.0001 to 25%. To assess the effect of heat treatment a second set of mixtures were autoclaved. DNA was extracted using the Wizard method [2]. Yield and purity of extracts were assessed by UV spectrophotometry. The detection of pork species was based on the PCR amplification of a mitochondrial DNA fragment. The results showed that it was possible to detect pork meat down to the lowest tested value of 1 mg/kg (0.0001%) in raw and 100 mg/kg (0.01%) in thermally treated binary mixtures, indicating that sensitivity of the technique was affected with processing. Considering that several commercially available Halal foods are canned meat products, the proposed technique represents a useful tool for their authentication.

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## Binary mixtures with phenolic compounds and BHT -Synergistic or antagonistic antioxidant effects-

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Butylhydroxytoluene (BHT) is an organic synthetic compound that is primarily used as an antioxidant food additive, as well as in cosmetics, pharmaceuticals, rubber, petroleum products, and embalming fluid. Its main purpose as a food additive is to prevent fats from going rancid, and to preserve food smell, colour, and flavour [1].

Polyphenols are the most abundant antioxidants in the diet. These secondary metabolites of plants are generally involved in defence against ultraviolet radiation or aggression by pathogens. In food, they may contribute to the bitterness, astringency, colour, flavour, and oxidative stability [2].

Some foods contain polyphenols of several classes. Our objective was to evaluate if the addition of a synthetic antioxidant like BHT to these foods could potentiate the antioxidant effect of polyphenols.

The DPPH<sup>•</sup> scavenging activity of four different polyphenols (Fig. 1) was evaluated: caffeic acid representing hydroxycinnamic acids; quercetin and kaempferol (flavonols) and eriodictyol (flavanone) representative of one of the most important classes of plant polyphenols, the flavonoids. The antioxidant potential of binary mixtures containing BHT was also evaluated in order to check for synergistic or antagonistic interactions between the compounds.

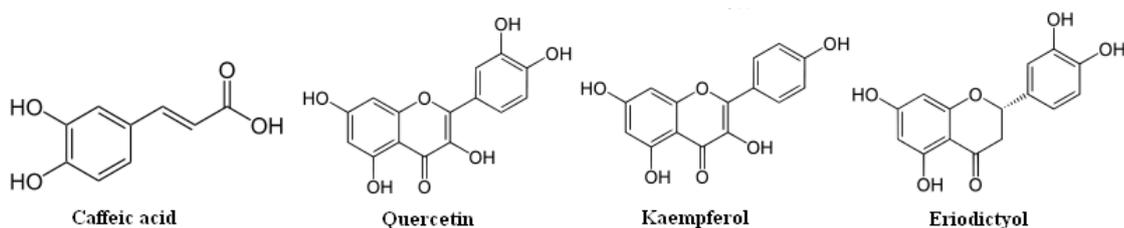


Figure 1 - Chemical structures of tested phenolic compounds.

The most active compound tested was quercetin. Kaempferol, which doesn't have the catechol group in B-ring, showed lower activity than quercetin. The double bond in C-ring is very important to the antioxidant activity and this explains the lower activity of eriodictyol when compared with quercetin. Caffeic acid was the less active compound.

Acknowledgements:

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# Black-billed Magpies (*Pica pica*) in an urban environment, the Oporto City Park

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The Black-billed Magpie, or simply the Magpie, is a CORVIDAE that occurs across much of the Northern Hemisphere [1]. In Portugal, it can be found in two main regions, the Western littoral area and the interior Alto Alentejo. The Magpie typically occurs in lowland with open natural or cultivated grasslands with some lightly wooded areas; populations spread in the last decades to urban environments due to Magpie adaptability and opportunism [1, 2].

In order to better understand Magpies distribution and behavior in an urban environment we selected the Oporto City Park as study site. The territory mapping method is used to obtain data on habitat use [3]. Video records are used to study Magpie behaviour (individual, socially and daily rhythms). For the characterization of Magpies in Portugal (sex ratio, age and biometric characteristics) [1, 2] an effort is directed to obtain carcasses of birds killed in hunting activities.

Magpies can be found in all the studied area, showing preferences for specific habitats during the day. Such preferences are related to their activities (sleeping, foraging or territory defense), but are also influenced the constant human presence in the park. Magpies were mostly observed alone or in pares; bigger magpies groups are less often (observed only once until now). These birds are always alert and spend a little time feeding each occasion they are in the ground, so tree-top-sitting and feeding are the most observed behaviors. Magpies top-sit for their own protection and to defend their territory; top-sitting is associated with natural or artificial objects.

Our results are preliminary but they confirm that the Black-billed Magpie is a quite generalist bird which easily adapts to an urban environment. It is also a quite artful bird, probably as result of previous human persecution. Human persecution may also be the reason behind the crescent aggressive behavior report in numerous papers, but never witnessed during our study.

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# Comparing the structure of benthic macroinvertebrate communities associated with *Corbicula fluminea* on the freshwater area of Lima and Minho estuaries

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The goal of this study was to compare the diversity and dynamics of the benthic macroinvertebrate assemblages of Minho and Lima estuaries associated to the non-native invasive species *Corbicula fluminea*. Because *C. fluminea* has shown different invasive behavior in these two estuaries<sup>[1][2]</sup>, this study may contribute to understand the factors that may influence the invasive behavior of this species in estuarine freshwater tidal areas contributing to find ways of controlling and mitigating the negative ecological and economic impacts of these bioinvasions. Sampling was carried out monthly from February to April 2012. In the laboratory, samples were sieved, the benthic macroinvertebrates collected were counted and identified to the lowest taxonomic level possible. The communities found in different sites were compared by a cluster analysis based on the Bray-Curtis similarity matrix and diversity indexes. Some differences between Minho and Lima benthic communities were found. The Minho river biodiversity was always generally greater than that of the Lima river. The population's density of *C. fluminea* was always higher in the Minho river, where it was in all months the most abundant species in all the sampling sites in good agreement with previous studies carried out by other authors. Thus, despite the several negative impacts of *C. fluminea*, including its advantageous competition with native bivalves that is believed to be one of the causes contributing to the severe decline of their populations in the Minho river estuary, its presence may benefit some species (e.g. by providing refuges, substrate, food, etc).

Keywords: *C. fluminea*, Minho and Lima estuaries, biodiversity, benthic communities

## Acknowledgments:

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# Culture optimization of the marine sponge *Hymeniacidon perlevis*, for toxicological studies: Evidences of the influence of luminosity on sponge and cyanobacteria associations

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Sponges are invertebrates known to produce bioactive compounds with pharmaceutical and toxicological relevance. Some of these compounds may result from associations between sponges and other organisms, being cyanobacteria one of the most relevant microorganisms. Thus, in this research it was intended to optimize techniques of culturing *Hymeniacidon perlevis* in laboratory, in order to study the association between this species and cyanobacteria, more precisely the influence of luminosity in this type of associations.

The species *Hymeniacidon perlevis* was selected because it is one of the most common intertidal sponge species of northern Portuguese coast and because it demonstrated a greater ability to adapt to laboratorial conditions. Two assays were performed, one to analyse the relevance of light intensity and another to study the influence of light privation, in the sponges as well as in their associated cyanobacteria. Initially, presence of cyanobacteria in sponges was detected through chlorophyll a and phycobiliproteins quantification<sup>1</sup>. After confirmation, it was used molecular techniques such as PCR (16S rRNA gene<sup>2</sup> (and sequencing) and of the phycocyanin operon<sup>3</sup>) and DGGE<sup>2</sup>, in the attempt to identify the cyanobacteria genera and their diversity.!

The obtained results suggest that an increase in the luminous intensity promotes the amount of cyanobacteria present in the specimens. The privation of light seems not to interfere in the amount of cyanobacteria associated with *Hymeniacidon perlevis*, during the study. The molecular analysis seems to point that the genus *Synechococcus* as the most frequent cyanobacteria in the analysed specimens.

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# ***Cystoseira tamariscifolia* (Hudson) Papenfuss ethyl acetate fraction: neuroprotective effects?**

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Due to the exhaustive study of terrestrial ecosystems in recent decades, researchers have changed their route of investigation into marine systems, known by their vast and unexplored biodiversity. Seaweeds, in particular, produce several metabolites as chemical defense and, therefore, their bioactivities need to be evaluated.

This study aimed to evaluate the neuroprotective effect of an ethyl acetate fraction of *Cystoseira tamariscifolia* (Hudson) Papenfuss (Phaeophyta) obtained from an aqueous extract. This fraction was tested against monoamine oxidase-A (MAO-A) and acetylcholinesterase (AChE), enzymes involved in depression and Alzheimer's disease, respectively. Its effect in the modulation of serotonin receptors (also implicated in depression) and GABA-benzodiazepine receptors (involved in epilepsy) was also assessed.

The results show that the ethyl acetate fraction did not inhibit the reuptake of serotonin using the [<sup>3</sup>H]citalopram binding assay and did not display any affinity for the benzodiazepine site of GABA<sub>A</sub> receptors using the <sup>3</sup>H-Ro 15-1788 (flumazenil) binding assay. Regarding the enzymatic assays, the extract showed moderate AChE inhibition (39% at 1.08 mg/mL) and a strong activity against MAO-A (IC<sub>50</sub> = 16 µg/mL, Fig. 1).

In order to correlate this *in vitro* antidepressant activity with the chemical composition, we proceeded to the GC-MS analysis of this fraction, which revealed mainly the presence of fatty acids, fatty acid derivatives and phloroglucinol.

These results showed that *C. tamariscifolia* is an important source of MAO-A inhibitors.

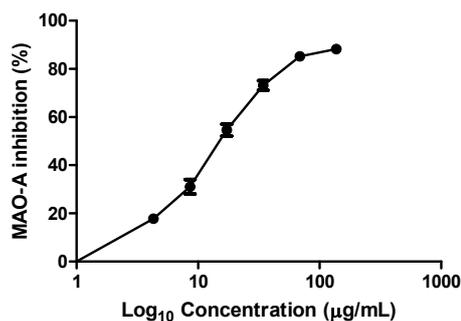


Fig. 1 – MAO-A inhibition activity of *C. tamariscifolia* ethyl acetate fraction.

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# Development of novel tools for *Cynara cardunculus* L. transformation and the study of protein intracellular trafficking

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***Cynara cardunculus* L. (cardoon)** is a flowering plant whose flowers are traditionally used in the manufacture of ewe's cheese in the Iberian Peninsula due to their milk clotting ability. This ability is a result of the presence of **cardosins** [1,4]: a family of plant aspartic proteinases (APs) which includes cardosins A and B, both present in large amounts in cardoon flowers. Despite being very similar, they share particular characteristics that allow their distinction: while cardosin A is essentially a vacuolar protein [2,4], cardosin B is mainly secreted to the extracellular matrix [3,4]. Cardosins have been considered a good model for the study of sorting and intracellular trafficking events given their specificities in terms of intracellular accumulation and distribution along development. However, little is known about cardosins trafficking in the native system mainly because of the lack of protocols for its transformation and long life cycle. Until now these studies have been made using heterologous systems (*Arabidopsis thaliana* and *Nicotiana tabacum*), given cardoon's constraints [4]. The main goal for this project is, therefore, the development of different methodologies to achieve transformation of cardoon cells, in order to map their endomembrane system and study these APs in their native system. To do this, we are currently testing 3 different transformation methodologies: Agrobacterium-mediated transformation of seedlings, cell suspensions and *calli*, either by co-culture, vacuum-infiltration and electroporation - using several fluorescent markers, and posteriorly chimeric fusions of cardosins with fluorescent proteins. Results will be analyzed through fluorescence and laser scanning confocal microscopy, as well as western blotting.

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# Ectoparasites of the Red Fox, *Vulpes vulpes*, in Portugal

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The Red Fox, *Vulpes vulpes*, is a common and widespread carnivore in Portugal. Foxes can carry and be affected by a variety of parasites, both internal (tapeworms, nematodes) and external (fleas, mites); they are also reservoirs of such parasites and may be vectors of diseases like rabies that can affect other wild animals [1, 2]. Direct contact and ensuing transmission is possible with our pets, in the countryside and in urban environments, as well as with man during hunting activities [3]. So it is of indisputable importance the knowledge of fox parasites and, in our case, of those that can be found in Portugal.

In order to evaluate ectoparasite diversity and to determine the prevalence and intensity of the most common parasites of the Red Fox we analyzed 148 foxes carcasses killed during the 2010-11 and 2011-12 hunting seasons in Portugal. The carcasses, offered by the hunters, were placed in plastic bags as soon as possible and frozen prior to their analysis. Ectoparasites were removed later (by combing foxes fur with a fine tooth comb) and preserved in alcohol. All parasites were identified and photographed.

Only half of the samples were infected but such a result is certainly an underestimate; in fact most carcasses probably were not placed in a bag immediately after the kill and ectoparasites are known to leave the carcasses as they cool down. Most of the parasited foxes carried ticks (83 %) but only 45 % carried fleas. Six foxes affected by sarcoptic mange were excluded from our analysis. The 266 ectoparasites identified included specimens of four tick (*Ixodes*, *Rhipicephalus*, *Dermacentor* and *Haemaphysalis*) and two flea (*Pulex* and *Ctenocephalides*) genus. *Ixodes* showed the highest prevalence (23.2 %) among ticks and *Pulex* fleas were the more prevalent (7 %). The very low intensity values of the two parasite groups (1.5 ticks and 2.5 fleas per fox) found are undoubtedly a consequence of parasite leaving the carcasses as they cool down.

Apart from biological and ecological issues, the identification of mange and of a number of anthropophil ectoparasite infestations (occurring at a synanthropic environment) may anticipate an increase of the rate of flea, tick and accidental mite infestations affecting pets and their owners, and enhances the potential transmission rate of vector-borne diseases.[4] Our results emphasize the need of further research to understand the dynamic of the Red Fox ectoparasite infestations and their health risks.

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# Effect of environmental variables on catch rates in three different bivalve grounds off the Portuguese coast

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The knowledge on the effects of environmental variability on marine production could be of great value if integrated into management of coastal fisheries. Understanding the effect of climatic and fishery at regional scale is therefore important for local management actions. Thus, the effect of environmental variables and fishing pressure on commercial catch rates of *Spisula solida* was studied in three areas off the Portuguese coast (Northwestern, Southwestern and South).

In Portugal dredge fleet target directly on *S. solida* clam along three distinct zones of the coast, each zone showed distinct environmental characteristics. Analysis of time series data using Dynamic Factor Analysis (DFA) included response variables such as annual landings per unit effort (LPUE), and explanatory variables such as annual and winter North Atlantic Oscillation (NAO) index, annual and seasonal sea surface temperature, annual and seasonal upwelling index, yearly and seasonal wind magnitude and direction and river discards.

Distinct sensitivity of *S. solida* populations to environmental variables was found between study areas. Variations in *S. solida* catches were determined by upper and lower environmental regional limits at a particular period (season). In the Northwestern, where annual temperature is on average lower than in other regions, increase in temperatures during spawning, larval development and settlement (winter-spring) increase stocks lag one year. In the Southwestern region, where high wind speed is observed, during spring (wind direction from South and Southeast) wind vectors were the dominant factors explaining catch variation, affecting larvae settlement in sediment. In the South, freshwater discharges are low comparatively to other regions, and during the winter (peak of discharge) river discards negatively affected *S. solida* catches, presumably by affecting the physiological condition of the species. Annual sea surface temperature in the South is consistently higher than that in other regions, and during summer had a deleterious effect on *S. solida* (pre-spawning phase, gametogenesis). Results show that “extreme” changes in environmental parameters operating seasonally between regions were the main causes of short-term variations in bivalve stocks. These regional differences result in different biological responses of the stocks by influencing life cycle processes. The magnitude of these impacts is determined by the “timing” of interaction between specific stages of the life cycle and local environmental conditions.

It is concluded that variations in commercial stocks of *S. solida* reflect mainly local climatic features off the coast (regional signature). However, “extreme” environmental regional changes at particular timings/seasons were the main drivers of short-term (lag 1 year) variations in the population dynamics of this bivalve. Consideration should be given to the role of local environmental pressures on *S. solida* populations when managing this commercially important resource.

# Effects of water acidification on the morphology of the green crab *Carcinus maenas* (Crustacea: Portunidae)

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Actual ocean water acidification is caused by an increase of atmospheric CO<sub>2</sub>. The dissolution of CO<sub>2</sub> in the seawater increases ion H<sup>+</sup> concentration, decreasing the pH level of the water. With this reaction there is a reduction of the carbonate available in the seawater, which limits the growth of calcifying organisms, such as the green crab, *Carcinus maenas* (Linnaeus, 1758) [1]. *Carcinus maenas* is a common littoral crab profusely found in Minho estuary. Like all crustaceans, *C. maenas* has to lose his calcified exoskeleton to grow in a process known as ecdysis or moulting and later form harden a new exoskeleton.

Knowing these facts, the aim of this project is to determine the effects of water acidification on the morphology and behavior of *C. maenas*, by comparing the carapace thickness of the exoskeleton left during the process of ecdysis (exuvia), between crabs growing in non-acidified water and animals growing in acidified water.

From a *C. maenas* population sampled in Minho estuary, 56 crabs were measured for carapace width, identified and placed individually in 4 closed circulation aquaria system kept at controlled temperature (20°C), salinity (28 PSU) and pH level (±8.0). The two consecutive moults of each animal were measured for carapace width and thickness after dried for several days at ambient temperature. This last parameter was measured at three points, and three times, because the carapace has an irregular surface.

Since the crabs take a long time to suffer two moults (minimum of about 10 months at the present conditions), so far we have the results of the group that grew and moulted in non-acidified water, hence being our “control group”. So, the next step is to repeat the same procedures with a set of crabs, from the same population, in acidified water (pH level of ±7,0) and compare the carapace thickness of the two consecutive moults for each animal and the difference in carapace thickness between the two pH conditions.

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## **Enzymatic and histological alterations in liver and gills of *Salmo trutta fario* after acetylsalicylic acid chronic exposure**

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The presence of pharmaceutical drugs in aquatic ecosystems has been widely reported. Aquatic organisms, namely fishes, in the presence of xenobiotics, display specific defense mechanisms, such as antioxidant enzymes and tecdular alterations. Acetylsalicylic acid, (ASA, the active substance of aspirin), is used in human medicine as an analgesic and antipyretic drug and shows also an activity in terms of platelet aggregation. In order to assess the ecotoxicological effects potentially elicited by ASA, in a freshwater animal model, brown trouts (*Salmo trutta fario*) were chronically exposed (28 days) to this chemical, in order to evaluate any enzymatic and histological damage caused by salicylic acid, in both gills and liver. The experiment was carried out under laboratory-controlled conditions, with 3 different salicylic acid concentrations (25, 50 and 100 µg/l). After the exposure period, the animals were sacrificed and dissected, and a portion of liver and gill tissues were removed for histological and enzymatic analysis. A qualitative and semi-qualitative evaluation of the liver and gills architecture were performed. Gills morphometry was also quantified. Oxidative stress was quantified trough the determination of glutathione S-transferases (GSTs), glutathione reductase (GRed), glutathione peroxidase (GPx) and Catalase (Cat) activities. The index of peroxidative damage (lipid peroxidation, TBARS) was also assessed. The here obtained data showed increasing levels of oxidative stress and histological changes, in both gills and livers of trouts, suggesting a close relationship with the levels of acetylsalicylic acid in water.

## Evaluation of adhesion and biofilm producing abilities of worldwide spread *Escherichia coli* A (ST10, ST23) and B1 (ST155, ST359) isolates

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Antibiotic resistant *Escherichia coli* isolates belonging to A and B1 phylogroups are increasingly recovered from extra intestinal human infections. Biofilm production might favour the persistence of these clonal groups. We aim to evaluate the ability of a diversity of isolates belonging to representative A and B1 *E. coli* (EC) clones from different settings to adhere on abiotic surfaces.

Fifty-one A (22 ST10, 14 ST23 complexes) and B1 (7 ST155 complex, 7 ST359) isolates from different countries (6 EU, 2 South American) were studied (1997-2008). They exhibited variable antibiotic resistance profiles (including ESBL or AmpC production) and were identified in nosocomial (52%) and community (12%) infections, healthy volunteers (14%) or animals (22%). Biofilm production was investigated by a modified quantitative assay. Adjusted bacterial cultures (0.5McF) were incubated in 96 well flat-bottomed polystyrene plates for 24h at 37°C. Adherent cells were washed with 1% PBS, stained with 2% crystal violet, washed with tap water and solubilised with 33% (v/v) glacial acetic acid. The optical density was measured in a spectrophotometer at 570 nm. *E. coli* strain CFT073 was used as positive control.

Isolates were classified as weakly adherent (n=22; 44%), moderately adherent (n=15, 30%) or strongly adherent (n=13, 26%). Moderately or strongly adherent strains were considered as presumptive biofilm producers. ST10 isolates were moderately adherent (n=9/22; 0.38<D.O<0.58), strongly adherent (n=8/22; 0.80<D.O<2.2) and less frequently weakly adherent (n=7/22; 0.18<D.O.<0.32). ST23 isolates were most frequently moderately adherent (n=7/14; 0.40<D.O.<0.64), weakly adherent (n=5/14; 0.24<D.O<0.32) and less frequently strong adherent (n=2/14; 1.64<D.O<2.7). ST155 isolates were weakly adherent (n=4/7; 0.21<O.D. <0.26) or strongly adherent (n=3/7;1.1<O.D.<1.9), whereas ST359 isolates were mostly weakly adherent (n=6/7; 0.18<O.D.<0.31) and less frequently moderately adherent (n=1; D.O.= 0.39).

Most of the isolates (n=28/50; 56%) were moderately or strongly adherent in the conditions tested. Higher adherence abilities and presumptive biofilm production was mostly observed among ST10 and ST23 *E. coli* isolates, which could explain their emergence as extra intestinal pathogens.

## *Ficus carica* L.: More than a fruit

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The regular consumption of fruits and vegetables is recommended by several nutritional studies for health protection. The fruit of *Ficus carica* L., commonly known as fig, is consumed in high amounts in our country and it has been traditionally used for its medicinal benefits, as laxative, cardiovascular, respiratory, antispasmodic and anti-inflammatory remedies [1].

For this study, aqueous lyophilized extracts of peels and pulps of three dark varieties of *F. carica* were prepared and analysed regarding their chemical composition and biological potential. Six phenolics compounds were identified by HPLC/DAD, quercetin-3-*O*-rutinoside being the main metabolite (Fig. 1).

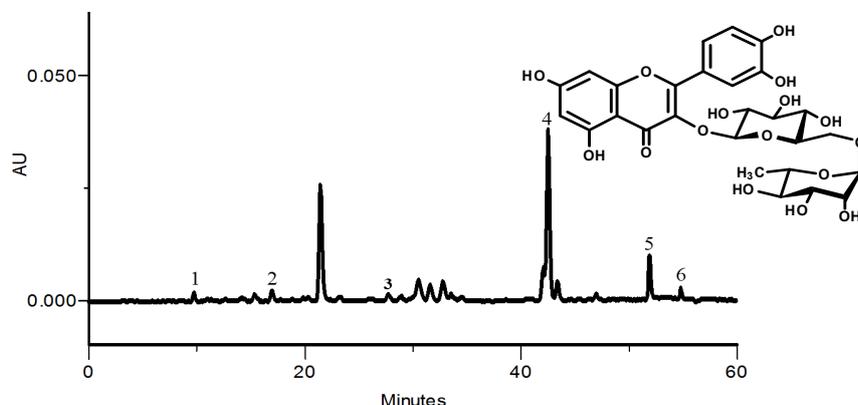


Fig. 1. HPLC-DAD phenolics profile of *F. carica* cv. "Preta Tradicional" peel and structure of the main compound. Detection at 350 nm. (1) 3-*O*-Caffeoylquinic acid; (2) 5-*O*-caffeoylquinic acid; (3) ferulic acid; (4) quercetin-3-*O*-rutinoside; (5) psoralen; (6) bergapten.

Six organic acids were determined by HPLC/UV. Fatty acids, sterols and triterpenes present in these extracts were determined by GC/MS, which enable the identification of 11 compounds. Stearic acid,  $\beta$ -sitosterol and lupeol acetate were the major compounds, respectively. GC/MS analysis allowed the identification of volatiles of different chemical classes.

The antioxidant potential was also checked against nitric oxide and superoxide radicals. Additionally, their capacity to inhibit  $\alpha$ -glucosidase and cholinesterases was evaluated. The fruits exhibited protective capacity only against nitric oxide ( $IC_{25}=596.4 \mu\text{g/mL}$ ) and  $\alpha$ -glucosidase ( $IC_{25}=2324.7 \mu\text{g/mL}$ ). Peels were always the most effective part, and this stronger activity may be related with the higher metabolites contents found in this material.

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# Flounder, *Platichthys flesus*, from the Baltic Sea: does genetics confirm the coexistence of two spawning ecotypes?

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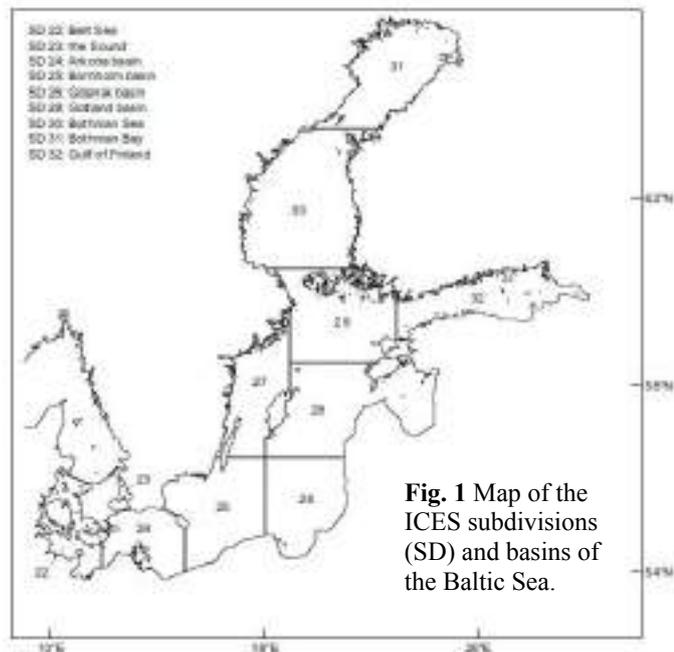
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In the Baltic Sea there are two different spawning ecotypes of European flounder, *Platichthys flesus*, one with denser demersal eggs and the other with pelagic eggs [1]. The first forms one distinct stock and may reproduce successfully far north as the Bothnian Sea (Fig. 1), up to the 6 psu isohaline. Opportunities for the pelagic eggs to obtain neutral buoyancy suggest that successful reproduction may occur in the southern basins. However, demersal eggs have been found in the Belt Sea (Fig. 1) (C. Petereit, pers. commun.). One consequence of egg density differences is that denser eggs are less buoyant and will end up near the seafloor, while less dense eggs will float and disperse more easily, originating different populations. Therefore, these two spawning ecotypes might be genetically distinguishable.

The purpose of this research is then to find if genetic diversity is associated with the Belt Sea *P. flesus*' eggs density. The flounder samples were obtained between February 2011 and April 2012 during both commercial and research campaigns in the area. A fraction of eggs were removed from live egg bearing females and their density tested. Another fraction of eggs, as well as finclips of the egg bearing females, was preserved in alcohol for later DNA analysis.

First, the best DNA extraction method was tested using Purelink<sup>®</sup> Genomic DNA kits, according to the Mammalian Tissue's protocol. At the moment, the best DNA amplification (PCR) methodology is under study to be applied to the eggs and finclips' samples. Genes to be sequenced will be COI, 18S and intron, with special interest on the last two which are less conservative. In the end, we expect to correlate the genetic diversity with the eggs' density.



**Fig. 1** Map of the ICES subdivisions (SD) and basins of the Baltic Sea.

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## A new inhibitor of p53-MDM2 interaction discovered using a yeast target-based screening approach

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The p53 tumour suppressor is a major regulator of cell proliferation and death. In tumours that retain a wild-type (wt) p53, the activity of this protein can be inhibited by the endogenous negative regulator MDM2. In this case, inhibitors of p53-MDM2 interaction have been considered promising drugs for cancer therapy [1]. In this work, yeast assays consisting of *Saccharomyces cerevisiae* cells co-expressing human wt p53 and MDM2 were used for the screening of inhibitors of p53-MDM2 interaction. In our previous work, it was shown that p53 induces yeast growth inhibition associated with S-phase cell cycle arrest [2], which is significantly reduced by MDM2. Moreover, it was shown that inhibitors of p53-MDM2 interaction, as Nutlin-3A, revert the inhibitory effect of MDM2 on p53-induced growth inhibition/cell cycle arrest. A chemical library of small-molecules synthesised by Santos's team was tested, and the small molecule NAP6 was identified as inhibitor of p53-MDM2 interaction. The molecular mechanism of action of NAP6 was validated in two human tumour cell lines derived from breast cancer (MCF7) and colon carcinoma (HCT116 p53<sup>+/+</sup>) by performing gene reporter assays and by analysis of p53, p21 and MDM2 protein levels as well as caspase activation by Western blot. The results obtained confirmed that 10  $\mu$ M NAP6 stimulated p53-dependent transcriptional activity, led to p53 protein stabilization, increased p21 and MDM2 protein levels, and induced caspase-7 activation in tumour cells. Notably, these effects were not observed in the HCT116 p53<sup>-/-</sup> derivative cell line.

In conclusion, using the established yeast screening approach a potential inhibitor of p53-MDM2 interaction was identified. Further studies in human tumour cells are underway to confirm the molecular mechanism of NAP6.

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# Alzheimer disease: development of an immunosensor for biomarker detection

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Alzheimer disease (AD) is a neurodegenerative pathology characterized by extracellular deposits of amyloid  $\beta$  ( $A\beta$ ) peptide (senile plaques), intracellular appearance of tau protein in neurofibrillary tangles and neuronal loss [1]. Since this disease is very difficult to diagnose, it is clinically important to find and detect accurately biomarkers for AD by new non-invasive methods that may facilitate the diagnostic process and identify patients at an earlier stage [1]. Thus, the aim of the proposed research is to develop a sensitive immunosensor for the detection of the main AD biomarker, i.e amyloid beta-peptide, in biological fluids.

Cyclic voltammetry (CV) and square-wave voltammetry (SWV) assays were performed using an Autolab electrochemical system (Eco Chemie, The Netherlands) equipped with PGSTAT-30 and GPES software. The electrochemical cell was assembled with a conventional three-electrode system: the developed biosensor as the working electrode, an Ag/AgCl/ KCl (3.0 mol/L) reference electrode, and a rod of glassy carbon as counter electrode. All experiments were performed at room temperature.

Several self-assembled monolayers (SAM) and experimental conditions, to modify the gold electrode, were tested, namely, cystamine, cystamine+mercaptoethanol, mercaptopropionic acid and mercaptopropionic acid+mercaptoethanol at different concentrations and immersion times. The best results were attained using 5 mmol/L mercaptopropionic acid and a time of immersion of 2 h. Gold nanoparticles (AuNPs) were synthesized by the Turkevich method. Hydrodynamic size and potential zeta values were characterized by dynamic light scattering and laser doppler velocimetry, respectively, using a Zetasizer Nano ZS (Malvern, UK), at 25°C. AuNPs exhibited a 37 nm mean hydrodynamic diameter and a -38 mV zeta potential. Electrochemical deposition of the AuNPs on the previously SAM modified electrode was carried out at - 0.2 V for 200 s. The deposition of the SAM and the AuNPs was evaluated by square-wave voltammetry using  $Fe(CN)_6^{3-/4-}$  as electroactive indicator. Different methods of immobilization of bioreceptor (antibody) will be tested and optimization of the immunosensor for the target biomarker will be performed.

Acknowledgements:

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# **$\alpha$ -Mangostin and gambogic acid: putative inhibitors of p53-MDM2 interaction revealed by yeast**

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In half of all human cancers, the wild-type (wt) p53 tumour suppression protein is inactivated due to the overexpression of endogenous negative regulators, such as MDM2. The reactivation of wt p53 activity by small-molecule inhibitors of the p53 interaction with MDM2 therefore represents a promising therapeutic strategy against these tumours [1,2].

Due to the high complexity of the p53 pathway in mammalian cells, yeast cell-based assays have been developed for a simpler and faster screening of inhibitors of p53-MDM2 interaction. As in mammalian cells [1], MDM2 significantly reduced the p53-induced yeast growth inhibition/cell cycle arrest, as well as the p53-dependent transcriptional activity in a yeast reporter gene assay (described in [3]). By testing a known inhibitor of p53-MDM2 interaction (Nutlin-3A), these yeast cell-based assays were validated for the screening of small-molecule inhibitors of this interaction. Using this yeast approach, the activity of two natural compounds,  $\alpha$ -mangostin and gambogic acid, was evaluated on p53-MDM2 interaction. The results obtained showed that both  $\alpha$ -mangostin and gambogic acid reduced the negative effect of MDM2 on p53-induced growth inhibition/S-phase cell cycle arrest, as well as on p53-dependent transcriptional activity in yeast.

In conclusion, using the yeast approach,  $\alpha$ -mangostin and gambogic acid were identified as putative inhibitors of p53-MDM2 interaction. This work provides new insights concerning the molecular basis of activity for these two compounds.

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## Antifungal activity and mechanism of action of synthetic compounds

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Fungal infections have been growing in the last decades. In our days, fungi represent a significant proportion of the etiological agents of serious infections especially in immunocompromised patients and they are responsible for high mortality and morbidity rates in hospitals [1]. Among the pathogenic species to humans, *Candida albicans* and *Aspergillus fumigatus* deserve our especial attention [2]. The existence of different available therapeutic alternatives in mycosis treatment has been changing the course of superficial and systemic fungal infections along the years. However, many of these therapies are associated with toxicity, fungistatic activity, poor bioavailability, and emerging resistance, which limit their use [3]. Hence, investing in the search for new compounds with antifungal activity to overcome the problems of the current therapy is a real and emergent need.

The antifungal activity of sixteen compounds, Ag1 to Ag16, obtained by chemical synthesis, was evaluated in eight strains of *Candida*, according to the reference method for microdilution antifungal susceptibility testing, approved by CLSI [4]. Antifungal activity was observed in seven of tested compounds, although the lowest MIC values were found for Ag1 and Ag3. These two compounds were also able to inhibit dimorphic transition, ergosterol biosynthesis and mitochondrial activity in *Candida albicans*, at the tested concentrations. In summary, Ag1 and Ag3 have potential as antifungal agents, being Ag1 the compound which revealed a broad spectrum of activity extended to all tested *Candida* strains.

Nevertheless, several additional studies are needed to enable them to become part of the set of antifungal alternatives in the future. The research of active molecules through chemical synthesis and their molecular optimization for activity improvement seems to be an important and necessary mean for the development of new drugs.

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## **Antihypertensive drugs and bone metabolism: *in vitro* study with human osteoclastic cells**

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Bone is a dynamic tissue that is in constant remodeling, by the action of the bone-resorbing osteoclasts and the bone-synthesing osteoblasts. It has been suggested that some antihypertensive agents might be associated to alterations in bone metabolism, but their effects on human bone cells remain to be elucidated. In this work, the effects of five calcium channel blockers, a class of antihypertensive drugs (AHDs), were investigated on osteoclastic differentiation.

Osteoclastic cell cultures were established from precursor cells isolated from human peripheral blood, and were maintained in the absence (control) or in the presence of  $10^{-8}$ - $10^{-4}$  M of different AHDs (amlodipine, felodipine, diltiazem, lercanidipine and nifedipine). Cell cultures were characterized throughout a 21 day period for tartrate-resistant acid phosphatase (TRAP) activity, number of TRAP+ multinucleated cells, presence of cells with actin rings and expressing vitronectin and calcitonin receptors, and apoptosis rate. Also, the involvement of several signaling pathways on the cellular response was addressed.

The tested AHDs revealed different abilities to modulate osteoclastogenesis. At low doses, amlodipine and felodipine caused an increase on osteoclastic differentiation, while the other drugs inhibited it. The different AHDs also differed on their effects on the tested signaling pathways.

In conclusion, AHDs seemed to cause a direct effect on human osteoclastic differentiation. Interestingly some of them increased while others inhibited the process. Unraveling the mechanisms beneath these observations might help to explain the adverse effects on bone tissue described for this drug class.

## Antimicrobial resistant bacteria in ready to eat “sushi” samples

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Food safety and food production are two major questions in actual societies. Bacterial resistance to antibiotics is a growing medical problem and food might play an important role in bacterial resistance spread. New nutritional imported habits might also play a role in fecal colonization of population. “Sushi” is recognized as a nutritive valuable way of preparing food with many adherents in our country, in recent years. Bacteria found in food not cooked (with no heating treatment) might represent a risk in terms of food safety in terms of pathogenic and antibiotic resistance threats. The quality of freshly prepared sushi strongly depends on the skills and habits of the preparation cooks, which may vary [1].

The aim of our work was the detection of antimicrobial resistant Gram negative bacteria, namely extended-spectrum producing (ESBL) coliforms. Three sushi meals purchased in different take away settings in the North of Portugal were analyzed. Different portions, crude butter fish, salmon, boiled rice and other constituents of the different meals were suspended separately in Tryptic Soy Broth (TSB) and incubated at 37° overnight. Isolates were selected by spreading 100 µl of broth on MacConkey agar with and without oxiiimino-beta-lactams. Colonies were randomly selected and susceptibility to antimicrobial agents was determined by the agar diffusion test, according to the Clinical Laboratory Standards Institute (CLSI) and screened for ESBL production by the double disc synergy test and clavulanic acid addiction to oximinobeta-lactam disks, according to the CLSI guidelines. Identification of the selected strains was achieved by ID 32 GN and API 20 E.

One sample obtained from a take away sushi restaurant, showed relevant results considering deficient hygiene and antimicrobial resistance. We detected ESBL producing *E.coli* from shrimp and surimi.

Microbiological quality of freshly prepared sushi in sushi bars take away might depend on the cooker skills and on the quality of food products used for preparation. Detection of ESBL producing *E.coli* is a worrying question in terms of population colonization and alerts for the hypothesis of contamination during food processing.

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# Antimicrobial Resistant Coliform Bacteria on River Douro Beach Sands

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Bacterial resistance to antibiotics is a growing medical and ecological problem, in which environmental bacteria may play an important role as reservoir of antibiotic resistance [1]. Bacteria found in beach sands, are often overlooked as a site where sensitive individuals can contact with pathogenic bacteria.

The aim of our study was the detection of antimicrobial resistant bacteria in sand samples, focusing on Extended Spectrum Beta-Lactamase (ESBL) producing coliforms.

Dry and wet sand samples were collected along three different points of river Douro, were suspended in Tryptic Soy Broth (TSB) and incubated overnight at 37°C. Isolates were selected by spreading 100 µl of broth on MacConkey agar and on MacConkey agar with ceftazidime, cefotaxime, aztreonam, and meropenem. At the same time, the samples were also suspended in sterilized water and the supernatant was submitted to a ramp vacuum filtration, followed by placement of the filters in the same medium as used for the TSB samples. Colonies were randomly selected and susceptibility to antimicrobial agents was determined by the agar diffusion test, according to the Clinical Laboratory Standards Institute (CLSI) and screened for extended-spectrum beta-lactamase (ESBL) production by the double disc synergy test and clavulanic acid addition, according to the CLSI guidelines. Identification of the selected strains was achieved by ID 32 GN and API 20 E.

Our results showed that bacteria were preferentially recovered from wet samples showing influence of water or sediments in their presence in the samples. ESBL producing *Escherichia coli* isolates were found in different samples.

In view of these results and considering that beach visitors tend to spend most of the time in contact with the sand, the presence of these bacteria might represent a risk to their health. This might represent a threat in terms of antibiotic resistant isolates environmental dissemination with consequences in human colonization and risk of infection, important in terms of public health and environmental protection. This might represent a relevant contribution for human colonization and community spread of ESBL producing *E.coli*.

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## Assessment of ability of drugs to prevent protein oxidative damage

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Drugs that scavenge reactive oxygen species (ROS) and inhibit the protein and lipid peroxidation have beneficial clinical effects in the prevention and/or treatment of several oxidative-stress diseases. In fact, the current therapeutic strategies are based on the design of multifunctional drug candidates that are able to interact with multiple disease related targets. Nevertheless, the scavenging capacity of drugs against ROS has not been adequately investigated because the analytical methods are based on the scavenging of stable non-biological radicals (e.g., DPPH<sup>•</sup> and ABTS<sup>•+</sup>) and/or on competitive assays that use target molecules (e.g., fluorescein in ORAC assay) that do not represent any biomolecule found in living organisms [1].

Therefore, the main aim of the present work is to determine the scavenging capacity of drugs by an analytical methodology that apply reactive species (peroxyl radicals) found at biological milieu and biotargets (endogenous protein) in reaction conditions (pH, temperature) similar to those found in vivo [2]. To achieve these goals, myoglobin was selected as protein model, while peroxyl radicals were generated from thermo-decomposition of 2,2'-azobis (2-methylpropionamidine) dihydrochloride (AAPH). The changes in protein structure induced by peroxidation were monitored spectrophotometrically by the absorbance decrease at 409 nm. Drugs that have the ability to prevent protein peroxidation caused a delay of absorbance decrease and their scavenging capacity was assessed by the increase of the area under curve (AUC). Several drugs used for cardiovascular pathologies, such as  $\beta$ -blockers (atenolol, labetalol, propranolol) and statins (pravastatin, fluvastatin, lovastatin and simvastatin), were tested and their ability to prevent protein peroxidation was compared to that obtained with endogenous antioxidants (glutathione, uric acid and taurine). Among the drugs tested, labetalol and fluvastatin were those that presented higher ability to prevent protein oxidative damage, indicating that their clinical effects may be potentiated by this pleiotropic effect.

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# Automatic quantification of cell outgrowth from neurospheres

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**Introduction:** This study was aimed at developing an automatic approach to quantify cell outgrowth from neurospheres. For this purpose, neurospheres were seeded on surfaces physisorbed with two different laminin isoforms, and cell outgrowth from neurospheres quantified. The automatic approach developed is expected to be an important tool for the screening of novel synthetic peptides in terms of ability to mediate neural stem migration, ultimately contributing for the design of more efficient polymeric vehicles for the transplantation of neural progenitors.

**Methods:** Neural progenitors were derived from a mouse ES cell line (46C), under adherent monoculture and in serum-free RHB-A medium. Neural progenitors (>95% Sox1<sup>+</sup>) were cultured under suspension conditions in the presence of basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) to induce the formation of neurospheres. Neurospheres (150-200 µm in diameter) were isolated and seeded at 4 neurospheres/cm<sup>2</sup> on [(Poly-D-lysine)-(Laminin 111)]-coated coverslips (PDL-LN 111) or on TCPS wells coated with recombinant human laminin 511 (LN 511). After 24h of cell culture in RHB-A medium supplemented with bFGF, the samples were fixed, permeabilized, and incubated with DAPI for DNA labelling. Samples were finally mounted with Fluoromount™ and observed under phase and fluorescent microscopy (Zeiss AXiovert 200M, Carl Zeiss). The neurosphere and outgrowth boundaries were estimated using fluorescence and phase images, respectively, using Matlab. To estimate the neurosphere boundary, the fluorescence image was filtered with a Gaussian filter and then segmented using automatic Otsu thresholding. The largest segmented region was considered to be the neurosphere, allowing the definition of its boundary. The outgrowth boundary was obtained based on the phase symmetry algorithm. Phase symmetry and phase orientation images were obtained and non-maximum suppression was carried out. Then, two sequential dilations followed by one erosion allowed the detection of neurosphere outgrowth, defining its area and boundary. The ratio between the outgrowth area and the neurosphere area (areas ratio) was computed, and the distance between each outgrowth boundary point and the neurosphere boundary was assessed, enabling maximal and average outgrowth distance computation.

**Results:** Neurospheres seeded on LN 511-coated surfaces showed significantly higher maximal and average outgrowth distance than neurospheres seeded on PDL-LN 111-coated coverslips (p<0,0001). Significantly higher areas ratio was also found for LN 511-coated surfaces as compared to neurospheres seeded on PDL-LN 111-coated coverslips (p<0,013). Results showed that LN 511-coated surfaces are more permissive to migratory outgrowth as compared to PDL-LN 111-coated coverslips. By making possible the detection of low-contrasted morphological features, the developed approach enabled the quantitative assessment of cell outgrowth from neurospheres in samples merely processed for DNA fluorescent staining. Its measures are in high agreement with the ones obtained manually and the image analysis time was reduced in more than 95%.

**Conclusions:** The automatic approach developed can be used as an alternative to manual measures which are time consuming and prone to human errors and subjectivity.

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# Calcium and magnesium in human brain: age-related changes and anatomic region specific differences

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Calcium, directly or indirectly, regulates several biological processes in the central nervous system (CNS) including intracellular signaling, neuronal gene expression and neuronal secretion of neurotransmitters into synapses. Magnesium also plays important roles in brain function regulating processes such as energy production, synthesis of essential molecules, cell signaling and ion transport across cell membranes. Changes in calcium and magnesium homeostasis in the brain may contribute to the cognitive decline associated with normal ageing and possibly to the development of neurodegenerative disorders. For a better understanding of the calcium and magnesium age-related changes and its role in the brain diseases mechanisms, it is essential to clarify their normal distribution and accumulation within the human brain. The main objectives of this work were to study: 1) the regional anatomic differences of calcium and magnesium levels within the brain, 2) the changes on calcium and magnesium levels in human brain in relation to age and 3) the differences in calcium and magnesium levels between individuals with and without evidence of ND.

From each neurologically and psychiatrically healthy individual submitted to autopsy ( $n=44$ ) the following 14 areas were sampled: (1) frontal cortex; (2A) superior and (2B) middle temporal; (3A) caudate nucleus, (3B) putamen, (3C) globus pallidus; (4) cingulated gyrus; (5) hippocampus; (6) inferior parietal lobule; (7) occipital lobe; (8) midbrain; (9) pons; (10) medulla; and (11) cerebellum. Samples from individuals with previous diagnosis of Alzheimer's ( $n=2$ ) and Parkinson's disease ( $n=1$ ) were also collected. After microwave-assisted acid digestion of samples, calcium and magnesium were quantified by Flame Atomic Absorption Spectroscopy.

Results showed that, considering the whole brain, magnesium (mean  $\pm$  sd:  $517 \pm 44$   $\mu\text{g/g}$ ) is more abundant than calcium ( $211 \pm 52$   $\mu\text{g/g}$ ). It was found that calcium distribution within brain tissue is not homogeneous: highest levels were found in frontal cortex ( $289 \pm 110$   $\mu\text{g/g}$ ), occipital lobe ( $276 \pm 157$   $\mu\text{g/g}$ ) and parietal lobule ( $272 \pm 160$   $\mu\text{g/g}$ ), while the lowest levels were found in medulla ( $147 \pm 71$   $\mu\text{g/g}$ ), cerebellum ( $150 \pm 37$   $\mu\text{g/g}$ ) and pons ( $165 \pm 78$   $\mu\text{g/g}$ ). It was observed a tendency for calcium levels increase with age in majority of studied brain regions. On the contrary, magnesium has a quite homogeneous distribution and remains quite unchanged with advanced age. Calcium and magnesium homeostasis seems to be unaffected in studied individuals with neurodegenerative diseases.

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## ***Cecropia catarinensis*: A plant with antitumoral potencial**

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The *Cecropia* genus is distributed throughout Latin America being frequently used in traditional medicine as diuretic, hypoglycemic and to treat asthma. Studies showed for different species of *Cecropia* some other activities such as antimalarial [1], antidepressant-like [2]. In this study our main goal was the chemical and biological studies in order to verify the antitumoral potential of *C. catarinensis* (synonym *C. pachystachya*). The crude methanolic extract (CME) was obtained by exhaustive extraction with methanol of *C. catarinensis* leaves, collected in Brazil. The CME purification by extraction resulted in two fractions: F1 (CH<sub>2</sub>Cl<sub>2</sub> and AcOEt) and F2 (MeOH). Samples of CME, F1 and F2 were evaluated for their effect on the *in vitro* growth of two cell lines namely, breast adenocarcinoma (MCF-7) and non-small cell lung cancer (NCI-H460), using the procedure followed by the National Cancer Institute (SRB assay). Preliminary results showed that CME was active for the two cell lines tested, being more active for the breast cancer cell line presenting values of GI<sub>50</sub>= 58.8 µg/ml. An interesting activity was observed for F1 being the best compound tested presenting values inferior to 50 µg/ml (MCF-7; GI<sub>50</sub>= 31.8 µg/ml, NCI-H460; GI<sub>50</sub>= 36.1 µg/ml). F2 was also active for MCF-7 (GI<sub>50</sub>= 80.9 µg/ml) and NCI-H460 (GI<sub>50</sub>= 85.4 µg/ml). F1 was purified by column chromatography and resulted in 14 fractions (G1-G14). The purification of G3, by column chromatography, led to the isolation of compound C. Preliminary structural data concerning the compound C indicates the presence of a steroidal scaffold being the chemical details under study. To the best of our knowledge we are the first team to study the antitumor potential of *C. catarinensis* for MCF-7, NCI-H460 and A375-C5 tumor cell lines.

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## ***Cecropia catarinensis*: a plant with biological properties against *Aedes aegypti***

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Dengue fever, a tropical disease caused by an arbovirus that account annually for several million cases and deaths worldwide, is transmitted by *Aedes (Stegomyia) aegypti* L., 1762 (Diptera: Culicidae)[1]. Natural products have played a dominant role in the development of new drugs. Our main goal was the evaluation of *Cecropia catharinensis* larvicidal potential against *A. aegypti*. The crude methanolic extract (CME) was obtained by exhaustive extraction with methanol of *C. catarinensis* leaves, collected in Brazil. The CME purification by extraction resulted in two fractions: F1 (CH<sub>2</sub>Cl<sub>2</sub> and AcOEt) and F2 (MeOH). CME and F1 were dissolved in DMSO and diluted at 1:3 in NaCl at final concentrations of 10-50µg /mL. *A. aegypti* eggs were obtained from Vector Research and Support Center/ NapVe/IOC, FIOCRUZ, RJ. The bioassays were performed at Vector Insect Laboratory/USS. The substances were applied in glass containers, which contained mineral water (20 mL) at 10, 30 and 50 µg/mL concentrations. Larvae were used (n=20) at the third-instar (L3) per group test. Control (untreated solution) and testimony control (DMSO solution without CME and F1) were also used in this study. After treatment, the larvae were maintained on normal diet in climatic chambers (BOD) at 27 ± 1° C and 70 ± 10% RH observed for 35 days, concerning the development and mortality. The results were analyzed using the Tukey test. CME interfered on the period of pupal development (days) (p<0,001) while F1 interfered on the period of larval (p<0,001), pupal (p<0,001) and L3-adult (p<0,001) development period. CME caused 8% pupae mortality (50 µg/mL) while F1 caused 25% pupae mortality. The data suggest that *C. catharinensis* can act on the lifecycle of the mosquito. The experiments are still in progress.

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# Chemical and *in vitro* studies of growth inhibitory effects on human cancer cell lines of an extract of the marine fungus *Neosartorya spinosa*

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Natural products are the major source of novel structures that lead to anti-cancer drugs [1] namely marine natural products [2]. Considering marine-sponge associated fungi, the production of bioactive compounds with antitumor properties is very promising [1,3,4]. The aims of this work were the chemical and biological studies of the crude extract of the marine-sponge associated fungus *Neosartorya spinosa*. The *N. spinosa* was isolated from the sponge *Rhiabdermia* sp. collected from Mu Ko Similan, Pang-Nga Province, in the Andaman Sea, Thailand. The ethyl acetate extract of *N. spinosa* was treated with water (EAE-NS, 24.5 g) resulting in two fractions, the soluble (EAE-NS-1) and the insoluble one (EAE-NS-2). The fraction EAE-NS-2 was purified by column chromatography, using silica gel (100 x 4 cm; 350 g of silica). Four hundred fractions (200 mL) were collected and joined in 17 groups, according to the chromatographic behavior. The purification of group 7 and 9, using chromatography and crystallization, allowed the isolation of compounds 1, 2 and 3. The structure of compound 1 was established by NMR as Aszonalenin. The crude extract (EAE-NS) and compound 1 were evaluated by the SRB assay in order to study the growth inhibitory activity on three human tumor cell lines: breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and melanoma (A375-C5). Compound 1 [ $GI_{50} = 39.1 \mu\text{M}$  (MCF-7);  $33.2 \mu\text{M}$  (NCI-H460);  $27.1 \mu\text{M}$  (A375-C5)], as well the crude extract [ $GI_{50} = 55.7 \mu\text{g/mL}$  (MCF-7);  $60.3 \mu\text{g/mL}$  (NCI-H460);  $89.0 \mu\text{g/mL}$  (A375-C5)] were active in all three cell lines. The compounds 2 and 3 are under NMR analysis. This marine-sponge associated fungus needs further investigations, underlining that we are the first team studying its chemical composition and the antitumor potential.

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## Cinnamic acid conjugates of primaquine and chloroquine active against *Leishmania infantum*

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The management of life threatening visceral leishmaniasis (VL) depends principally on treatment with chemotherapeutics. However, the treatment of this infection is hindered by the limited efficacy, high costs, low bioavailability, high toxicity and parasite resistance to the action of many of the currently available drugs. Therefore, it is mandatory to develop new compounds without these disadvantages.

We have recently tested, as potential anti-leishmanials, cinnamic acid conjugates of primaquine (1) and chloroquine (3) recently reported as interesting antimalarial leads [1,2]. Primaquine and chloroquine are, respectively, 8- and 4-aminoquinolines with proven activity against diverse protozoans. Primaquine derivatives were obtained by coupling the parent drug's aliphatic amine to distinct cinnamic acids substituted at the aromatic ring (2) [1]. Similarly, the chloroquine heteroaromatic group was linked to differently substituted cinnamoyl groups through a flexible aminobutyl spacer (4) [2].

In the present work, we evaluated the activity of these derivatives of primaquine and chloroquine against both the promastigote and the intramacrophagic amastigote stages of *L. infantum*, the European agent of VL. We found that chloroquine and its derivatives were highly potent against the promastigote stage, being more active than primaquine and its derivatives and than the reference drug miltefosine. We also established that chloroquine derivatives had comparable or superior activity to miltefosine (IC<sub>50</sub> = 5.9 μM), when tested against the intramacrophagic amastigote stage. Notably, chloroquine and most of its derivatives did not exhibit cytotoxicity against bone marrow-derived macrophages.

Cinnamic acid conjugates of chloroquine display potent activity against *L. infantum*, serving as leads for the development of more effective and safer anti-leishmanials.

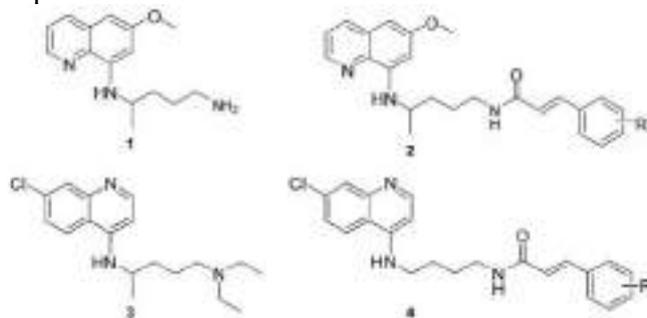


Fig. 1 – Chemical structures of primaquine (1), chloroquine (3) and their derivatives conjugated to distinct cinnamic acids substituted at the aromatic ring (R), respectively (2 and 4).

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# Ciprofloxacin resistant coliforms in the fecal flora of bovines

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The rate of community dissemination of antimicrobial resistant *E.coli* might suggest the occurrence of environmental reservoirs potentially leading to human contamination through water, food consumption or direct contact with animals. There are descriptions of fecal carriage of such organisms among food-producing animals especially poultry and pigs. The reports concerning livestock cattle are less abundant. There might be a cross-transmission between humans and animals or a common environmental source of human and animal contaminations. Meat can be a source of resistant bacteria, which could be spread through the food chain [1].

The aim of our study was the detection ciprofloxacin resistant coliforms in bovine fecal flora.

We studied five animals from two different producing facilities in the North of Portugal.

Four animals were from a production of thirty animals fed by corn, straw and industrially prepared animal feedlot. Another one was from a little facility fed by corn and grass.

Fecal samples were suspended in Tryptic Soy Broth (TSB) and incubated at 37° overnight. Isolates were selected by spreading 100 l of broth on MacConkey agar with and without ciprofloxacin. Colonies of lactose fermenters were randomly selected and susceptibility to antimicrobial agents was determined by the agar diffusion test, according to the Clinical Laboratory Standards Institute (CLSI). Identification of the selected strains was achieved by ID 32 GN and API 20 E.

We found ciprofloxacin resistant *E.coli* isolates in the fecal flora of all the studied animals. Ciprofloxacin resistance was associated to resistance to tetracycline. Fluoroquinolones are commonly used in veterinary treatment, suggesting a potential for selection of ciprofloxacin resistant coliforms in the fecal flora of production animals. This reality might contribute for fecal colonization of humans by those isolates, with the consequent risks in terms of infection by these opportunistic multi-resistant pathogens.

These results suggest that meat can be a source of resistant bacteria, which could potentially be spread to the community through the food chain.

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## Co-expression network analysis in preeclampsia vs normal pregnancy: looking for target genes

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Preeclampsia has been studied for many years, but until now the underlying mechanisms remain unknown. Similarly, it has not been identified a predictive diagnostic marker or a treatment effective to prolong the fetus and/or mother's wellbeing. The objective of this study is to identify groups of genes responsible for the differential expression between normal (N) and preeclampsia (PRE) through the analysis of the co-expression network obtained by microarray experiments.

We selected five projects from GEO database comprising 123 and 104 microarrays from PRE and N pregnant women, respectively. The microarray results correspond with placenta tissue analysis obtained at delivery. We mapped the probe arrays to the respective Entrez gene ID by manual observation and also using the updated manufacture annotation information for all the platforms. Only those common genes to all platforms were used in the subsequent steps (6816 genes).

Genes with more than one probe were combined by averaging of the intensity values using *collapseRows* and *intersect* the functions available in WGCNA package. The second normalization was performed in order to re-scale the intensity and also remove the cross-platform batch effects using a *Combat* function in SVA package. For the identification of statistically significant gene differentiated between N and PRE groups we used *lmFit* from Limma R-Package and only genes with p-value  $\leq 0.05$  (n=1146 genes). Genes differentiated between Normal and PRE groups were used to construct the network gene co-expression weighted in each group using WGCNA package. Network modules were detected using Dynamic Tree Cut algorithm. We also compared the modules obtained in each network (CoN and CoP), using the Fisher exact test, were we basically analyze the number of commons genes between modules, therefore, the modules (between both conditions), with an increased number of shared genes tend to be more unspecific.

Some network modules of co-expression were identified significantly related with gene differences in expression and poorly overlapped between N and PRE conditions. These module also revealed a highly centrality and were significantly related to angiogenesis, blood flow and inflammatory response. On the other hand, some well-known PRE related genes were also identified as ENG, FLT1, INHA, while others like, TPBG, NDRG1, were also involved in the same modules but remain little explored or unknown during pregnancy, suggesting that further experimental studies need to be done.

# Commercial smoothies containing fruit and fermented milk: stability of quality attributes during storage

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There is an expanding demand of consumers for natural food products that are fresh or minimally processed and convenient [1]. Smoothies are blended beverages containing fruits, fruit purées, pulps and/or fruit juices, yoghurt or milk that preserve the desirable characteristics of fruits [2]. The effect of processing and storage on commercial smoothies could affect quality attributes, like color and acidity, important parameters for the consumer acceptability of the product [3].

The aim of this work was to analyze the physical and chemical properties associated with quality attributes, in different smoothie formulations available in the Portuguese market ( $n=16$ ). Samples were divided into five groups, according to composition. The total titratable acidity, pH value and soluble solid content were determined using standard methods (NP EN 12147:1999, NP EN 1132:1996 and NP EN 12143:1999, respectively). The color was measured using a colorimeter, expressed in CIE units ( $L^*$ ,  $a^*$ ,  $b^*$ ) and converted to hue angle ( $h^0$ ) and chroma ( $C^*$ ). Analyses were performed into three moments after purchase (0, 14 and 21 days).

Data showed that there were no significant differences ( $p=0,678$ ) in acidity along storage, although differences in chemical composition were observed (the group of “juices, purées and pulps” had the highest malic acid content at the end of the storage). There was an increasing trend for soluble solid content along storage time, that could be related with the hydrolysis of polysaccharides into monosaccharides (no statistical significance  $p>0,05$ ). The samples containing fermented milk and fruit presented higher  $L^*$  compared with fruit smoothies. There were no significant differences ( $p>0,05$ ) for  $L^*$ ,  $C^*$  and  $h^0$  along storage.

In conclusion, data showed that the quality attributes analyzed in this study, were retained during storage.

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## Co-mobilization of quaternary ammonium compounds and antibiotic resistance genes among bacteria - a matter of concern?

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Diverse environmental stressors (e.g. biocides) can participate in the selection and maintenance of antibiotic resistant (AB<sup>R</sup>) strains and/or genetic elements. Quaternary ammonium compounds (QACs) are heavily used in hospitals, industry and household products. Our goal was to assess the spread of known QACs resistance genes occurring in Gram positive/Gram negative bacteria among *Enterococcus* spp from several sources, and to evaluate if the genetic elements linked to their mobilization also carry AB<sup>R</sup> genes.

We analyzed 120 *E. faecalis*, 176 *E. faecium* and 104 *Enterococcus* spp from hospitalized (82) or healthy humans (45), piggyeries environment/swine (197), aquacultures/trout (29) and sewage (47) (Portugal; 1997-2012). Susceptibility to AB was studied by disk diffusion/agar dilution and to benzalkonium chloride (BC) by microdilution methods. Genes codifying resistance to AB (*vanA*, *tetM*, *tetL*, *tetS*, *ermB*, *aac(6')-Ie-aph(2'')-Ia*, *aadE*, *blaZ*) or QACs (*qacA/B*, 2 *qacH* sequences, *qacEΔ1*, *qacGI*, *smr*) and the link of *IS1216* to *qac* genes were searched by PCR. Mating assays, MLST and analysis of the plasmid carrying *qac* genes (S1-PFGE, rep/rel typing, hybridization) were done.

A ST17 *E. faecium* strain (ampicillin, tetracycline, erythromycin, HLR-gentamicin/streptomycin resistant; BZ MIC=8mg/L) from hospital sewage carried the *qacH* gene previously identified in *Staphylococcus* spp and *E. faecalis* V583 (GenBank Y16945.1, AE016833.1). This gene was located in a 50kb plasmid also carrying a partial fragment of CTn6000 containing *tet(S)* as well as *tetM*, *aac(6')-Ie-aph(2'')-Ia*, *aadE* and *rep-pRE25* genes. Two copies of *IS1216* flanked a 3000bp region including *qacH*. This *qacH* plasmid was transferred to *E. faecium* BM4105RF using different AB as selective agents and transconjugants (n=4) were resistant to tetracycline, HLR-gentamicin, HLR-streptomycin (n=4) and erythromycin (n=2). Plasmids of variable size contained other AB<sup>R</sup> genes (*ermB* and *aadE*-30-60kb).

This is the first description of *qacH* in *E. faecium* which seems to be located in a composite *IS1216* transposon. The use of both biocides and AB might favour the spread and /or persistence of genetic elements carrying genes coding for resistance to several selective agents often used in different settings. The results also demonstrate the high genome plasticity of *E. faecium* clones to acquire different adaptive traits.

## COMT GENETIC POLYMORPHISMS MAY INFLUENCE OPIOID DOSE REQUIREMENTS IN THE TREATMENT OF CANCER-RELATED PAIN

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Opioid analgesics are the mainstay treatment for moderate to severe cancer-related pain. However, clinical studies suggest that genetic variability may result in significant differences in analgesic response to opioids. The  $\mu$ -opioid receptor (OPRM1) is the primary site of action for opioids. The polymorphism A118G is relatively frequent in Caucasians and causes an amino acid change from asparagine to aspartic acid. This polymorphism seems to influence opioids action, with homozygous for A allele requiring lower doses of opioids. Catechol-O-methyltransferase (COMT) is involved in the metabolism of catecholamines, which have a role in the nociception mechanism. The functional polymorphism Val(108/158)Met codes the substitution of valine (Val) by methionine (Met). Individuals with the Met/Met genotype have the lowest activity of COMT and have been reported to have increased pain sensitivity and lower  $\mu$ -opioid system activation during sustained pain. Polymorphisms in multidrug resistance protein 1 (MDR1) can have pharmacologic consequences after opioids administration. Two of the most frequent polymorphisms are C3435T and C1236T. Homozygous individuals for T allele of the C3435T have lower mRNA expression. C1236T was found to be in linkage disequilibrium with C3435T and was also related to different opioid doses, higher in T allele homozygous. Our purpose was to investigate the effects of these polymorphisms on several pain-related parameters in Caucasian cancer patients.

DNA samples from 30 cancer patients were genotyped for the polymorphisms in OPRM1 (rs1799971), COMT (rs4680), and MDR1 (rs1128503, rs1045642) with Real-Time PCR. Daily doses were re-expressed as oral morphine equivalents. We examined the relation between the polymorphisms and opioid dose, pain intensity, performance status, adverse effects, age, gender, bone or CNS metastases and breakthrough pain.

Total morphine consumption was related to the polymorphism Val(108/158)Met in COMT gene, with carriers of Met allele showing to be significantly associated with higher dose of opioids ( $p = 0.004$ , Pearson  $\chi^2$  test), which was also confirmed by logistic regression adjust to age and gender ( $p = 0.013$ ). All the other polymorphisms and parameters revealed no statistically significant association.

This preliminary result indicates that genetic variation at COMT enzyme may influence opioid dosing requirements in the treatment of cancer-related pain.

# Crosstalk between outgrowth endothelial cells and fibroblasts in vascularization

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Vascularization is a major challenge in tissue engineering. Different strategies exist, but cell-based approaches have emerged as a promising therapy to achieve successful vascularization. The use of endothelial cells, namely outgrowth endothelial cells (OECs), has been extensively investigated to engineer vascularized tissues. In previous works, it has already been demonstrated that co-cultures of endothelial cells with supporting cells, like osteoblasts, mesenchymal stem cells, fibroblasts, or smooth muscle cells, result in a beneficial effect concerning capillary-like structures formation. Therefore, the main goals of the present work are (a) to determine whether fibroblasts improve neovascularization over time, evaluating their potential to stabilize capillary-like structures formed by OECs, that have been shown to exhibit a higher proliferative capacity and increased regeneration potential – and (b) to characterize the extracellular matrix produced in these co-culture systems. Hence, OECs have been isolated from human umbilical cord blood samples by gradient centrifugation, recovery and plating of mononuclear cells (MNC) and characterized in terms of endothelial markers expression by immunocytochemistry (CD31+, vWF+, VEGFR2+), as well as in terms of their angiogenic potential by Matrigel assay. Co-cultures of endothelial cells (OEC, HUVEC or HMVEC) with fibroblasts were performed in order to evaluate the formation of capillary-like structures and the presence of collagen IV in the extracellular matrix by immunocytochemistry and western blotting. OECs expressed collagen IV, an extracellular matrix component present in basement membranes, and formed capillary-like structures in Matrigel assay. Fibroblasts influenced the formation of capillary-like structures by endothelial cells.

# Determination of bisphenol A in children desserts combining QuEChERS extraction with dispersive liquid-liquid microextraction followed by GC-MS

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Bisphenol A [2,2-bis(4-hydroxyphenyl)propane; BPA] is a synthetic compound with endocrine disrupting properties. It can be found in a diverse range of products including food containers such as returnable beverage bottles, infant feeding bottles and food cans. Small amounts of BPA can potentially leach out from food containers into foodstuffs and beverages and therefore be ingested. Numerous studies in both animal and humans have demonstrated association between BPA and an increased incidence of cardiovascular disease, diabetes, fertility problems and liver enzyme abnormalities. There is also evidence that low-dose exposure to BPA during early stages of development may lead to permanent hormonal alterations as well as changes in developmental or reproductive capacity as neurological and endocrine systems are developing and hepatic system is still immature, in particular to fetuses, infants and children. Currently, 50 µg/kg body weight per day is the maximum acceptable dose established by European and US authorities, although several authors stress the need to significantly lower this limit [1].

The purpose of this study was to determine bisphenol A in children desserts using as method a combination of QuEChERS extraction with DLLME (dispersive liquid-liquid microextraction) followed by gas chromatography-mass spectrometry (GC-MS) [2]. The DLLME procedure involved the use of tetrachloroethylene as extractive solvent while the own acetonitrile extract obtained from QuEChERS was used as dispersive solvent, and anhydride acetic as derivatizing reagent. Besides the great enrichment factor provided, the final DLLME extractive step allowed the simultaneous acetylation of the compounds required for their gas chromatographic analysis. Several samples purchased in Portuguese market including gelatines and fruit purées were analysed. All the samples analysed were free of BPA.

**Keywords:** bisphenol A, children desserts, QuEChERS extraction, DLLME, GC-MS

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## Development of a yeast model to search for activators of the executioner caspase-3, -6 and -7

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One of the hallmarks of cancer is its resistance to apoptosis. At the core of the execution phase of apoptosis are the caspase-3, -6 and -7. Therefore small-molecule activators of these executioner caspases represent promising anticancer drugs. In spite of this, to date, only few small-molecule activators were reported. The first described small-molecule activator of caspases was the procaspase activating compound-1 (PAC-1) that activates caspase-3 and -7 [1]. More recently, another compound named 1541, which activates caspase-3 and -6, has been reported [2]. Although the antitumor properties of these small-molecules via caspase activation have been demonstrated, the few templates for caspase activators described make urgent the search for new and more effective caspase activators. Besides the small number of assays available to search for executioner caspase activators, most of them are expensive cell-free biochemical assays. With these assays, relevant drug properties, such as cellular permeability, instability and cytotoxicity cannot be evaluated.

Yeast has been described as a powerful model system for genetic and chemical screenings [3]. It was previously reported that, although the human procaspase-3, -6 and -7 have no cytotoxic effects when expressed in yeast, their active (cleaved) forms cause growth inhibition [4]. As such, activators of these procaspases should induce growth inhibition in yeast expressing the procaspase-3, -6 or -7, having no impact on the growth of control yeast (transformed with the empty vector). Based on this, in the present work, a simple and reliable yeast phenotypic assay was developed to search for activators of procaspase-3, -6 and -7 in a cost-effective manner. For that, these human procaspases were individually expressed in *Saccharomyces cerevisiae* as confirmed by Western-blot. The absence of cytotoxic effects of human procaspase-3, -6 and -7 in yeast was also confirmed by growth analysis. The validation of the assay to the search for small-molecule activators of caspase-3, -6 and -7 has been performed by testing known small-molecule activators of these caspases, namely PAC-1 and 1541.

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# Development of smart carriers for delivering menadione to mitochondria

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Mitochondria play a central role in cell life and death and are known to be important in a wide range of diseases including the cancer, diabetes, cardiovascular events and the age-related neurodegenerative processes. These mitochondrial-related diseases are associated with increased oxidative stress, degradation of cellular macromolecules, and decreased ATP production. Oxidative damage to mitochondria has been shown to impair mitochondrial function and lead to cell death via apoptosis and necrosis. The unique structural and functional characteristics of mitochondria enable the selective targeting of drugs designed to modulate the function of this organelle for therapeutic gain.

While the molecular mechanisms by which mitochondrial oxidative stress triggers apoptosis are still under investigation attempts to achieve protection using antioxidant molecules have already been successful in several models of neuronal cell death. To increase the availability of antioxidant drugs at the mitochondrial level within cells, it has been proposed to covalently couple antioxidant molecules to a carrier that acts as a membrane-permeable lipophilic cation [1]. Lipophilic cations, composed by an antioxidant group, a linker and a penetrating cation (*e.g.* triphenyl phosphonium- TPP) can rapidly permeate phospholipid bilayers and accumulate in negatively-charged compartments, such as the mitochondrial matrix, driven by the membrane potential. Mitochondrial drug-targeting strategies will open up avenues for manipulating mitochondrial functions and allow for selective protection or eradication of damaged cells for therapeutic gain in a variety of diseases.

The objective of our project is the rational design and synthesis of analogues of the mitochondrial electron carrier vitamin k2. The modified antioxidant electron carriers are designed to suppress oxidative stress, and diminish the degradation of cellular macromolecules, in addition to supporting ATP synthesis. In that way they can be used as potent and selective antioxidant agents throughout the specific targeting of mitochondria. The synthesized compounds, methods and applications are in patenting process.

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# Diosquinone inhibits human acetyl- and butyrylcholinesterase

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Alzheimer's disease (AD) is the most common neurodegenerative disorder. Currently, a *N*-methyl-*D*-aspartate (NMDA) receptor antagonist (memantine) and cholinesterases (ChE) inhibitors are the only drugs available for symptomatic treatment of AD. Two principal ChE are responsible for the neurotransmitter, acetylcholine, hydrolysis: acetylcholinesterase (AChE) – which activity is unchanged or even decreased in AD - and butyrylcholinesterase (BChE) – which activity is normally increased in AD [1]. In this work, we studied the AChE and BChE inhibitory activity and the antioxidant properties of natural occurring naphthoquinones (NQ).

Tested NQ were two dimers extracted from the root barks of *Diospyros chamaethamnus* Dinter ex. Mildbr [diosquinone (DQN) and diospyrin (DPR)] and four monomers commercially available [menadione (MND), plumbagin (PLB), naphthazarin (NTZ) and juglone (JGL)]. The enzymes used were AChE and BChE from *Electrophorus electricus* (electric eel) and human neuroblastoma cell line (SH-SY5Y). The antioxidant properties were studied by lipid peroxidation inhibition and by DPPH<sup>•</sup> scavenging assays. Galantamine (GLT) was used as positive control.

DQN was the only NQ with activity against AChE and BChE from both human cells and electric eel (Table 1). Furthermore, DQN completely inhibited the activity of both human enzymes, at 100 $\mu$ M. DPR had the highest activity towards eel AChE, while JGL was able to inhibit 50% the human BChE activity (Table 1). NTZ was the compound with best antioxidant activity: DPPH<sup>•</sup> scavenging of 54.9 $\pm$ 3.50% with 1mM of NTZ. NTZ (100 $\mu$ M) inhibited the lipid peroxidation in 31.5 $\pm$ 4.59%.

DQN was the best ChE inhibitor, being more potent than GLT for human enzymes. The results obtained by DPR for human and eel AChE inhibition (higher activity for eel AChE and lower activity for human AChE) show the importance of evaluating the ChE inhibition using human enzymes. NTZ was the best antioxidant NQ, probably due to stabilization of its structure by sharing hydrogen atoms of hydroxyl groups with the carbonyl groups.

Table 1: IC<sub>50</sub> ( $\mu$ M) of GLT, DPR, DQN and JGL against human and eel ChE. Data are shown as the mean $\pm$ SEM (n=3-4).

Source	Enzyme	GLT	DPR	DQN	JGL
Electric eel	AChE	5.37 $\pm$ 0.410	0.861 $\pm$ 0.0389	10.6 $\pm$ 1.32	-
	BChE	48.7 $\pm$ 3.53	-	110 $\pm$ 5.21	-
Human	AChE	40.7 $\pm$ 6.48	-	32.8 $\pm$ 2.44	-
	BChE	572 $\pm$ 83.3	-	41.7 $\pm$ 4.54	84.4 $\pm$ 10.5

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# Discrimination of *Escherichia coli* clones by Fourier Transform Infrared Spectroscopy

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Fourier Transform Infrared Spectroscopy (FTIR) is a spectroscopic high-throughput method, which has proven to be highly discriminatory at different taxonomic levels. Our goal is to investigate the potential of FTIR to quickly discriminate clinically relevant *Escherichia coli* clones from B2 and D phylogenetic groups, mainly responsible for the worldwide spread of particular antibiotic resistance genes.

Seventy-five *E. coli* isolates belonging to B2 (32 ST131, 7 non-ST131 from diverse sequence types) and D (13 ST69, 12 ST393, 11 ST405) phylogenetic groups were studied. They represent a diversity of isolates from multiple countries, origins and periods (1980-2010), which have been previously characterized. Each clonal group includes diverse PFGE-types. Isolates were grown in Mueller Hinton agar at 37°C for 18h, colonies were directly transferred from the agar plates to the ATR crystal, air-dried and spectra were acquired from 4000-400 cm<sup>-1</sup> with a resolution of 4 cm<sup>-1</sup> and 32 scan co-additions. Each strain was tested in triplicate in three independent subcultures. Spectra were processed and analysed with supervised and unsupervised chemometric methods considering the carbohydrates absorption region.

FTIR demonstrated a reliable and reproducible discrimination of the *E. coli* clonal groups tested. The HCA model revealed a good differentiation between the B2-ST131 isolates, which were consistently grouped in one cluster, and all other B2 and D clones. Moreover, the PLSDA model correctly predicted 99.8% of ST131 isolates as ST131. HCA performed only with B2 isolates clearly discriminated ST131 isolates from all other diverse B2-non-ST131 isolates. Furthermore, when HCA was performed in phylogroup D isolates, three clusters corresponding to the three clonal groups tested (ST69, ST393, ST405) were identified.

FTIR spectroscopy showed a good discrimination ability of the four *E. coli* clonal groups tested. This low cost, rapid and simple spectroscopic method might be a reliable tool for the identification of clinically relevant *E. coli* clones with potential application at a large-scale basis.

## Distribution of adenosine receptors in the glomeruli of diabetic Spontaneously Hypertensive Rat

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Diabetes damages the kidney, being diabetic nephropathy a prime cause of end-stage renal disease [1]. Adenosine regulates a wide range of renal physiological functions [2]. Most diabetic patients also have hypertension, aggravating the prognosis [3]. The work aimed at studying the distribution of adenosine A<sub>1</sub> and A<sub>2B</sub> receptors in the glomeruli of spontaneously hypertensive rats (SHR) with and without streptozotocin (STZ)-diabetes.

On day 0, male SHR rats (12 weeks) were i.p. injected with STZ (65 mg·kg<sup>-1</sup>; STZ group) or vehicle (control group). On day 21, animals were anesthetized and the left kidney was removed, fixed in formalin and included in paraffin. Tissue sections (4 µm) were incubated with primary antibodies directed against adenosine receptors (anti-A<sub>1</sub> and anti-A<sub>2B</sub>), the resulting immunocomplexes detected with a biotinylated secondary antibody and the chromogenic reaction enhanced with ABC using DAB as substrate. DAB-immunostained sections were imaged using bright field optics on a microscope and acquired using a CDD camera connected to a computer.

In both experimental groups, the adenosine A<sub>1</sub> receptor immunoreactivity was located in mesangial cells and for the adenosine A<sub>2B</sub> receptor it was mainly observed in podocytes but also in mesangial cells. Immunoreactivities for both receptors were more marked in superficial than in deep glomeruli. In all glomeruli, the STZ group showed lower immunoreactivity for the adenosine A<sub>1</sub> receptor than the control group. However, an increased immunoreactivity for the adenosine A<sub>2B</sub> receptor was observed both in podocytes and mesangial cells, the increase being more marked in the later cell type.

The results of the present study, although only qualitative for now, suggest that in SHR animals, STZ-induced diabetes alters the renal expression of adenosine receptors which might be triggered by the diabetes-induced higher concentration of endogenous adenosine described in the literature.

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# Effect of GRK4 Gene Variants on Dopamine Receptor function

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In the kidney, dopamine promotes natriuresis by inhibiting both proximal and distal tubule NaCl reabsorption through its action on renal dopamine receptors. In human essential hypertension, single nucleotide polymorphisms of a G protein-coupled receptor kinase, GRK4 $\gamma$ , increase G protein-coupled receptor kinase (GRK) activity and cause the serine phosphorylation and uncoupling of the dopamine D1 receptor (DRD1) receptor from its G protein/effector enzyme complex in the renal proximal tubule. DRD3 promotes natriuresis, especially during salt-replete states. The involvement of DRD3 dysfunction in the etiology of hypertension has been demonstrated through pharmacologic and genetic interventions. While its gene locus at 3q13.3 has been linked to hypertension, the signal transduction pathway of DRD3 has not been fully ascertained.

The aim of the present study was to determine the physiological effects of the GRK4 $\gamma$  sequence variants on DRD3 function *in vitro*, elucidate whether GRK4 $\gamma$  sequence variants (A142V and A486V) promote DRD3 hyper-phosphorylation that results in receptor dysfunction.

For this purpose, Chinese hamster ovary cells stably transfected with the tetracycline-inducible GRK4 splice variants (GRK4 $\gamma$ -A142 and GRK4 $\gamma$ -A486V), or empty vector as control, were transfected with human DRD3. After serum starvation, transfected cells were stimulation with the highly selective DRD3 agonist PD128907 (1  $\mu$ M) for 30 min and total cell lysates were collected. The heterologously expressed DRD3 was pulled down from uniform amounts of protein (500  $\mu$ g) using a His pulldown kit and resolved in 10% SDS-PAGE. In CHO cells expressing GRK4 $\gamma$ -A142 and GRK4 $\gamma$ -A486V variants, stimulation with PD128907 resulted in an increase in receptor phosphorylation. To determine whether the MAP kinase pathway is involved in GRK4-mediated mitogenesis upon DRD3 stimulation, total cell lysates were immunoblotted for phosphorylated p44/42 and for a panel of phosphorylated proteins thereafter. DRD3 stimulation increased the amount of phosphorylated p44/42 MAP kinases and the downstream target p90RSK, in both GRK4 $\gamma$  variants and wild type. Contrasting, S6 ribosomal protein showed a decrease in phosphorylation upon DRD3 activation.

In conclusion, these results indicate that these GRK4 variants phosphorylate and possible desensitize DRD3, suggesting an impairment of its function.

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# Acute toxic effects of cadmium on wild *Pomatoschistus microps*

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The common goby (*Pomatoschistus microps*) is an important intermediary predator in several European estuaries where it plays a determinant role in ecosystem functioning. In early juvenile phases it feeds on zooplankton thus contributing to control this community that is closed equilibrium with the phytoplankton. Several stress factors can disrupt zooplankton-zooplanktivorous fish interactions by different ways, including chemical environmental contaminants through toxic effects of zooplanktivorous fish. Several zooplankton populations playing a crucial role. Thus, the objective of the present study was to investigate the toxic effects of cadmium, which is a common environmental contaminant in estuaries, on wild juvenile *P. microps*. Fish were collected in the Lima River estuary (NW Portugal) and after an acclimation period to laboratory conditions they were used in 96h acute bioassays performed in a photoperiod (16h light: 8h dark) and temperature (20±1°C) controlled chamber. Fish were randomly distributed in different groups that were exposed to artificial sea water (control) or to different cadmium concentrations (1.5 to 50 mg/L of Cd). Additional air was supplied during the bioassay and no food was provided. Mortality and several biomarkers indicative of fish health were used as effect criteria. The median lethal concentration after 96h of exposure was determined and several alterations on the biomarkers were found, indicating that in the range of concentrations tested cadmium was able to cause acute toxicity to juvenile *P. microps*. The concentrations tested in the present study are considerably higher than those occurring in the most part of real estuary scenarios. Thus, in the next phase of the study, the long-term effects potentially induced by the exposure of juvenile fish to ecologically relevant cadmium concentrations will be investigated.

**Keywords:** cadmium, juvenile *Pomatoschistus microps*, zooplankton-zooplanktivorous fish interactions

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## Assessment of volatile organic compounds in the indoor air of an Oporto pre-school

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Among the most important groups of indoor air pollutants are the volatile organic compounds (VOCs), ubiquitous organic compounds that present a boiling point between 50°C and 260°C [1]. Traffic and industrial emissions are the major outdoor sources of these compounds while building and furnishing materials, arts and crafts materials, mechanical and electronic equipments, cleaning agents and personal care products are some of the most common indoor sources [2-3].

Children spend the most part of their day in school. High population density and poor ventilation occur commonly at school environments [3]. Moreover, children are a sensitive group because their lung structure and immune system are not fully developed, and they also present higher breathing rate relative to their body size. VOCs are associated with the increase of allergic reactions and asthma in children [2-3].

The levels of total VOCs in the indoor air of an Oporto school, located closed to a highway existing point and influenced by intense traffic emissions, were assessed. The measurements were done at a common playroom during five weeks at the beginning and at the end of the school day, with 25 to 90 (aged 3 to 6 years) children present in the room. The indoor air VOCs levels ranged from 0.26 to 1.93 mg/L ( $1.15 \pm 0.41$  mg/L). The determined levels largely exceeded the maximum reference limits settled by the Portuguese legislation for the indoor air quality (0.6 mg/m<sup>3</sup>, considering isobutylene as standard references).

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## **Biomonitoring and seasonal variability of *Corbicula fluminea* biomarkers in the Minho River**

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Biomonitoring is an important tool in ecotoxicology, as it allows evaluating the health status of a species along time and may also detect changes in the ecosystem. The objective of the present study was to perform a preliminary study of the variation of the health status of the exotic invasive bivalve *Corbicula fluminea* inhabiting downstream in the Minho River but before the entrance of main sources of contamination in the estuary. The population was sampled seasonally from the winter 2011 to the autumn 2012, and biomarkers indicative of oxidative stress, biotransformation, neurotoxicity and alterations in the pathways of energy production were determined. Seasonal variation was found for some biomarkers in agreement with water flow changes in the Minho River and other environmental factors, highlighting the importance of considering natural variability of biomarkers in biomonitoring studies in wild populations.

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# Detection of ABC transporter and tumour markers proteins in zebrafish (*Danio rerio*) liver after a single exposure to Benzo(a)pyrene

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The ABC superfamily is a highly conserved family of transmembrane proteins [1], related with the phenomenon of multidrug resistance (MDR), characterized by the cells' low accumulation of anticancer drugs, due to increased expression of P-glycoprotein (P-gp; ABCB1 subfamily). P-gp also plays a role in environmental toxicology since is present in barrier tissues of aquatic organisms, acting as efflux pumps of toxicants, in a phenotype called multixenobiotic resistance (MXR) [2]. An important group of environmental pollutants are the polycyclic aromatic hydrocarbons (PAHs), being benzo(a)pyrene (BaP) a typical and widely spread PAH in the aquatic environment with recognised carcinogenic properties [3], therefore was selected as the model contaminant. The model species used was the zebrafish (*Danio rerio*), which is an excellent model for toxicological studies. Juvenile zebrafish (3 weeks old) were exposed to waterborne BaP (100 ppb) for 48h, the control group was exposed to the vehicle acetone. In both groups there were a media renewing at 24h, due to the BaP volatility. Liver tissues were collected three months after exposure. The main objective of this work was to study the induction of zebrafish liver neoplasia by BaP and the expression of specific proteins in hepatic tissue. The proteins evaluated includes the described P-gp and the proliferating cell nuclear antigen (PCNA) which is an auxiliary protein in the S-phase of cell cycle, used as tumour marker. The analysis of these proteins was achieved by immunohistochemistry (IHC) on paraffin liver sections, using 2 different commercial antibodies against P-gp (C219 and C494) and the PC10 clone for PCNA proliferative marker. The results of this work are preliminary, but so far demonstrate enhanced levels of P-gp-like proteins on liver, verified 3 months after BaP exposure, suggesting the relevance of these proteins in the defence against environmental toxicants. Increased levels of PCNA expression were also measured, indicating high levels of hepatic cellular proliferation. The overall data of this work suggest that one single BaP exposure is able to induce long-term effects in zebrafish, namely the up-regulation of the hepatic protein expression, specifically the P-gp transporter involved in cellular efflux process and the PCNA marker of tumorigenesis.

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# Ibuprofen effect on the nitrifying bacteria *Nitrosomonas europaea*

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*Nitrosomonas europaea* are ammonia-oxidizing bacteria (AOB) and are used in wastewater treatment to catalyze the first step of nitrification (conversion of NH<sub>3</sub> to NO<sub>2</sub><sup>-</sup>). AOB are a key group of microorganisms in wastewater treatment in the removal of the high nitrogen levels that has become a growing concern [1]. It has been described that the presence of some pharmaceutically active compounds (PhACs), namely ketoprofen, naproxen, carbamazepine and gemfibrozil, may affect AOB activity and subsequent nitrogen removal in wastewater treatment [2]. The present work arises from the need to assess the impact of other PhACs on nitrifying bacteria such as *N.europaea*. Ibuprofen (IBP) is one of the most widely used non-steroidal anti-inflammatory drug [3] and it was been highly detected in wastewater and soils [4], therefore, it was selected as the first compound to examine in this study.

Nitrite production was measured in minimal growth medium (ATCC 2265) amended with 2.5 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and with the PhAC. IBP was added to flasks to yield a final concentration of 0.1, 1 or 10 μM. Triplicates of each treatment condition were prepared. Flasks were also prepared tested without IBP and used as controls. Nitrite concentrations and biomass (*N.europaea*) were determined.

It was found that the tests with higher concentrations of IBP have a lower nitrite production than the control tests. The maximum observed nitrite production inhibition was 17% for the test with 10 μM of IBP after 6 hours of monitoring. These results suggest that IBP may impact *N.europaea* nitrite production and therefore the removal of ammonia in wastewater treatment.

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## ***In vitro* exposure of *Ostrya* spp. and *Carpinus* spp. pollen to atmospheric levels of SO<sub>2</sub>, NO<sub>2</sub>, O<sub>3</sub> and CO**

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*Ostrya* e *Carpinus* (Fig. 1) are plants of the *Betulaceae* family used for ornamental purposes and mainly due to high traffic concentration in these areas they are subject to atmospheric pollutants. The pollen of these plants constitutes an important risk factor to human health due to its potential allergenicity and due to the increase of these health effects as consequence of the exposition to air pollution [1]. In this project the pollen of *Ostrya* e *Carpinus* were fumigated with CO, O<sub>3</sub>, SO<sub>2</sub> and NO<sub>2</sub> in order to assess the effect of these air pollutants on the viability of the pollen and on the potential increase of human allergenicity.



Fig.1 *Ostrya* (left) and *Carpinus* (right) plants.

Pollen fumigation was done with a homemade camera with the temperature, humidity, artificial solar light and pollutant concentration control [1]. After fumigation the viability, germination, total protein content and polypeptide profile of the pollen samples were determined using standard procedures as well as the immunodetection assays [1].

Pollen exposed to a relatively lower concentration of the studied air pollutants shows marked effects. In this communication the main results will be presented and discussed as well as the possible impacts of pollen exposed to air pollutants at levels considered safe for human health.

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## Lipids assessment of octopus from distinct marine environments

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Lipids and lipophilic biomolecules, such as fatty acids, sterols and vitamin E, are determinant for the equilibrium of innumerable biological functions in humans, varying from molecular signaling to membrane integrity. However, these compounds are only acquired through diet and dietary supplements, thus their assessment in commonly consumed food products is imperative.

Commercial octopus was selected in this preliminary study due to its importance in the Portuguese nutrition. Therefore, three common species were analyzed: *Octopus vulgaris*, *Octopus maya* and *Eledone cirrhosa* (fresh and frozen) from different locations: Northeast Atlantic (NEA), East and West Central Atlantic (ECA and WCA), Pacific Ocean (PO) and Mediterranean Sea (MS) (n = 4, each). The edible parts were separated, minced, and frozen at -20 °C for further lyophilization. Lipids were extracted, followed by cholesterol and  $\alpha$ -tocopherol analysis by HPLC-DAD-FLD, while fatty acids profile was assessed by GC-FID, according to Cruz and co-workers [1].

Total fat content varied between 2.39 g/100g and 4.30 g/100g dry basis (DB), corresponding to *O. vulgaris* from WCA and *E. cirrhosa* from NEA, respectively. Among all *O. vulgaris* origins, those from PO showed significantly higher amounts of cholesterol ( $1503 \pm 282$  mg/100g DB) and  $\alpha$ -tocopherol ( $9.30 \pm 4.13$  mg/100g DB) ( $p < 0.001$ ). Regarding polyunsaturated fatty acids (PUFA), there were no significant differences between species, excepting *O. vulgaris* from NEA, which showed the highest values ( $62.7 \pm 3.23\%$ ). Additionally, these samples also revealed the highest relative amounts of eicosapentanoic acid (C20:5n-3; EPA) and docosahexaenoic acid (C22:6n-3; DHA), the latter with similar amounts in *E. cirrhosa*, both of recognized health benefits. A high variable n-3/n-6 ratio was observed, varying from 1.3 to 10.1, again higher in the NEA *O. vulgaris* samples.

In conclusion, the marine environment seems to exhibit a preponderant impact on the chemical composition of octopus species, and therefore their nutritional quality.

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# **Oxidative stress assessment in octopus (*Octopus vulgaris*) by measuring antioxidant enzyme activities and oxidative damage**

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Aquatic organisms are exposed to many pollutants that are released into the aquatic environment. Some of these chemicals, like metals, can be toxic and have the ability to produce reactive oxygen species (ROS). ROS can cause oxidative damage to important biomolecules affecting the health of exposed organisms. Oxidative stress status can be evaluated by the use of biomarkers that measure biochemical changes [1]. This study aims to assess oxidative stress status in wild *Octopus vulgaris*, by measuring antioxidant enzymes activity and oxidative damages in the digestive gland. In the arm is also targeted oxidative damage as a measure of food quality for human consumption [2]. *O. vulgaris* was selected for this study as a bioindicator species for being ecological relevant due to its abundance in the NW Portuguese coast, territorial nature and top position in the trophic chain. Octopus were caught in September in the north coast of Portugal, sampled after arrival and after 14 days in captivity. Spectrophotometric measurements were performed to evaluate the antioxidant enzymes activity in digestive gland and oxidative damage in both tissues. Levels obtained were compared to the levels observed in octopus collected in different periods of the year to evaluate the seasonality of these biomarkers and to estimate the degree of metal contamination. Generally, the biomarker levels were comparable to the levels found in other seasons on *O. vulgaris*. Catalase activity remained similar to the activity measured in different periods, being the most stable antioxidant enzyme throughout the year while superoxide dismutase and glutathione s-transferase had some fluctuations. LPO and PCO had slight variations throughout the year in both tissues. The 14 day depuration period had no significant effects on any of the biomarkers studied. The octopus seems to have a good antioxidant system able to maintain the oxidative damage within certain levels. Moreover, we might estimate that the metal levels did not change significantly in this season since significant correlations between metal levels and the biomarkers studied were previously found in this species [3].

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# Relationships between microplastics properties and their ingestion by zooplankton-zooplanktivorous fish

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Microplastics are emerging environmental contaminants of concern in estuarine and marine ecosystems. They have been found at high amounts in the marine environment and to induce adverse effects on aquatic organisms. An important adverse on organisms potentially affecting the whole ecosystem is their ingestion as food by zooplankton and small fish that may lead to starvation and death. Thus, the objective of the present study was to investigate the relationships between some basic properties of microplastics and their ingestion as food by the common goby *Pomatoschistus microps*. This fish is an important intermediate predator in estuarine food webs that feeds in zooplankton in its early juvenile phases. Wild fish were collected in estuaries of the NW coast. After an acclimatization period in the lab, they were maintained in artificial sea water for 96h without food, at 20±1°C and 16h light:8h dark. Then, a feeding experiment was conducted offering different types of microplastics and/or the microcrustacean *Artemia parthenogenetica* to individual specimens. The results indicate that the common goby ingested preferentially *Artemia parthenogenetica* to microplastics, and some microplastics than others apparently depending on colour and size mainly. Furthermore, this study indicates that microplastics are confounded with food and thus that the presence of microplastics in estuaries may impair the health condition of small *P. microps* juveniles due to physical effects causing starvation.

**Keywords:** microplastics, physical effects, *Pomatoschistus microps*, estuarine fish

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## Siderophore production by *Bacillus megaterium*: effect of growth-phase and cultural conditions

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The chelating agents, such as EDTA, are used in a wide variety of industries, for instance, textile, pulp and paper, food, cosmetic or detergent industries. However, the commonly used synthetic complexing agents are not biodegradable. Due to this reason, they are accumulated in the environment, which is a matter of concern. Thus, there is a growing interest in replacing these compounds by similar, biodegradable and environment- friendly chelating compounds.

Some microorganisms are able to produce molecules with ability to capture metals. Siderophores are one of these types of molecules. Siderophores are compounds produced by bacteria, fungi and grass plants, able of forming very stable complexes with iron. This work aimed to study the effect of different cultural and nutritional conditions on the siderophore production by the bacterium *Bacillus megaterium*.

Siderophore production was detected using chrome azurol S (CAS) method [1], in an iron-deficient culture medium, during the exponential growth phase, prior to the sporulation, in the presence of glucose. Growth of bacteria in the presence of various carbon sources showed that siderophore production is affected by the type of carbon source. The growth on glycerol promoted an increased production of siderophore; opposite effect was observed in the presence of mannose. *B. megaterium*, when grown in the presence of fructose, galactose, glucose, lactose, maltose or sucrose, produced similar concentrations of siderophore. The increase of arginine concentration, in the culture medium, did not increase the production of siderophore. Aeration had a positive effect on the production of siderophore; growth under static conditions delayed and reduced the production of siderophore.

In conclusion, siderophore production is influenced by the carbon source used but is not affected by catabolite repression or the amount of arginine and is not related with the triggering of sporulation. Glycerol appears to be an alternative carbon source, to monosaccharides and disaccharides, for the production of siderophore. Aerobic metabolism seems to be involved on the production of the siderophore by *B. megaterium* ATCC 19213.

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## The fatty acid variability of canned sardines

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Canned sardines are regarded as a healthy food in a diversified diet, particularly due to their high content in long-chain eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids. However, canned sardine fat content and fatty acid composition are known to be variable, particularly regarding the sardine harvest season and the sauce used for canning [1].

In order to study canned sardines variability regarding these important nutritional features (fat content and composition) while attempting to understand the extension of the exchanges with the sauce, 35 duplicated samples of canned sardines from the same producer, marinated in various sauces (water, tomato, vegetable oil and olive oil) were analysed [2]. For an effective nutritional comparison, all samples were previously drained (5 min.).

As regards to the total fat amounts, the lowest average content (5.9%), was observed in sardined canned in water or brine, with values ranging between 2.0% and 9.0%. For sardines in tomato sauce, the average results were higher (10.3%), and ranged from 2.5% and 13.5%. For sardines marinated in vegetable oil an average of 13.3% was observed (8.9% and 16.8%), similar to olive oil, with 15.2% (11.9% - 17.5%). Although already expecting higher fat content in oil canned sardined, in opposition to the plain ones with fish lipids only, the high variability observed within all the sauces seems to be a direct consequence of the initial sardine composition.

Regarding the EPA and DHA content, strictly of fish origin, the results were also highly variable within each sauce. Still, on a comparative basis, an average of 2g was found per 100g of sardine in olive oil or tomato sauce, being slightly lower in vegetable oil (1.6%) and in sardines canned in water or brine (1.3%). These results might be strong indicators that the sauce has an important part in the preservation of these long-chain polyunsaturated fatty acids, as both olive oil and tomato sauce are rich in natural antioxidants, being reduced in refined vegetable oil. In comparison, preservation in water, despite resulting in low fat sardined from the nutritional point of view, seems to be responsible for a reduction in long-chain omega-3 fatty acids. As to whether this reduction is a result of hydrolysis or drainage should be further explored.

These preliminary results highlight that the nutritional benefits of canned sardines are highly influence by the sauce, particularly when preserved in water or brine, but other factors must also be further explored, including harvest date and sardines size.

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# The influence of air pollution on *Ligustrum lucidum* pollen productivity. Fertility and allergenicity changes after in vitro exposure to carbon monoxide

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This work aims to investigate the effects of air pollution in the pollen of *Ligustrum lucidum*. Several characteristics were taken into account namely the number of pollen produced by anther, fertility and the allergenicity of the pollen.

To ascertain if the number of pollen per anther would change when the tree was exposed to air pollution, branches of different *Ligustrum* trees were collected. These trees were divided in to two categories: Polluted trees, that grow near a highway and less polluted trees. The pollen quantity per anther was calculated in three branches of each tree, by selecting three flowers of each branch, and two anthers of each flower. Later the pollen of each anther was counted with the help of an optical microscope (x10). The results were submitted to statistical tests using the software SPSS. This test demonstrated that there were significant differences between the number of pollen per anther from trees that were non polluted and the trees that were polluted, the last with the lower pollen count.

To ascertain the effects of air pollution on pollen fertility and allergenicity, *Ligustrum* pollen previously collected was exposed in vitro to different concentrations of Carbon Monoxide (CO). Four fumigations were performed with CO concentrations of 3.66 ppm; 6.18 ppm; 9.30 ppm; 20.44 ppm. After exposure pollen proteins were quantified and the molecular weight of the polypeptides was determined by SDS-gel electrophoresis and the immunoreactivity was assessed by Western blot using patient sera sensitized to pollen. Also, pollen fertility was determined by means of pollen viability using the Trypan Blue Dye test.

The results showed that, in high level of CO, the quantity of protein and the IgE recognition by patient sera lowered. However to the level of 3.66 ppm the IgE recognition increased in comparison to control sample (non-exposed pollen).

The pollen viability tests showed that the higher the CO concentration exposed the lower was the pollen viability.

This study indicated that the air pollution has an important influence in pollen production, viability and allergenicity.

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## Toxic effects of nickel on juveniles of *Pomatoschistus microps*

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Nickel is a heavy metal widely used in several anthropogenic activities, resulting in their presence above “natural” levels in several ecosystems, including estuaries, where it can induce toxic effects in wild organisms. It is present in water, sediments and organisms of the NW Portuguese coast estuaries.

Thus, the objective of the present study was to investigate the acute toxic effects of nickel on the common goby, *Pomatoschistus microps*, a species with a relevant role in trophic webs lagoons and estuaries of Europe.

Juveniles from wild populations were acclimatized to laboratory conditions. After the acclimatization period, groups of animals, randomly selected, were individually exposed to nickel for 96h. Treatments were: a control without nickel; 1,6; 3,1; 6,2; 12,5; 25,0 and 50,0 mg Ni/L). At the end of the bioassay, several biomarkers were determined. The results are discussed in relation to the effects of nickel on fish health.

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# Toxicity of nickel to the invasive clam *Corbicula fluminea* (Mollusca: Bivalvia) – first findings

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*Corbicula fluminea* (Mollusca: Bivalvia) is an exotic invasive species native from Asia that is now present in different continents, including in Europe, causing considerably negative ecological and economic impacts. The species has been showing a different invasive behaviour in the estuaries of Minho and Lima Rivers that have different levels of pollution [1]. Therefore, the comparison between the population of Minho estuary inhabiting a relatively low impacted estuary with that one of Lima estuary where the chemical contamination levels are higher may provide important knowledge on the influence of pollution on the invasive behaviour of this species. Such knowledge is very important to control and mitigate the negative impacts of *C. fluminea* invasions. Thus, a preliminary study was carried out to assay the acute toxicity of nickel to this species. Clams were collected in the wild and after a two weeks acclimatization period to laboratory conditions, groups of 8 clams randomly selected were exposed to tap water (with no chlorine) or to nickel treatments (0.98, 3.91, 15.63, 62.5, 250, 1000 mgL<sup>-1</sup> of Ni) for 96 hours in a temperature and photoperiod controlled room. No food was provided. Mortality and other adverse effects were observed and the results are discussed in relation to the needs of further research.

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## about *design*

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This poster tells us a story about the evolution of Design. It takes several moments in history, various ideas and principles from some of the designers who are a reference to us, in order to explain what this discipline really means and how it has evolved so far.

Vitruvius<sup>1</sup> (I century BC) identified three architectural principles, which later influenced the principles of design. They are: *utilitas*, *venustas* and *firmitas* (utility/functionality, aesthetics and structure/durability).

With the Industrial Revolution (mid-eighteenth century), Design comes with the need to organize, plan, project and rationalize for the development of mass production. The Bauhaus (1919–33) continues the idea that all objects of design shall be designed to be easily adapted for industrial production, but also applies the key principle of functionalism: *form follows function*<sup>4</sup>. Mies van der Rohe<sup>2</sup> became known for his sentence ‘less is more’.

Later, Dieter Rams<sup>3</sup> summarizes ‘good’ Design on ten principles: ‘good design is innovative; makes the product useful; is aesthetic; makes a product understandable, is unobtrusive, is honest; is long-lasting; it is thorough down to the last detail, is environmentally friendly, design is as little design as possible.’

Design is a discipline that involves the concept, creation and production of a project which main goal is to find a solution for a problem, or improving an existing one, taking into account all the characteristics mentioned above. We would add ethical and moral responsibility to that.

[1] Marcus Vitruvius Pollio was a Roman architect and engineer who lived in the first century BC and wrote a ten-volume work entitled ‘De Architectura’, which is the only European treaty from the Greco-Roman period and is the source of inspiration to many texts on architecture since the Renaissance.

[2] Ludwig Mies van der Rohe, (Aachen, 1886– Chicago, 1969) was a German-American architect. He is widely regarded as one of the masters of modern architecture and one of the developers of the International Style.

[3] Dieter Rams (Wiesbaden, 1932) is an industrial designer who collaborated with the German company Braun for over four decades (1955-98).

[4] *form follows function*: Louis Henri Sullivan (Boston, 1856-1924) was an architect and a pioneer of the American Modernism, Bauhaus.

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# **Development and psychometric studies of a scale of occupational sources of stress in Portuguese call centre operators**

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The occupational stress is a feature of modern life: the phrase "be under stress" has become part of the dialogue of most of us and we hear it often in work contexts. This concept takes on added relevance in the context of the work of call center operators. Since this is a population increasingly large - in Portugal are about 55,000 players - and one of the occupations of the current economy that suffers most from stress, became necessary to identify the sources of occupational stress in this specific population. The self-report questionnaires are commonly used to evaluate the sources of stress at work, however, and despite the recent development of the phenomenon of call centers, there are no specific scales to categorize sources of stress in call center workers in particular. Thus, we intend to identify the main sources of occupational stress perceived by call center operators Portuguese, and to develop and validate a scale of Occupational sources of stress in Portuguese Call Centre Operators. For this, the present investigation was divided into two sequential and complementary phases. Based on the results of a focus group and literature review, we developed initially a scale with 29 items. Then, we proceeded to the application thereof to operators of several call centers ( $n = 121$ ), for further validation. We identified six factors that explain 73.8% of the total variance, all with very high consistency values: characteristics of the task and the work ( $\alpha = .93$ ), working conditions and hierarchical relationship ( $\alpha = .89$ ), lack of training / team / autonomy ( $\alpha = .87$ ); relationship with colleagues and demands ( $\alpha = .83$ ), physical conditions ( $\alpha = .86$ ), and d customer attitude ( $\alpha = .85$ ). However, some results of the Factorial Analysis were difficult to interpret, especially the factor number three, what makes us believe that further psychometric studies are needed in future. With the development of a scale that allows us to identify the sources of occupational stress in Portuguese call center, we hope to encourage studies designed to improve working conditions in these contexts

# Impact of Divorce on Self-esteem and Intergenerational Solidarity

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Divorce is a topic that has been increasingly focused on psychological research during the last decade <sup>[1]</sup>. The number of divorces has significantly increased in Western societies <sup>[2]</sup>. At the end of the last century, about half of marriages were predicted to end in divorce <sup>[1]</sup>. Divorce has a considerable impact on other family transitions and dynamics, and may have, thereby, a considerable influence, on emotional, cognitive, social and relational development of children. Research demonstrates that divorce may reduce children's self-esteem, in particular when parental and other relatives' (in particular grandparents') support is compromised <sup>[1, 2]</sup>. This study aims to investigate the impact of divorce on children of divorced couples' self-esteem. Furthermore, it explores the impact of divorce on the amount and kind of support (emotional or instrumental) provided by grandparents to their grandchildren. The sample of the study was composed by 250 participants, adolescents (46,4%) and emerging adults (53,6%) with ages ranging from 14 to 32 years old. 20,2% were children of divorced couples. Data was collected through two instruments: the *Rosenberg Self Esteem Scale* (Rosenberg, adapted by Santos & Maia, 1999), to measure the construct of self esteem ( $\alpha=.74$ ), and the *Intergenerational Solidarity Index* (Bengtson & Roberts, 1991, adapted by Monteiro,2010) to measure the construct of intergenerational solidarity/support ( $\alpha=.91$ ).

Surprisingly, children of divorced couples showed a higher self-esteem ( $M=4.22$ ,  $SD=.581$ ) compared to the children of continuously married couples ( $M=3.98$ ,  $SD=.734$ ) ( $t_{215} = - 2.173$ ,  $p=.031$ ). It was also observed that the children of divorced couples perceived more emotional support ( $M=2.966$ ,  $SD=1.341$ ) than instrumental support ( $M=2.592$ ,  $SD=1.122$ ) by their grandparents ( $t_{48}=2.717$ ,  $p=.005$ ). This result, that was also observed among children of continuously married ( $M=2.621$ ,  $SD=1.098$  versus  $M=2.284$ ,  $SD=.945$ ) ( $t_{161}=5.901$ ,  $p < .001$ ), is probably due to the relationship pattern that grandparents traditionally established with their grandchildren, and/or to grandparents' economical deficiency. Furthermore, children of continuously married couples, perceived less support from their grandparents ( $M=2.526$ ,  $SD=.975$ ) compared to the children of divorced couples ( $M=2.857$ ,  $SD=1.124$ ) ( $t_{209} = - 2.008$ ,  $p=.046$ ). It is important to mention that this study takes place in a specific cultural context – the Portuguese society –, with a specific age group (adolescents and emerging adults) and has methodological limitations, in particular the unequal number in both main groups represented on the sample (divorced and married couples' children). However, results suggest that, nowadays, the impact of divorce is less detrimental and that grandparents' support plays an important role in buffering the potential negative effects of divorce and in preventing the decrease of adolescents' and emerging adults' self-esteem.

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# Schooling Effects on Students' Lives: The Implicit Discourse of Narratives

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**Abstract:** This project discusses the effects of schooling throughout the life of students from eight different Portuguese schools, located in the North of the country. Along with the Centre for Research Education (CIIE) of the Faculty of Psychology and Education Science, these institutions make up the Observatory of Life in Schools (Observatório da Vida nas Escolas - OBVIE). The research project aims at understanding the effects on the students' lives by the institution, in which they experience their scholar component. Once we recognize students as an important asset on the educational system, we are able to attribute meaning to their voices [1] through a methodology that sets biographical narratives as a possibility to interpret the reality and build knowledge [2]. This work focuses on the implicit discourse observed in biographical interviews. The university and the schools involved in the project decided the topics of the interviews, which were carried out with students of the ninth year, who had a record of five years in the institution. The project relevance relies, especially, on the fact that it displays understanding on the importance of students' voice, on the grounds of the polyphony present at schools, in order to help grasp the meaning they attribute to the institution in its different perspectives. As initial results of an ongoing research, now associated with the Faculty of Letters through the course of Sociology as proposed in the terms of IJUP 2012 projects, we highlight that the students' *scream* is based on a search for integration and a humanized work in all of the school departments, drawing attention to the fact that, in addition to a physical structure, curriculum and methodologies, the school is made up of people, and only the successful management of these relations can result in scholar development [3]. Through the students' voice, we are enabled to discuss the preliminary results of this project through a theoretical basis that privileges the tension between the evolvement of relationships among the educational actors, in a perspective of transformation, and the enforcement of old pedagogical traditions, methodologies and discipline codes, which usually affect in a negative way on how students experience the school environment [4].

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# Aqueous-carbonic fluids associated with quartz crystallization in granitic pegmatite from Companheiro (Central Portugal)

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Pegmatites display aspects of both igneous and hydrothermal systems [1]. The origin of pegmatites continues to be debated and there is little doubt that hydrothermal metassomatism occurs namely in rare element pegmatites [2].

The studied granitic pegmatite is located at "Companheiro" (N40°;43'48"; W 7°; 37'32"), in the central region of Portugal, in the Penalva do Castelo municipality. This pegmatite is part of the important "Aplite-pegmatite field of the Iberian Massif". The pegmatite vein, N110° subvertical, occurs embedded in porphyritic biotitic granite with coarse grain belonging to the "Beiras Batholith." It consists primarily of quartz, potassium feldspar, albite, muscovite and beryl. In areas richer in quartz, the pegmatite exhibits alternations of milky and clear quartz parallel to a N30°E fracturing system.

Petrographic, microthermometric and Raman microspectrometric studies in hyaline quartz were performed utilising Chaixmeca and a Linkam stages and a Horiba Jobin-Yvon LabRaman spectrometer interfaced to Olympus microscopes. Raman Spectra were obtained using the 632.8 nm emission line of HeNe laser (20mW).

The quartz studies revealed the existence of three-phase primary fluid inclusions (Fig 1 a) and two-phase secondary fluid inclusions.

The primary fluid inclusions, contemporaneous of quartz crystallization contain aqueous-carbonic fluids (Fig. 1), from the system H<sub>2</sub>O-CO<sub>2</sub>-(CH<sub>4</sub>-N<sub>2</sub>-NaCl), with minimum P-T conditions estimated 330°C and between 190-240 MPa. These conditions for fluid entrapment are well below to wet granite solidus and similar those described for Variscan hydrothermal systems in Central Iberian Zone [3] and confirm the role of hydrothermal fluids in the pegmatite history.

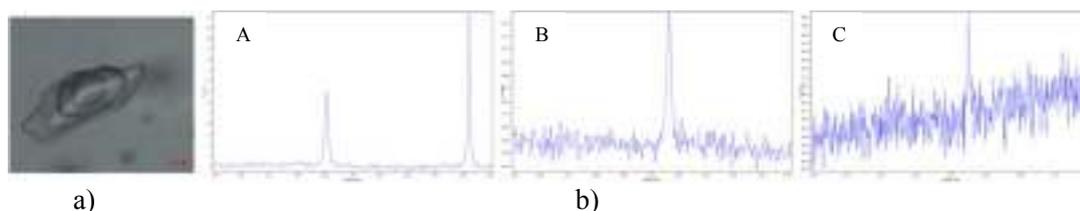


Figure 1: a) Three phase aquo-carbonic fluid inclusion; b) Raman spectra of volatile phase on the three-phase fluid inclusion. A: of CO<sub>2</sub>; B: of CH<sub>4</sub>; C: of N<sub>2</sub>.

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# Sulphides from Borralha tungsten deposit (Northern Portugal)

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Borralha is a tungsten hydrothermal quartz vein deposit located in Northern Portugal, on the boundary between the ZCI and ZGTM, is in the contact zone between Palaeozoic metasediments and Variscan granites.

Previous studies (1) showed that after an oxide stage (wolframite+scheelite) occurs a main sulphide stage essentially characterized by pyrite, chalcopyrite, sphalerite, molybdenite, marcasite and bismuthinite. However the concentrate of chalcopyrite resulting from ore treatment was characterized by a content of 30g of Ag by ton of chalcopyrite (1). The present contribution aimed to realize a detailed study giving special attention to the presence of rare metals associated to the main sulphides.

In this work was used reflected-light polarizing microscope; scanning electronic microscope (SEM) for observation and characterization of minerals by energy dispersive spectra (EDS) for qualitative analysis; and electron microprobe for observation and characterization of minerals through spectra WDS for qualitative and quantitative analysis.

A sphalerite ((Zn,Fe)S<sub>2</sub>) with Co, Pb, Mo, Cu, Sb, Cd and Bi as trace elements, however there is another generation of sphalerite which is less rich in Fe but with Ge associated with Mo, Cu, Cd but without Bi Co.

In pyrite (FeS<sub>2</sub>) were analysed trace elements such as Co, Ag, Zn, Pb, Mo, Cu, Sb, and Bi.

Especially in combination with chalcopyrite ((Cu,Fe)S<sub>2</sub>) with Co, Ag, Zn, Bi and Pb, occurs galena (PbS<sub>2</sub>), with Se, Ge, Cd, Bi, Ag, Cu, Co, Fe, closely associated with native bismuth (Bi), with Sb, Cu and Co, especially in contact with quartz.

Included in chalcopyrite were also identified sulphides with rare metals: matildite ((AgBi)S<sub>2</sub>) with Cu, Fe and Co as trace elements; pavonite ((AgPb<sub>2</sub>Cu<sub>2</sub>Bi<sub>3</sub>)S<sub>8</sub>) with Co, Fe, Se, Sn and aikinite ((PbCuBi)S<sub>3</sub>) with Zn and Fe. These minerals namely matildite and pavonite can justify the Ag contents in chalcopyrite concentrates.

Acknowledgments: To CEMUP by EDS analysis facilities and to LNEG by WDS analysis.

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# The K feldspars and lithiophilite from the granitic pegmatite from Mesquitela (Mangualde - Central Portugal)

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The studied pegmatite is part of the important "Aplite-pegmatite field of the Iberian Massif," in Central Iberian Zone. It is located at Mangualde and more concretely at Mesquitela in "Chão do Castanheiro Mine" belonging to the important "Mangualde Pegmatitic Field".

At Mesquitela, a sub-vertical pegmatite vein (N130°) occurs spatially associated with sinorogenic two-mica granites, consisting primarily of quartz and potassium feldspar with subordinated albite and muscovite. Beryl is the main accessory mineral.

By their mineralogical and economic interest we must also emphasize the occurrence of several Li-Mn phosphates, as accessory phases, being the lithiophilite, the main and more abundant phosphate phase, firstly described by Jesus [1] in Cabeço do Seixo area (now corresponding to "Poço 1"). After that, many other phosphates were described as presents at Mesquitela pegmatite but they are considered as secondary minerals [2].

Petrographic and mineralogical studies using XRD, SEM, MEB, XRF analysis were performed utilising equipment from CGUP, LNEG and CEMUP laboratories in order to characterize potassium feldspar and the main phosphate phase.

The K feldspar, the most important mineral, occurs, usually, as *White Feldspar* (WF) but *Reddish Feldspar* (RF) was also observed. The microscopic study of both revealed the presence of a perthitic orthoclase with late albitization. However, RF exhibited a troubled aspect not affecting the late albite plates. The chemical analysis showed that RF has higher values of total Fe and LOI (0.26 and 1.01 %) than WF (0.04 and 0.5 %). For K<sub>2</sub>O and Na<sub>2</sub>O there are smaller differences, as 12.38 and 2.42 for RF and 11.43 and 3.06 for WF. RF and WF have 1230 and 2320 ppm of Rb. However, RF and WF have anomalous contents in U (24 and 34ppm), Sn (17 and 24 ppm) and W (12 ppm in RF). Petrography on transmitted light of the studied phosphate showed a mineral colourless to pale yellow very rich on fluid inclusions with birefringence on grey colour. This mineral is crossed by a brown mineral with higher birefringence. SEM-EDS analyses revealed a different composition (Fe, Mn, Mg, Ca, K, Cl) compatible with a late phosphate phase, having more complex composition than the primary one (Fe, Mn). XRD analysis showed a spectrum with peaks of lithiophilite and triphylite. XRF analysis revealed scarcity of Rb (<3) and Pb (8 ppm); Sr and Ba with 20 and 30 ppm and a high content on Zn (1311 ppm).

Our results confirm the initial study [1] that considers the colour of RF related with Fe content and the most important phosphate corresponding to a member of the triphylite-lithiophilite series.

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## Antioxidant capacity evaluation of *Eruca sativa*

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Dietary antioxidants, such as water-soluble vitamin C and phenolic compounds, as well as lipid-soluble vitamin E and carotenoids, present in vegetables contribute both to the first and second defense lines against oxidative stress. As a result, they protect cells against oxidative damage, and may therefore prevent chronic diseases, such as cancer, cardiovascular disease, and diabetes [1].

Brassicaceae family comprises more than 350 genera and had been described as an important part of a healthy diet. This family of vegetables is associated with a reduction of several types of cancer. *Eruca sativa* commonly named rocket salad is a member of this family and is widely used all over the world. This vegetable is usually consumed fresh and it is described as containing several health promoting agents including fibers, vitamin A and C, carotenoids and flavonoids [2], some of these agents are known as powerful antioxidants.

The aim of this study was to evaluate the total antioxidant capacity (TAC) of rocket leaves using conventional optical methods. In order to optimize the antioxidant extraction from leaves, two extraction procedures were carried out: i) 0.2 g of rocket leaves sample was blend with 5 mL of ethanol and shaken for 20 min, then the mix was centrifuged at 4245 rpm for 15 min. The supernatants were collected, dried under nitrogen gas and resuspended in 1.5 mL of ethanol [3]; ii) The second extraction procedure was performed using a solution containing ethanol and water in the ratio (1:4) and 0.5 g of rocket leaves sample. This mixture was shaken for 1 hour [4], then the extracts were filtered and stored at 4 °C. The antioxidant profile of the extracts was assessed by the measurement of the total phenol and flavonoid contents, reducing power and DPPH radical scavenging activity [5].

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# Antioxidants from eucalyptus by-products: Optimization of the extraction conditions using factorial design

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Phenolic compounds are commonly found in both edible and nonedible plants, and they have been reported to have multiple biological effects, including antioxidant activity. Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolics are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food [1].

Agro-industrial by-products are good sources of phenolic compounds, and have been explored as source of natural antioxidants. While the use of naturally occurring phenolic compounds as food antioxidants is particularly interesting, practical aspects that need to be considered include extraction efficiency, availability of sufficient raw material, and toxicity or safety considerations. Therefore, the phenolic compounds profile of these by-products has to be determined [2].

Because of its fast growth and increase in woody biomass, the genus *Eucalyptus* is extensively cultivated in the Mediterranean area. Its leaves have been used as traditional remedies for treatment of various diseases such as pulmonary tuberculosis, influenza, fungal infections and diabetes. Because of their antioxidant activity, leaf extracts of *E. globulus* have been used as food additives [3].

The purpose of this study was to establish the best extraction conditions for antioxidant compounds and to determine the antioxidant capacity of different samples of eucalyptus leaves. A factorial design (2<sup>3</sup>) was used to find the optimal extraction parameters (extraction time, extraction temperature, and solvent composition).

Total phenolic content (TPC), total flavonoid content (FC), DPPH radical scavaging activity, and ferric reduction activity power (FRAP) were determined in order to assess the antioxidant capacity of the extracts.

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# Assessment of matrix effects of green vegetables in gas chromatography with electron capture detector in organochlorine pesticide analysis

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Pesticides are used to protect crops before and after harvest from infestation by pests and plant diseases. Organochlorines pesticides (OCP) are chemicals in which carbon and chlorine are combined. These compounds are chemically very stable and that means they break down very slowly and can remain in the environment for a long time. The OCP are also known to be toxic to humans and animal life. Despite being banned from agricultural use many years ago, these chemicals were still detected at varying levels in different food and environmental samples [1,2]. OCP such as DDT, DDE and methoxychlor were long ago banned in Europe but due to their persistence there are still detected in vegetables [3]. Therefore, methodologies capable to determine pesticides simultaneously with satisfactory sensitivity and selectivity are highly required. One of the most important approaches to reduce pesticide exposure is the monitoring of pesticide residues in foods. A multiresidue analysis method was developed for monitoring low levels of 14 OCP using a gas chromatography with electron capture detection in parsley and spinach samples. In this study, we present solid-phase extraction techniques using disposable pipette extraction (DPX) and dispersive solid phase extraction (d-SPE) both coupled with modified QuEChERS methodology. Comparisons of the two techniques were discussed. The study of different types of matrix effects (signal suppression or enhancement) was the main objective of this work. The signal suppression effects were observed during the determination of  $\alpha$ -,  $\beta$  - hexachlorocyclohexane and hexachlorobenzene in spinach and parsley samples using DPX and d-SPE QuEChERS methodologies. The results showed that the degree of matrix effects is dependent of the analyte, sample type and extraction methodology.

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## Authentication of herbal infusions containing *Hypericum androsaemum* and *Hypericum perforatum* by DNA barcoding

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Over the last decades, the use of medicinal plants has been growing not only in developing countries but also in developed countries. This kind of products may however contain undeclared species as a result of misidentification or even intentional substitutions, potentially leading to serious health issues and to commercial fraud. In Portugal, two *Hypericum* species are widely consumed for their medicinal properties: *H. perforatum*, the most commonly used, with proved antidepressant properties [1], and *H. androsaemum*, used mainly for its hepatoprotective qualities [2]. The later, sold at prices 3 to 4 times higher than *H. perforatum*, is a potential target for adulteration. Therefore, the detection of this particular adulteration is of extreme importance, especially for patients taking prescribed medicine, in order to avoid interactions with other drugs. General approaches for herbal identification depend on morphological, anatomical and chemical analysis. However, in plant mixtures, and particularly in powdered preparations, identification is difficult to achieve. Hence, molecular genetic methods have become widely employed for rapid identification of herbal medicine.

This work aimed at searching the potential use of two DNA barcode candidates (ITS1 and matK) for the differentiation of the two *Hypericum* species in herbal infusions. Specific PCR primers were designed targeting ITS1 and matK sequences to produce fragments of 298 bp and 358 bp, respectively. Their sequencing results were on the basis for the development of a real-time PCR assay using EvaGreen dye combined with High Resolution Melting analysis, as a simple approach for the reliable discrimination of both species. The proposed approach targeting the matK locus was successfully applied to commercial herbal infusions, enabling to easily identify the two *Hypericum* species under study. Regarding labeling compliance, the results showed that two samples were mislabeled. One allegedly containing only *H. androsaemum*, included also *H. perforatum*, and the other declaring only *H. perforatum*, also contained *H. androsaemum*. This finding highlights the importance of genomic tools for authentication of medicinal plants.

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## Chemical and morphological characterization of pine nuts commercialized in Portugal

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Pine nut is the edible seed of the pine tree (*Pinus* spp.) and is an integral part of diet in the Mediterranean region. It is a dried fruit with great economic and social importance, but its commerce is being threatened by the introduction of pine nuts of lower quality, mainly of Asiatic origin. This work intended to evaluate the nutritional quality authenticity of different pine nuts sold in Portugal. For the purpose, a detailed morphological and chemical characterization was performed on 15 commercial samples.

The morphological characterization showed fruits high homogeneity, with an average length of 12.9 mm, 5.4 mm width, 4.3 mm thickness and 0.18 g mass, with a characteristic and homogenous color. The chemical assays showed that pine nuts are mainly composed of lipids, followed protein, with mean values of 48.9% and 30.6%, respectively. The fatty acid profile was found to be composed mainly of polyunsaturated fatty acids, with linoleic present with an average value of 47.2%. As to the antioxidants present, vitamin E was present (22.8 mg/100g on average) and the total phenolic compounds were estimated as being equivalent to 670 mg gallic acid per 100g on average.

These preliminary results revealed a highly homogeneous product. The morphology and chemical composition of the samples studied was also in accordance with literature data, being both probable indicators of high quality standards for the samples commercialized in Portugal.

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# Coffee residues as soil amendment: influence on crop's mineral profile

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Huge amounts of coffee residues are obtained after beverage preparation, without specific disposal strategies, being usually mixed with common garbage. Hence, recent studies focused on new possible reuse approaches for coffee by-products are being developed [1,2]. Due to recognized bioactivity of minerals, the effect of espresso spent coffee grounds, composted or not, in lettuce elemental profile was assessed in the present work, in order to define its potential applicability as soil amendment.

For the purpose, five spent coffee/vegetable soil mixtures were prepared: 2.5%, 5%, 10%, 15% and 20%. In parallel, after composting at Lipor, other five mixtures with composted coffee/vegetable soil were prepared: 5%, 10%, 15%, 20% and 30%, all on a volume basis. *Lactuca sativa* L. var. capitata cv. "Four Seasons" was used, using 15 lettuce plants per treatment and using plain vegetable soil as control (0%). Plants were harvested after 32 days, and both plants and soil samples were analyzed. After dry ashing and nitric acid/hydrochloric acid digestion, high-resolution continuum source atomic absorption spectrometry with flame atomization was selected for all mineral analyses (potassium, magnesium, calcium, sodium, iron, manganese, zinc, and copper), except for phosphorous which was analyzed by a standard vanadomolybdophosphoric acid colorimetric method.

The soil analysis revealed a progressive decrease of total mineral content and of all individual mineral elements, with the increase of spent coffee percentage, excepting potassium (which revealed an increment up to 13.7%). On the other hand, there was a notable increment of major elements in lettuce, particularly potassium, when low amounts of composted spent coffee, enhancing its nutritional features. These observations reinforce the viability of reutilizing this residue, in small amounts, for horticultural purposes.

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# Development of a flow-through extraction system for monitoring the release of antioxidants from solid food samples

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The assessment of antioxidant capacity of solid food samples is based on the extraction with a water-miscible organic solvent (methanol, ethanol or ethanol/water) and further determination of the antioxidant properties of the extracts by several chemical-based assays described in the literature [1]. The kinetic profile of the release of antioxidant compounds from solid food matrices is difficult to assess by batch-wise procedures because it requires stopping the agitation, followed by collection of an aliquot of the extraction solvent and its centrifugation prior to further analysis. Moreover, the reproducibility of time events is not straightforward when a large number of samples are analysed simultaneously. Therefore, these extraction procedures are limited to endpoint measurements, without any information about the release profile of antioxidants.

In this context, the main goal of the present work is to develop a flow-based dynamic extraction protocol to evaluate the kinetic profile of the release of antioxidant compounds from complex food matrices. For this, the food sample (particle size >250 and <355 µm) is placed inside a disposable extraction device and via software control, the extraction solvent percolates the sample at a fixed flow rate. Aliquots of the leachate are collected at different time intervals during 60 min and the antioxidant capacity is analyzed off-line by the spectrophotometric ABTS<sup>•+</sup> assay. Different cereal samples were tested as model of solid food samples, namely, corn, wheat, oatmeal, sesame seeds and other commercial breakfast cereals. Several extraction solvents were tested, namely, ethanol/water (50/50, v/v), water and solvents with chemical composition similar to those found in stomach and intestinal environments. The kinetic profile of antioxidants released from food samples in the first 10-30 min and the total antioxidant capacity determined was dependent on the composition of the extraction solvent used. Hence, the developed flow-through system based on a dynamic extraction and using a disposable extraction chamber has been shown as a useful tool to study the effect of the matrix composition in the release of bioactive compounds.

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# Evaluation of caffeine, 5-hydroxymethylfurfural and 5-O-caffeoylquinic acid contents of coffee silverskin

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Coffee silverskin is a thin tegument of the outer layer of green coffee beans. It is a major by-product of coffee roast industry since it is detached from the beans during torrefaction. This product is currently used as fuel (by direct burning), for composting and fertilization [1]. Some authors suggest an alternative use of coffee silverskin as a functional ingredient, based on its low concentration in lipids, high content in soluble fiber (~60%) and antioxidant activity [2].

The aim of this study was to ascertain caffeine, 5-O-caffeoylquinic acid (5-CQA) and 5-hydroxymethylfurfural (5-HMF) contents of coffee silverskin in order to contribute to a better knowledge of this by-product.

Silverskin from three industrial coffee batches (a pure robusta and two blends with both arabica and robusta coffees) were kindly provided by a national coffee torrefaction industry (BICAFÉ).

The compounds were extracted with a mixture of ethanol:water (1:1) at 40°C, for 60 min and analysed by RP-HPLC-DAD. The chromatograms were recorded at 280 nm for caffeine and 5-HMF, and at 330 nm for 5-CQA. The compounds were identified based on their UV spectra and by comparison with standards.

Coffee silverskin from the blends presented ~7.5 mg 5-HMF/100 g, while the remaining sample contained a significantly lower ( $p < 0.05$ ) amount (5.5 mg/100 g). Differently from what is described for coffee beverages [3], robusta silverskin also showed decreased amounts of 5-CQA (87 mg/100g) against ~245 mg/100g, for the other samples. Silverskin caffeine contents ranged from 1.1 to 1.5 g/100 g.

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## Flavonoids content and radical-scavenging activity in Portuguese onions: influence of storage time and freezing

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Onions (*Allium cepa* L.) are one of the world's oldest cultivated vegetables and can be regarded as exceptional dietary source of flavonoids, especially anthocyanin and flavonol glycoside forms. Flavonoids have been found to possess antioxidant, anticarcinogenic, antiasthmatic and antibiotic effects. Biochemical changes occur during storage of onions, however, little information is available regarding the effects of storage or exposure to specific stress conditions on changes of the flavonoids composition [1].

The aim of the present work was to compare the flavonoids composition in two Portuguese onions cultivars, "Branca da Póvoa" and "Vermelha da Póvoa". The effect of storage time, at room temperature in a ventilated hangar, on flavonoids composition and on radical-scavenging activity was evaluated. In addition, the effect of freezing on flavonoid content and radical-scavenging activity was assessed. Analyses of flavonoids were carried out by HPLC-DAD. The radical-scavenging activity was evaluated using the DPPH method and expressed as EC50.

Significant differences in total flavonoid levels were obtained between the two varieties of onions. Anthocyanins were detected mostly in red onions and their total concentration was statistically lower after 120 days of storage ( $p < 0.05$ ). Total flavonol content was statistically higher in red variety, and no significant differences were observed in flavonol levels during storage time of the two onions varieties. Radical-scavenging activity was statistically higher ( $p < 0.05$ ) for red onions, and no significant changes were observed during storage time. Total flavonoids content increased significantly after freezing the two onions varieties and their radical-scavenging activity was not significantly affected by storage time at low temperature. Cutting operation may cause a stress on vegetables increasing the production of flavonoids. In addition, freezing changes the structure of the tissue which makes flavonoids extraction easier, particularly those that are more non-polar.

The storage conditions are a relevant parameter on stability of flavonoids and subsequent nutritional quality of the onions.

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## Isoflavones in soy-based dietary supplements

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Isoflavones are a class of phytoestrogens, natural plant-derived compounds structurally similar to 17- $\beta$ -estradiol, capable of binding to estrogen receptors (ERs) and modulating hormone-dependent processes [1]. Epidemiological evidences suggest that they have positive effects in prevention of some forms of hormone-dependent cancers (endometrium, breast and prostate cancers), cardiovascular disease, osteoporosis, and adverse menopausal symptoms. Because of the possible health effects, a large number of isoflavone-based products are now available on the market, particularly for the management of menopausal symptoms. Soybean (*Glycine max* (L.) Merr.) is the main source of isoflavones for dietary supplements preparations. Soy products contain primarily daidzein, glycitein and genistein. Several studies have shown a great variability in marketed products regarding the concentration and source of isoflavones, stressing the need of standardization and quality control of these products, considering its therapeutic use.

The aim of this work was to quantify the isoflavones contents in soy-based dietary supplements and evaluate compliance of data supplied by the producers.

Eight different products (tablets and capsules) were purchased from local retail and health stores. Samples were ground to a fine powder and dispersed in C18 sorbent, cleaned-up with water and isoflavones eluted with methanol–water (9:1, v/v) [2]. Isoflavones were analyzed by RP-HPLC/DAD and quantified by the internal standard method. Daidzin, genistin, daidzein, glycitein and genistein were determined.

In most of the assayed samples, the amounts of isoflavones found were not in accord with the labeled values. The soy extracts in the supplements are standardized for total isoflavones; however, the amounts of individual isoflavones are reported in only three preparations. Since differences in the biological activity of the individual isoflavones are recognized, these variations may have a serious impact on the efficacy of soy-based supplements.

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# Optimization and Validation of a DLLME-GC-MS Method for Monitoring of Pesticides Residues in Tropical Fruits

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The world demand for tropical fruits is increasing each year due to their nutritional and exotical properties. According to Food and Agriculture Organization (FAO) the production of tropical fruits (excluding banana) in 2008 was 82,5 million tones and predicts an average growth of 2,4% per year until 2019 [1]. That way, it is convenient to control/monitor these commodities concerning to pesticides residues.

The key step in the analytical methods for determination of pesticide residues is the sample preparation, due to the low legally established levels and the complex nature of the matrices in which the target compounds are present typically in low amounts. Thus, the aim of this work is to optimize a gas chromatography-mass spectrometry method using a combination of QuEChERS and dispersive liquid-liquid microextraction (DLLME) as sample preparation. There are few works centered in pesticides residues analyses using QuEChERS procedure with DLLME [2], but none applied to monitorization of multiresidues analysis in tropical fruits. Initially, two sample preparation procedures were compared i) original QuEChERS based [3] and ii) QuEChERS-DLLME [2]. As expected the combination QuEChERS-DLLME resulted in higher peak areas for all the pesticide residues than those obtained by the QuEChERS procedure. This was expected because of the higher enrichment factor of DLLME. Special attention was then given on the optimization of the DLLME parameters namely the volume of dispersive and extractive solvents and procedure of agitation (e.g. ultrasounds versus hand). The developed method was used to assess the occurrence of 21 different pesticide residues in mango and banana.

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# Optimization of a GC-MS method for determination of type A and B-trichothecenes in Cereals and Cereal-based Products

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Trichothecenes (TRC) are a large group of mycotoxins, mainly produced by molds belonging to *Fusarium* genera. They are divided into four groups (types A, B, C and D) according to their characteristic functional groups. The most relevant in food and feed belongs to type A, such as T-2 toxin (T-2), HT-2 toxin (HT-2) and diacetoxyscirpenol (DAS), and type B, like deoxynivalenol (DON) and nivalenol (NIV) [1]. These toxins infect agricultural crops throughout the world, particularly cereals and cereal-based products and are recognized as a potential hazard for human and animal health, responsible for a wide range of toxic effects, such as immunosuppression, mutagenicity, neurotoxicity, gastrointestinal toxicity, anemia, etc. [2].

The aim of this work was the optimization of an analytical procedure based on GC-MS for the simultaneous determination of 12 TRC, belonging to type A and B. In order to improve the derivatization step, various reagents were tested, with different reaction conditions: i) HFBA (heptafluorobutyric anhydride) at 80°C during 40 min; ii) TFAA (trifluoroacetic anhydride), 80°C 30 min; iii) PFPA (pentafluoropropionic anhydride) at 60°C for 60 min and iv) Tri-Sil TBT(BSA(bis(trimethylsilyl)acetamide):TMSI(trimethylsilylimidazole):TMCS(trimethylchlorosilane),(3:3:2)) at 80°C from 30 min. The best results were obtained with the mixture of silylating agent (iv), which allowed the derivatization of all mycotoxins of interest contrary to the other reagents studied. Then, to lessen the time required for the derivatization step, a household microwave was tested at medium potency with different reaction times (3, 5 and 7 min). All reaction times tested are able to derivatize all the analytes, but the best signal was obtained with 3 min.

In conclusion, it was successfully reached an improvement on derivatization step of trichothecenes, which allows the reduction of time spent in sample pre-treatment process before GC-MS analysis.

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## Senegalese sole fed experimental diets with fish oil replacement: flesh lipid content and fatty acid profile

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Senegalese sole (*Solea senegalensis* Kaup, 1858) is a highly appreciated fish and a strong candidate for intensive aquaculture in Mediterranean countries. The current demand for fish oil has resulted in a high pressure for dietary substitution by alternative lipid sources, but the incorporation of vegetable oils has never been tested in experimental diets for market-size Senegalese sole. The main goal of the present work was to assess the impact of fish oil replacement by vegetable oils in fish diets on the lipid content and fatty acid (FA) profile of Senegalese sole muscle, mainly its nutritional value regarding omega-3 polyunsaturated fatty acids (PUFA).

Fish were fed with 4 experimental diets, containing several degrees of fish oil replacement by vegetable sources (FO diet containing fish oil; a diet with a 50% substitution of fish oil by vegetable oils (VO50); a diet with a 100% substitution of fish oil by vegetable oil (VO100) and a diet with a concomitant replacement of 50% fish meal and 50% fish oil (VO50 PP50), during 140 days. After that, a period of “washout” was achieved by feeding all groups with FO diet (control) during 26 days in order to standardize their FA profile. Muscle, liver and skin lipid content was determined and muscle FA profile determination was achieved by GC-FID after sample methylation [1].

By the end of the trial, total lipids in muscle (2.0-2.8%) were similar among the different groups while in liver (15.6-27.8%) and skin (2.9-4.8%) the fat content was significantly different among groups ( $P < 0.05$ ). Despite some significant differences, no major changes were found in muscle fatty acid profile, which means that the main edible portion of the fish preserves its lipid composition, remaining as a very important source of PUFA, such as the long chain n-3 fatty acids, EPA (20:5 n-3) (1.61-2.83%), DPA (22:5n3) (3.44-4.70%) and DHA (22:6 n-3) (15.11-17.76%). After the “washout” period few differences were found among FA’s between groups and higher levels of n-3 PUFA were noticed. In conclusion, Senegalese sole can reach the market-size with blends of vegetable oils without compromising muscle lipid content and fatty acid profile.

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# Translation and exploratory application of a food and nutrition knowledge questionnaire

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Good nutrition knowledge would help consumers make more informed food choices. This knowledge may promote consumers health, considering that a good nutrition contributes to general well-being and disease prevention. Therefore, it is important to measure dietary knowledge by developing nutrition knowledge tools or evaluating existing nutritional knowledge questionnaires to predict nutrition knowledge accuracy and prevent nutrition-related health problems. The present study aimed to translate into Portuguese and validate a food and nutrition knowledge questionnaire, proposed by Turrell and Kavanagh [1]. This questionnaire comprises 20 statements requiring true/false answers which included a “don’t know” response option. These statements cover a diverse range of issues pertaining to food, nutrition and health, and their interrelationships, to reflect a person’s general knowledge. Correct answers were coded as “1” and incorrect and ‘don’t know’ responses to “0”. The 20 statements from the original questionnaire were independently translated into Portuguese by the investigators and a consensus version was generated. Subsequently, back translation was performed and compared with the original English version. Discrepancies were discussed and solved. A pilot study, for general interpretability was carried out with a convenience sample of 29 respondents. Since there were no doubts or questions during the administration, a final Portuguese version was established. Aiming for future validation of the translated questionnaire, it was applied to a sample of Portuguese consumers (n = 146) (64% female and 36% male), aged between 18 and 71 years old. Respondents in general, have shown a high nutrition knowledge value (mean total score  $16.6 \pm 2.3$ ), with no significant effect of gender or age. 3.4% of the sample answered correctly all the 20 questions. Concluding, the questionnaire was successfully translated into Portuguese and the exploratory administration showed that respondents had a generally high knowledge regarding food and nutrition. This study will be further developed through the application within a larger sample and repeated application to evaluate the stability and validity of the questionnaire.

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Posters

**Thursday, 14<sup>th</sup>**



## Effects of antiepileptic drugs on human osteoclastogenesis

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Bone is a rigid and dynamic mineralized tissue that is constantly being remodeled. Bone remodeling is important for the regulation and maintenance of skeletal mass, and it involves resorption and formation events, accomplished by the osteoclasts and osteoblasts, respectively. Epilepsy is a neurologic condition, which may affect bone health in a number of ways. It has long been observed that treatment with antiepileptic drugs (AEDs) might be associated with changes in bone mineral density. Nevertheless, although this is one of the adverse effects of AEDs, the knowledge regarding their effects on bone cells are still very scarce, particularly on osteoclastic behaviour. In this context, the aim of this study was to investigate the effects of five different AEDs on human osteoclastic cells.

Osteoclastic cell cultures were established from precursor cells isolated from human peripheral blood, and were maintained in the absence (control) or in the presence of  $10^{-8}$ - $10^{-4}$  M of different AEDs (valproate, carbamazepine, gabapentin, lamotrigine and topiramate). Cell cultures were characterized throughout a 21-day period for tartrate-resistant acid phosphatase (TRAP) activity, number of TRAP+ multinucleated cells, presence of cells with actin rings and expressing vitronectin and calcitonin receptors, and apoptosis rate. Also, the involvement of several signaling pathways on the cellular response was addressed.

It was observed that the tested drugs exhibited different profiles on their osteoclastogenic modulation properties, with a global tendency to inhibit the process. Furthermore, the signaling pathways involved in the process also seemed to be differentially affected by the AEDs.

In conclusion, the present study showed that the different AEDs had the ability to negatively modulate the osteoclastogenesis process, shedding new light towards a better understanding of how these drugs can affect bone tissue.

# **Effects of chronic alcohol consumption and withdrawal on NPY-containing neurons and cholinergic innervation of the hippocampal dentate hilus**

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It is well known that chronic alcohol consumption induces profound morphological alterations in several brain regions, and the hippocampal formation appears to be particularly vulnerable. Furthermore, withdrawal from ethanol, instead of stopping these alterations, tends to aggravate the ethanol-induced effects. In the present work, we have analyzed the effects of prolonged alcohol consumption and withdrawal on neuropeptide Y (NPY) expression and on the cholinergic innervation of the hippocampal dentate hilus. We choose NPY because it has important roles in the regulation of various functions, including circadian rhythms and cognition. In addition, there is evidence that the expression of this neuropeptide depends on the trophic support provided by the cholinergic system. The hippocampus receives a strong innervation from basal forebrain cholinergic neurons, which depend on the nerve growth factor (NGF) for phenotype maintenance. Therefore, we have also evaluated if the administration of NGF would interfere with the content of NPY and the cholinergic innervation of the hippocampal dentate hilus.

Male Wistar rats were assigned to control (n=5), ethanol-treated (n=15), withdrawn (n=5) and NGF-treated withdrawn (n=5) groups. At the end of the experiments, rats were transcardially perfused and the brains serially sectioned in the coronal plane. From each brain, three adjacent series of sections were separately collected. The first and the second series were used for NPY and vesicular acetylcholine transporter (VACHT) immunostaining, respectively, whereas the third was used for Nissl staining. The densities of NPY containing neurons and of cholinergic varicosities were estimated in the hilus of dentate gyrus. The handling and care of the animals followed the Principles of Laboratory Animal Care (NIH Publication No. 86-23, revised 1985) and the European Communities Council Guidelines in Animal Research (86/609/UE). All efforts were made to minimize the number of animals used and their suffering.

It was found that withdrawal, but not chronic alcohol consumption, increased the number of NPY-containing neurons in the hilus when compared to control rats. Conversely, chronic alcohol consumption markedly reduced the density of VACHT varicosities in the hilus, and withdrawal aggravated it. Interestingly, NGF treatment augmented the density of VACHT varicosities to values higher than in controls, but did not increase the density of NPY-containing neurons.

These results show that chronic alcohol consumption reduces the density of cholinergic varicosities but does not affect the density of NPY-containing neurons in the hilus. Furthermore, it was also demonstrated that alcohol withdrawal aggravates the ethanol-induced reduction of the cholinergic varicosities density and increases the density of NPY neurons. This study also reveals that NGF administration to withdrawn rats increases the cholinergic varicosities density to super-normal values and reestablishes NPY density.

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# Effects of Chronic Ethanol Treatment and Withdrawal on the Neuropeptide Y Content and Cholinergic Innervation of the Rat Somatosensory Cortex

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It is widely accepted that chronic ethanol treatment (CET) and withdrawal (W) can modify the morphology and disturb the function of the central nervous system. Among the brain systems vulnerable to ethanol intake and withdrawal are the neuropeptide Y (NPY)-ergic and the cholinergic systems. NPY regulates several physiological functions such as learning and memory, circadian rhythms and sleep, feeding behavior and emotion. It is abundantly expressed in various brain regions, including the cerebral cortex. The cerebral cortex receives a dense cholinergic innervation from projection neurons of the basal forebrain and there are studies suggesting a trophic dependence of NPY cortical neurons on this input. Forebrain cholinergic neurons are dependent on nerve growth factor (NGF) for phenotype maintenance and there is evidence that exposure to ethanol and W alters the NGF neurotrophic support. Thus, we examined the effects of CET and W on the density of NPY-immunoreactive (NPY-ir) neurons and cholinergic varicosities in layers II/III, V and VI of the primary somatosensory cortex (SI) of the rat. We have also investigated if the administration of NGF would interfere with the content of NPY and the cholinergic innervation of this cortical area.

A total of 20 Wistar male rats were used. Rats were assigned to control (n=5), ethanol-treated (n=15), withdrawn (n=5) and NGF-treated withdrawn (n=5) groups. At the end of the experiments, rats were perfused transcardially and the brains serially sectioned in the coronal plane. From each brain, four adjacent series of sections were separately collected. The first and the second series were used for NPY and vesicular acetylcholine transporter (VAcHT) immunostaining, respectively, whereas the third was used for Nissl staining. The brain sections were analyzed in order to estimate the areal density of NPY-ir neurons and of cholinergic fiber varicosities in the SI.

Our estimates show that the density of NPY-ir neurons and cholinergic varicosities in the SI are altered neither by CET nor by W. Our data also show that NGF treatment of withdrawn rats significantly increases the expression of NPY in layers V and VI relative to control, ethanol-treated and withdrawn rats. Furthermore, we have also found that the density of cholinergic varicosities is significantly higher in NGF-infused withdrawn rats than in control, ethanol-treated and withdrawn rats.

Our results might point toward the existence of region specificity in the effects of CET and W on the cortical NPY-ergic and cholinergic systems. This study also reveals that NGF, administered to withdrawn rats, increases to super-normal levels both the NPY content of layers V and VI and the density of cholinergic varicosities of all analyzed layers of the SI. Our results might be of importance for understanding the still cryptic role of the cortical NPY-ergic and cholinergic systems in various functional and behavioral alterations associated with CET and W as well as the potential therapeutic role of NGF in the treatment of these changes.

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# Effects of proton pump inhibitors on human osteoblastic cells

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Proton pump inhibitors (PPIs) are widely used in many pathological conditions, like, for example, gastroesophageal reflux, dyspepsia and peptic ulcers. Their mechanism of action relies in the inhibition of H<sup>+</sup>/K<sup>+</sup> ATPases, responsible for gastric acid secretion. It has been suggested that the consumption of this class of drugs might be associated with alterations in normal bone metabolism. Although the osteoclast might represent a possible bone target of PPIs action, osteoblasts may also be affected by the presence of these drugs. In this context, this work intended to characterize the effects of three PPIs on human osteoblast differentiation.

Human bone marrow cell cultures, performed in conditions that favor osteoblastic differentiation, were treated with different concentrations (10<sup>-7</sup>-10<sup>-3</sup> M) of omeprazole, esomeprazole and lansoprazole. Cell cultures were characterized throughout a 21 day period for cell viability/proliferation, alkaline phosphatase (ALP) activity and histochemical staining and apoptosis rate; cell response to PPIs was also addressed regarding several intracellular signaling pathways (MEK, NFκB, PKC and JNK).

The tested PPIs elicited a dose-dependent inhibition of osteoblast proliferation and differentiation, at levels higher than 10<sup>-6</sup> M. The percentage of inhibition was higher for ALP activity compared to that on cell growth. The inhibitory effect was more pronounced for omeprazole and lansoprazole. PPIs showed differences on their effects regarding the affected intracellular signaling pathways.

Taken together, PPIs have the ability to decrease human osteoblastogenesis, in a dose-dependent way, an effect that might contribute to the eventual deleterious effects of these drugs in the bone tissue.

## Endogenous and exogenous adenosine and the distribution of adenosine receptors in the glomeruli of SHR diabetic rats

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Adenosine regulates a wide range of physiological functions in the kidney [1]. Diabetes damages the kidney [2]. Most diabetic patients also have hypertension, aggravating the prognosis [3]. This work aimed at studying the role of endogenous/exogenous adenosine in the distribution of adenosine A<sub>1</sub> and A<sub>2B</sub> receptors in the glomeruli of spontaneously hypertensive rats (SHR) with streptozotocin (STZ)-induced diabetes.

On day 0, male SHR rats (12 weeks) were i.p. injected with STZ (65 mg/kg). On day 14, diabetic animals were anesthetized with pentobarbital sodium (65 mg/kg), osmotic minipumps were i.p. implanted for continuous infusion (10 µl/h; 7 days) of 2-choroadenosine (5 mg/kg/d; CADO group) or 1,3-dipropyl-8-sulfophenylxanthine (90 µg/kg/h; DPSPX group) or underwent a sham-operation (STZ group). On day 21, animals were reanesthetized and the left kidney was removed, fixed in formalin and included in paraffin. Sections (4 µm) were incubated with anti-A<sub>1</sub>/anti-A<sub>2B</sub> primary antibodies, the resulting immuno-complexes detected with a biotinylated secondary antibody, revealed using ABC/DAB and imaged using bright field optics on a microscope and acquired using a CDD camera connected to a computer.

In all groups, adenosine A<sub>1</sub> receptor immunoreactivity was located in mesangial cells whereas adenosine A<sub>2B</sub> receptor immunoreactivity was mainly observed in podocytes but also in mesangial cells. Immunoreactivities for both receptors were more marked in superficial than in deep glomeruli. CADO treatment only slightly decreased the immunoreactivities in mesangial cell of deep glomeruli. DPSPX treatment didn't alter adenosine A<sub>1</sub> receptor immunoreactivity but decreased A<sub>2B</sub> receptor immunoreactivity in podocytes and particularly in mesangial cells.

Results, only qualitative for now, suggest that exogenous adenosine doesn't influence adenosine receptors' expression in the glomeruli. However, endogenous adenosine is relevant for adenosine A<sub>2B</sub> receptor expression both in podocytes and mesangial cells.

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# Estradiol and progesterone effects on the total number of estrogen receptor $\alpha$ -immunoreactive neurons in the MPN of female rats

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The medial preoptic nucleus (MPN) is a sexually dimorphic nucleus responsible for the coordination of behavioral and physiological functions related with reproduction. It is well established that a great proportion of MPN responses are modulated by gonadal steroids, namely estradiol and progesterone, through their cognate receptors [1]. MPN neurons express both types of nuclear estrogen receptors (ER),  $\alpha$  and  $\beta$  [2]. In this study we focus on the ER $\alpha$ , the activation of which in the MPN is known to modulate reproductive and non-reproductive functions. Very few studies have investigated ER $\alpha$  expression in response to hormonal administration in the MPN of female rats. Furthermore, most of the reports have measured mRNA ER $\alpha$  levels by in situ hybridization techniques and, therefore, it is not known if the number of ER $\alpha$ -positive neurons varies as a function of hormonal levels. The aim of this study was to evaluate the effect of estradiol and progesterone on the total number of ER $\alpha$ -immunoreactive (ER $\alpha$ -ir) neurons in the MPN of female rats. In order to accomplish such goal, 3 months-old female Wistar rats were ovariectomized and then ascribed to one of the following treatments: (1) oil, (2) estradiol-benzoate (EB) and (3) estradiol-benzoate plus progesterone (EB+P). The rats were perfused and the brains were sectioned and processed for immunohistochemistry using a rabbit anti-ER $\alpha$  antibody. ER $\alpha$ -ir neurons in the MPN were counted using a stereological method – the optical disector. The results were statistically analyzed, using a one-way ANOVA. Our results show a slight increase in the total number of ER $\alpha$ -ir neurons in the MPN of the EB group, relatively to the oil and EB+P groups. Interestingly, the number of ER $\alpha$ -ir neurons in the oil and EB+P groups did not differ. The results indicate that EB seems to have the capacity of inducing the expression of its own receptor in the MPN. On the other hand, the subsequent administration of P seems to induce the down-regulation of the ER $\alpha$ , as opposed to what happens in other the regions of the brain that also express the ER $\alpha$ . Because the MPN is involved in the modulation of the female sexual behavior, this may be a subcellular mechanism of hormone action underlying this behavioral answer. In conclusion, this work may shed new light on the influence of exogenous estradiol and progesterone administration on the expression of ER $\alpha$  in neurons involved in the coordination of a particular component of an important behavioral answer. These results may help unravel the underlying cellular mechanisms of important female behaviors.

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# Estrogen effects on the total number of ER $\alpha$ -immunoreactive neurons of the principal division of the bed nucleus of the *stria terminalis*

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The bed nucleus of the *stria terminalis* (BNST) is a relay nucleus of the rostral forebrain closely related to the amygdala. It is extensively connected to some preoptic and hypothalamic nuclei that are involved in the neuroendocrine regulation of the reproductive behavior [1]. The effects of estrogens on the principal division of the BNST (BNSTpr) are essential for its role in the regulation of sexual and defensive behaviors. The BNST is known to express abundantly both types of nuclear estrogen receptors (ER) and their expression is modulated by hormone levels [2,3].

The aim of this study was to evaluate the effect of steroid hormones on the total number of ER $\alpha$ -immunoreactive (ER $\alpha$ -ir) neurons in the BNSTpr of young adult female Wistar rats. Ovariectomized rats were treated with vehicle, estradiol benzoate (EB) or estradiol benzoate plus progesterone (EB+P). After these treatments, the animals were perfused and the brains sectioned. The BNST-containing sections were processed for immunohistochemistry using an anti-rabbit ER $\alpha$  antibody. The estimates of the total number of neurons were obtained by using stereological methods. The results were statistically analyzed using a one-way ANOVA.

After the administration of estradiol there was a tendency towards an increase in the total number of ER $\alpha$ -ir neurons in the BNSTpr. Interestingly, the administration of estradiol followed by progesterone did not significantly change the total number of ER $\alpha$ -ir neurons relative to the estimated in rats that were treated only with estradiol.

These results suggest that ovarian hormones have the ability to modulate the expression of ER $\alpha$  in the neurons of the BNSTpr and, in so doing, they may regulate neuronal inputs delivered to the circuits that control the behavior. This may be a way to the hormone control of the relay mechanism of the BNSTpr for the olfactory information to the hypothalamic nuclei involved in the control of sexual behavior.

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## Evaluation of oral *Staphylococcus* colonization in chronic kidney disease patients undergoing Peritoneal Dialysis.

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Infections related with peritoneal dialysis (PD) continue to be a serious complication for PD patients.<sup>[1]</sup> The source of these infections is continuously studied and some relation is described with microorganisms of the normal microbiota.<sup>[2-4]</sup> To evaluate the oral, nasal and peritoneal catheter exit-site staphylococci colonization of PD patients and identify a possible relation between staphylococci peritonitis and exit-site infections' agents and normal staphylococci microbiota. Also, the prevalence of oral staphylococci colonization in a chronic kidney disease (CKD) population undergoing PD was compared to a healthy population.

Clinical and demographic information was collected from 27 PD patients and 18 family member controls. Also a non-invasive intra-oral examination was performed and saliva was collected for microbial analysis. In addition nasal and exit-site swabs were collected from PD patients for microbial analysis. Microbial analysis consisted in isolating staphylococci by culturing samples in Mannitol-Salt agar and identifying by API 32 STAPH multi-test system.

The patients evaluated were on average on PD for 13 months, 37% had previously one peritonitis episodes and 48.1% had previously one or more exit-site infection episodes. PD patients as well as controls had a low education level, bad or regular oral hygiene and high DMFT index. In comparison to controls, PD patients presented higher saliva pH but similar salivary flow. Regarding colonization, all PD patients and controls presented saliva staphylococci colonization. Additionally, in PD patients, staphylococci were present in 96% of nasal and 37% of exit-site swabs. The saliva staphylococci load and species did not differ between PD patients or controls. Interestingly, *S. aureus*, *S. warneri* and *S. epidermidis* were the most frequent colonizers in saliva, nose and exit-site catheter mucosa. No previous peritonitis agent was current a colonizer of PD patients whereas 21.4% of previous exit-site catheter agents were current part of normal microbiota of the patient.

Oral health and saliva staphylococci colonization is similar between PD patients and healthy controls. The most common staphylococci colonizers in saliva, nasal cavity or exit-site catheter mucosa of PD patients are in correspondence to the most frequent staphylococci agents of exit-site catheter infections and peritonitis.

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# Evaluation of phytochemicals and antioxidant activity of aqueous, ethanolic-aqueous (50:50) and ethanolic extracts of thirteen species of *Medicago*

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**Introduction:** By-products of plant food processing represent a major disposal problem for the industry concerned, but they are also promising sources of compounds with favourable technological or nutritional properties [1]. A great deal of effort has been expended to utilize different by-products wastes in biogas and energy production, as food ingredients, and speciality chemicals. *Medicago* is an example of this kind of by-products. *Medicago* genus includes about 56 species distributed in the Mediterranean climatic conditions. The most known species is commonly called Alfafa (*Medicago sativa*) and is the main *Medicago* species widely grown throughout the world, predominantly as a source of high quality forage for livestock, renewable energy production, phytoremediation and a source of phytochemicals. The aim of this work is the evaluation of the antioxidant activity of ethanolic, ethanolic-aqueous and aqueous extracts of 13 *Medicago* species by different methodologies. The antioxidant properties of these extracts were evaluated using DPPH radical, FRAP assay, Folin and flavonoids.

**Methodology:** *Medicago* species (*M. litorallis*, *M. minima*, *M. murex*, *M. orbicularis*, *M. tornata*, *M. truncatula*, *M. arabica*, *M. doliata*, *M. polymorpha*, *M. rigidula*, *M. Scutelata*, *M. segitalis* and *M. sativa*) were sown and collected. The powdered leaf (1 g) were submitted to solvent extraction by maceration with ethanol, ethanol:water (50:50) or water (20 ml each solvent) at a temperature of 40°C for 30 minutes. Extracts were filtered through a Whatman No. 1 filter paper and the filtrates collected. Aqueous filtrates were freeze-dried and organic filtrates reduced to residue using a rotary evaporator set to 37 °C. Samples were stored at 4 °C until required for analysis. The antioxidant activity of the extracts was assessed by standard methods based on electron transfer reactions, such as Folin-Ciocalteu (F-C), 2,2-diphenyl-1-picrylhydrazyl (DPPH•), Ferric Reducing Ability Plasma (FRAP) and flavonoids. . The evaluation of the reaction was carried out by spectrophotometry.

**Results:** The antioxidant activity of the thirteen different species of *Medicago* was determined. The highest antioxidant activity and the higher amounts of total phenols were observed for different species. The most efficient solvent was the mixture ethanol:water (50:50) for most species. This study confirmed the antioxidant potentials of different species of *Medicago*.

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## Evaluation of the structure-activity relationship of rifabutin and analogues: a drug-membrane study

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Tuberculosis (TB), an infectious disease caused by *Mycobacterium tuberculosis* (MTb), is still a major health concern, partly due to its impact in HIV-positive people. According to the World Health Organization (WHO), in 2011 there were 8.7 million incident cases of TB, 1.4 million deaths from TB among HIV-negative people and an additional 0.43 million deaths from HIV-associated TB [1]. Consequently, the development of new drugs is required, especially compounds that are able to fight multi drug resistant bacteria (MDR-MTb). Rifabutin (RBT) is one of the most efficient antibiotic used in TB/HIV co-infection and has been useful against some isolates of MDR-MTb. Hence the development of RBT analogs, such as N<sup>7</sup>-acetyl-rifabutin (RFB2) and N<sup>7</sup>-(butyl-3'-enoyl)-rifabutin (RFB3), is a promising way to reveal new and more effective MDR-MTb therapeutics [2]. In this work, the influence of RFB and its analogues (RFB2 and RFB3) on the biophysical properties of the lipid membranes was evaluated. For that purpose, monolayers and multilamellar vesicles (MLVs) composed of egg L- $\alpha$ -phosphatidylcholine (EPC):cholesterol (CHOL) 4:1 (molar ratio) were chosen to mimic the biological membranes. Several accurate biophysical techniques were used to establish a putative relationship between the chemical structure of the antimycobacterial compounds and their activity on the membranes. Therefore, a combination of *in situ* experimental techniques as Langmuir isotherms, Brewster angle microscopy (BAM), polarization-modulated infrared reflection-absorption spectroscopy (PM-IRRAS) and small-angle X-ray scattering (SAXS) were carried out to assess the drug-membrane interaction. The establishment of a relationship between drugs' effect on the membranes and their chemical structure were found and might be useful in the development of new drugs with a higher efficacy and less toxic effects.

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## Expanding our therapeutic options: adrenergic beta-blockers for colon cancer?

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Colon cancer is a leading cause of cancer and cancer deaths in the Western world, and its incidence is increasing. Stress response has been linked to the increased incidence and development of cancer [1]. The catecholamines (CA), adrenaline (AD) and noradrenaline (NA), are crucial mediators of stress response, exerting their effects through interaction with alpha- and beta- adrenergic receptors (AR). The expression of beta-AR has been identified on colon cancer cells and their activation has been implicated in carcinogenesis and tumor progression [2]. Recently, interest in the efficacy of beta-AR blockers as possible additions to cancer treatment paradigms has been gain strength. The aim of this work is to investigate the effects of several adrenergic agents, including beta-blockers, upon cellular proliferation and viability, and on CA handling by human colon adenocarcinoma cell line HT-29. For this purpose, in the first phase of this work, cells were incubated in the absence (control) or in the presence of the AR-agonists, AD (0.10-10  $\mu$ ), NA (0.1-10  $\mu$ M) or isoprenaline (ISO) (0.1-10  $\mu$ M) for 12h or 24h. To assess the effect of these agents on HT-29 cell proliferation, we have used both <sup>3</sup>H-thymidine [3] and 5' bromodeoxyuridine (BrdU) [3] incorporation assays and also the MTS test [3]. For comparison between two groups, Student's *t*-test was used. Differences were considered to be significant when  $P < 0.05$ .

<sup>3</sup>H-thymidine assay showed that chronic treatment with AD led to a significant increase of cell proliferation by 18% ( $p=0.016$ ;  $n=8$ ) and 29% ( $p=0.009$ ;  $n=9$ ), when used at 0.1 $\mu$ M and 1 $\mu$ M, respectively, whereas treatment with NA (10  $\mu$ M) increased the proliferation by 34% ( $p=0.010$ ;  $n=6$ ), relatively to controls. The most potent AR agonist in this effect was ISO, enhancing proliferation by 39% comparing to control ( $p=0.010$ ;  $n=7$ ). The BrdU assay confirmed that AD and NA (both at 0.1 $\mu$ M) induced a significant increase of cell proliferation to about 131% ( $p=0.010$ ;  $n=9$ ) and 119% ( $p=0.001$ ;  $n=9$ ) of controls, respectively. Similarly to the above proliferation tests, MTS also showed that AD (1 $\mu$ M) and NA (10  $\mu$ M) induced a significant increase of cell proliferation to about 134% ( $p=0.010$ ;  $n=6$ ) and 150% ( $p=0.037$ ;  $n=9$ ) of controls, respectively. These results suggest that adrenergic activation may play an important role in colon cancer carcinogenesis. Next step will be the evaluation of beta-blockers effects upon proliferation previously induced by AR activation. The elucidation of which of these drugs are the most effective in reverting CA-induced proliferation in colon cancer cells might contribute to reveal promising strategies in cancer treatment.

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# Extended-spectrum beta-lactamase producing coliforms in the fecal flora of bovines

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Food production is one of the major questions in actual societies. Bacterial resistance to antibiotics is a growing problem and animal production might play an important role in bacterial resistance spread. Animal husbandry represents an important veterinary challenge in terms of animal welfare and economic profit. Milk production in association with meat production represents a relevant activity of some regions of the country. Animal infectious diseases pose serious threats to animal producing facilities. Bacterial infections need appropriate therapeutics that includes antimicrobial agents of the same type of those used for humans. The concept one health includes animals in this approach, to antimicrobial resistance spread. Specific third generation cephalosporins are common veterinary antimicrobials that might contribute for extended-spectrum beta-lactamase (ESBL) producing isolates selection in bovine fecal flora. This reality might create a risk for community spread of ESBL producing *Escherichia coli* [1].

The aim of our study was the detection of extended-spectrum producing coliforms in bovine fecal flora.

We studied three animals from two different producing facilities in the North of Portugal. One of them was a familiar production, located in a rural region, with only two animals, which lived independently. The sample collected from this facility belongs to an animal without a story of illness or antibiotherapy. The other one was a production with around thirty bovines. We analyzed two samples from different randomly selected animals from this facility.

Fecal samples were suspended in Tryptic Soy Broth (TSB) and incubated at 37° overnight. Isolates were selected by spreading 100 µl on MacConkey agar and on MacConkey agar with ceftazidime, cefotaxime, aztreonam, and meropenem. Colonies were randomly selected and susceptibility to antimicrobial agents was determined by the agar diffusion test, according to the Clinical Laboratory Standards Institute (CLSI) and screened for extended-spectrum beta-lactamases (ESBL) production by the double disc synergy test and clavulanic acid addition, according to the CLSI guidelines. Identification of the selected strains was achieved by ID 32 GN and API 20 E.

We found ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* isolates, in the fecal flora of two of the screened animals belonging to the bigger production.

This finding might influence colonization of facility staff and meat contamination by these isolates in abattoirs with the consequent impact in human colonization by meat consumption and manipulation.

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# Extended-spectrum beta-lactamase producing coliforms in the fecal flora of companion animals in the North of Portugal

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The change in the relationship between humans and companion animals is marked by the increasing intensive care provided to companion animals in veterinary medicine. Recent reports emphasize that human contact with pets increases the chance to be colonized with extended-spectrum beta-lactamase (ESBL) producing *E. coli*. This might contribute to the expansion of a reservoir of ESBL producing *Enterobacteriaceae* in the community [1]. The emergence of nosocomial infections in small animal clinics might be one of the reasons for this evolution, especially in terms of multidrug-resistance and potentially zoonotic pathogens [2].

The aim of our study was the detection of ESBL producing coliforms in the fecal flora of companion animals in the North of Portugal.

For that purpose, fecal samples from three dogs and two cats were analyzed. Samples were inoculated in TSB (tryptic soy broth) and incubation at 37°C overnight. From these broths, 100 µl were plated on MacConkey agar with and without oxiiimino-beta-lactam antibiotics. Selected bacterial colonies were analyzed for their morphology and lactose fermentation. Lactose fermenters were subjected to susceptibility testing according to the CLSI guidelines and ESBL production was screened by the double disk synergy test. ESBL producers were studied for associated resistance by testing antimicrobials of other families. Bacterial identification was achieved by traditional methods, API 20E and ID 32 GN.

We found three dogs and one cat colonized with ESBL producing *E.coli*. Of those, one dog was a domestic one, without veterinary contacts in the last years. Fecal samples of the three other animals were obtained from a veterinary clinic.

These findings alerts for a potential source of community human colonization with antimicrobial resistant opportunistic pathogens of unknown consequences in community spread and infection risk for colonized people.

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## Fetal gender and perinatal outcome. The Portuguese situation.

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**Introduction:** Knowledge of the influence of fetal gender in fetal and neonatal mortality and morbidity may lead to more adequate gender-specific healthcare interventions. It may also help to understand how sex-ratio-specific socio-demographic changes may occur [1]. However, although there is a consensus that there is a general perinatal female advantage over males, there is contradictory data, among different centres and countries, pertaining to some situations, such as fetal growth restriction, among others [1]. The aim of this communication was the assessment of the influence of fetal gender in perinatal outcome, in Portugal.

**Material and Methods:** Fetal,  $\geq 22$  weeks and  $\geq 28$  weeks, neonatal  $< 7$  days and perinatal mortality rates and main perinatal disease rates were assessed in the Portuguese population using a database produced by the Portuguese Institute of Statistics – Instituto Nacional de Estatística (INE), pertaining to year 2005 [2]. Calculations were performed both at the national and regional level. For statistical analysis, relative risks (RR) with 95% confidence intervals (95% CI) were also estimated.

**Results:** Overall, fetal mortalities  $\geq 22$  weeks and  $\geq 28$  weeks were higher among males than females (3.7/1000 *versus* 3.3/1000 and 2.9/1000 *versus* 2.7/1000, respectively), whereas perinatal and neonatal mortality rates, were lower among males than females (5.0/1000 *versus* 5.1/1000 and 1.6/1000 *versus* 1.9/1000, respectively). However, the RR of male *versus* female fetus, perinatal and neonatal deaths were not statistically significant: RR ranging between 0.89 (95% CI:0.66-1.20) for neonatal death, and 1.09, (95% CI:0.90-1.34) for fetal death  $\geq 22$  weeks. The RR of fetal death was higher among males in Madeira, Alentejo, Centre of Portugal and Lisbon: RR ranging between 2.42 (95% CI:0.86-6.80) in Madeira and 1.09 (95% CI:0.78-1.50) in Centre. Whereas it was lower in the North of Portugal, Azores and Algarve: RR ranging between 0.95 (95% CI:0.65-1.39) in the North, and 0.58 (95% CI:0.26-1.28) in Algarve. Fetal malformations, prematurity and fetal growth retardation were more common among females than males, whereas perinatal asphyxia was more frequent among males: RR=0.70, (95% CI:0.39-1.23) and RR=1.30, (95% CI:0.41-4.11), respectively. **Conclusions:** The study findings should be interpreted with caution, as they are based on a single year retrospective national registry, with limited data, that should be confirmed in future prospective studies. Nonetheless, the Portuguese situation evidences that there may be significant national and regional differences regarding fetal gender and perinatal outcomes. This finding should be in the mind of the perinatal care providers and researchers.

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## Flavonoids as potential small-molecule inhibitors of p53-MDM2 interaction revealed by a yeast-based assay

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The p53 tumor suppressor protein has a crucial role in the regulation of cell cycle and apoptosis. Therefore, p53 is considered a key protein in cancer development. The activity and stability of p53 are regulated by the endogenous negative regulator, MDM2. Hence the discovery of activators of p53 and inhibitors of p53-MDM2 interaction is considered a promising strategy for cancer treatment [1].

Flavonoids represent an outstanding subclass of plant secondary metabolites, which have long been recognised for their pharmacological properties, being the antitumor activity one of the most extensively studied [2]. Previous studies from our research group have shown potent growth inhibitory effects of several flavonoids against distinct human tumor cell lines. Additionally, it was demonstrated that the growth inhibitory effect of some of these compounds was associated with the induction of cell cycle arrest and apoptosis [3]. Given the central role of p53 in the cell cycle arrest and apoptotic cell death, the modulatory effect of these flavonoids on p53 activity and on p53-MDM2 interaction was investigated. For this purpose, phenotypic screening assays using yeast cells expressing only human p53 and co-expressing human p53 and the negative regulator MDM2 were performed. Among the tested compounds, promising inhibitors of the p53-MDM2 interaction were identified.

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# Immunofluorescent detection of SIRT6 in human corpus cavernosum

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**Introduction:** SIRT6 belongs to the family of mammalian sirtuins (SIRT 1-7), seven homologs of yeast silent information regulator (Sir)2, isoenzymes that present NAD<sup>+</sup>-dependent deacetylase and ADP-ribosylation activities. It has been described that SIRT6 is mainly expressed in the cell nucleus, where it regulates histone acetylation, chromatin structure and DNA repair, presenting an important role in biological process of aging in human tissues [1]. In fact, it was previously demonstrated that SIRT6-depleted cells present Werner syndrome phenotype, a premature aging disorder, and that upregulation of SIRT6 could counteract physiological aging [2]. The expression of each isoenzyme of sirtuin family present tissue-specificity, and recently was reported the expression of SIRT1 in Human corpus cavernosum (CC) [3]. Based on this evidence, we hypothesized that SIRT6 could also be expressed in this high-vascularized tissue that present an important age-dependent loss of function. Indeed, aging is considered the most important independent risk factor for erectile dysfunction. The aim of this study was to determine whether SIRT6 is present in human CC, employing samples obtained from young and elderly individuals.

**Methods:** Samples of human corpus cavernosum (CC) were collected from healthy organ donors without erectile dysfunction (ED) or known ED risk factors at the Hospital of S. João Porto and divided in two groups according to the donor age (Young - 16 to 35 years and Aged – 59 to 74 years). The samples were excised and immediately fixed in formalin solution. Immunolabeling of SIRT6 and specific marker of smooth muscle cell ( $\alpha$ -actin) was performed employing appropriate antibodies. Images were acquired in an Apotome microscope (Carl Zeiss System) connected to an Axiocam MRm camera.

**Results:** SIRT6 was detected in the fusiform smooth muscle in the CC of human co-localizing with  $\alpha$ -actin. A strong labelling was observed in the nuclei.

**Discussion:** Our results demonstrate for the first time the expression of SIRT6 in the human corpus cavernosum tissue. Further on course molecular studies will clarify how age regulates its expression.

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# **Influence of sex steroids in the anatomy and neurochemical organization of the lateral posterior parvocellular division of the hypothalamic paraventricular nucleus**

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It is well established that sex steroids regulate glucocorticoid production by modulating the activity of key elements of the hypothalamic-pituitary-adrenal axis (HPA axis). Female rats are known to produce more corticosterone than males, both in basal and stress conditions, and this difference has been ascribed to sex-related features of the HPA axis. However, there is clinical and preclinical evidence of dissociation of adrenocorticotrophic hormone (ACTH) and glucocorticoid production, particularly during disease, which suggests that the activity of the adrenal cortex is regulated by factors other than ACTH produced by the pituitary gland. The lateral posterior parvocellular division of the PVN (PVNlp) originates one of the autonomic-related descending projections of the PVN and its neurons express receptors for sex steroids. In this study, we have examined this PVN division in order to find out if its neurons display gender-related differences that might influence the apparent dimorphic physiology of the adrenal cortex. The studies were carried out in male and female Wistar rats aged 6 months. Throughout the experiments rats were maintained under standard laboratory conditions and had ad libitum access to solid food and water. The estrous cycle was monitored by daily collection of vaginal smears and histological examination. Only females exhibiting regular (4-5 days) estrous cycles were used. Fifteen days before the end of the experiment, males and females were randomly assigned to two groups each. Rats from one group were submitted to gonadectomy and the remaining to sham-gonadectomy. Thirteen days later, half of the rats in each of the four groups were stereotaxically injected in the lateral ventricle with colchicine. Two days later, rats were anaesthetized and perfused with a fixative solution, and their brains were processed using either conventional histological procedures or immunocytochemistry techniques. The total number of PVNlp neurons and the total number of neurons immunoreactive for corticotrophin-releasing hormone (CRH), vasopressin (VP) and oxytocin (OXT) were estimated using the optical fractionator method. Data were analyzed by using a 2-way analysis of variance with sex and gonadectomy as the independent variables. Whenever significant effects were detected, the Tukey HSD post hoc test was performed. There were no sex differences in the PVNlp volume nor in the total number and mean somatic volume of its neurons. No sex differences were found in the total number of VP and OXT neurons. However, and in contrast to OXT neurons, the number of VP neurons was significantly reduced in gonadectomized males and females. The total number of CRH neurons was sexually dimorphic, with males containing approximately twice the number in females. The number of CRH neurons was significantly reduced by gonadectomy in males, but not in females. These results show that the neurochemistry of the PVNlp differs between male and female rats, and that these sex differences are dependent on the circulating levels of sex steroid hormones. This indicates that the PVNlp might play a significant role in the establishment of the sexually dimorphic pattern of corticosterone production.

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# Internalization of Nanostructured Lipid Carriers by Primary Human Dendritic Cells.

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Dendritic cells (DC) are key players in immunity and tolerance, link innate and adaptive immune responses, and are able to establish crosstalk with many different cells, such as lymphocytes. DC integrate signals from pathogens and surrounding milieu, produce cytokines, and are capable of driving different immune responses. Thus, being able to target DC with specific drugs for modulating their function towards immunity or tolerance is a promising strategy, already being explored in some areas of research. In this study Nanostructured Lipid Carriers (NLC) were loaded with the fluorochrome FITC to study their internalization by DC, envisaging their future use as drug carriers.

NLC were produced using a modified hot homogenization technique. Lipid and aqueous phases were warmed up 5-10 degrees above the lipids melting temperature and subsequently mixed to promote formation of oil/water emulsion. The mixture was then submitted to high-speed stirring in an Ultra-Turrax T25, followed by sonication in order to form nanoscale particles. Cooling to room temperature allowed lipid crystallization and formation of lipid nanoparticles. The formulation without FITC appeared white, while with FITC it was yellow. Both were milky and had low viscosity. To access formulations stability they were stored at room temperature, protected from light, and periodically analyzed. NLC were characterized for their average diameter, and polydispersity index (PI) using dynamic light scattering (DLS), and zeta potential determined using electrophoretic light scattering (ELS). Primary human monocytes, negatively isolated from peripheral blood of healthy donors, were differentiated to DC for 7 days, with GM-CSF and IL-4. Several volumes of FITC-loaded or unloaded NLC were added to the cells and allowed to internalize for different times. Cells were harvested and stained for DC lineage marker CD1a and analyzed using ImageStream<sup>x</sup>, imaging flow cytometry.

Lipid nanoparticles had a mean diameter of 200 nm and a PI of around 0.2, suggesting an acceptable monodispersity distribution, with low variability and no aggregation. The highly negative zeta potential, around -30 mV, suggested electrostatic repulsion of the lipid nanoparticles and, consequently, their physical stability. Also, results obtained show that NLC were internalized by DC and their fluorescence was specifically detected by ImageStream. Encapsulating more FITC beyond 2mg did not result in higher detectable fluorescence inside the cells. Also, an incubation time of 1h was sufficient for nearly 100% of DC to internalize NLC. Increasing NLC amounts added to cultures led to increasing fluorescence detectable inside DC, from 25µl in 2ml of culture with 2x10<sup>6</sup> cells leading to about 36% of cells with detectable internalized NLC, and up to 99% of DC having detectable internalized NLC, when 200µl were added to the same volume and cell number. Finally, NLC internalization did not lead to significant cell morphological changes or visible cell death. These results indicate that NLC are a suitable system to deliver drugs to DC.

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## Low-dose acetylsalicylic acid improves the antihypertensive efficacy of losartan in spontaneously hypertensive rats

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**Introduction and aims:** The addition of low-dose acetylsalicylic acid (ASA) to angiotensin II receptor blockers (ARB)-based antihypertensive therapy appears to reduce cardiovascular morbidity and mortality among high-risk hypertensive patients when compared to patients not treated with ASA. Besides its well known anti-thrombotic and anti-inflammatory effects, ASA also induces the synthesis of 15-epi-lipoxin A<sub>4</sub> (15-LXA<sub>4</sub>) through a pathway involving cyclooxygenase 2 (COX-2) and 5-lipoxygenase (5-LO). 15-epi-LXA<sub>4</sub> has anti-inflammatory and antioxidant effects and may contribute for the overall benefits of ASA. Renal COX-2 derived-prostaglandins regulate hemodynamics and excretory function in a close relationship to the renin-angiotensin system. However, little is known about renal lipoxins and their modulation by low-dose ASA or ARB. Therefore, we aimed at evaluating the effects of the association of low-dose ASA with an ARB (losartan) on blood pressure control, renal function, oxidative stress and lipoxins synthesis in spontaneous hypertension.

**Methods:** Twelve week-old male spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY) were assigned to 4 groups: control, losartan (15 mg/kg/day, 8 days), ASA (10 mg/kg/day, 8 days), and losartan+ASA (8 days). Systolic blood pressure (SBP) was measured by the tail-cuff method. H<sub>2</sub>O<sub>2</sub>, 8-isoprostane, LXA<sub>4</sub> and 15-epi-LXA<sub>4</sub> were evaluated by commercial kits. Renal COX-2, myeloperoxidase (MPO) and 5-lipoxygenase (5-LO) expression was evaluated by Western Blotting. The other markers of renal function were evaluated using an automated biochemical analyzer.

**Results:** Losartan or losartan+ASA reduced SBP both in WKY and SHR (day 7: 123.1±0.6 mmHg in WKY control; 125.3±0.7 mmHg in WKY ASA; 114.8±0.8 mmHg in WKY losartan; 113.8±0.9 mmHg in WKY losartan+ASA; 204.9±0.9 mmHg in SHR control; 207.0±1.1 mmHg in SHR ASA; 186.3±1.4 mmHg in SHR losartan; 174.5±0.9 mmHg in SHR losartan+ASA). However, in SHR, the combined treatment with ASA and losartan improved the antihypertensive effect of losartan ( $p < 0.001$  vs SHR losartan). All SHR groups had reduced creatinine clearance, decreased urinary sodium excretion, increased plasma urea and augmented proteinuria ( $p < 0.05$  vs WKY groups). Treatment with ASA, losartan or losartan+ASA did not significantly alter these markers in SHR, although proteinuria was tendentially lower in the losartan+ASA group. No significant changes were observed in urinary excretion of H<sub>2</sub>O<sub>2</sub> or isoprostanes. SHR groups had lower urinary excretion of LXA<sub>4</sub> and 15-epi-LXA<sub>4</sub> compared to WKY. COX-2 was reduced in the renal cortex of SHR compared to WKY. Interestingly, treatment with losartan, but not with losartan+ASA, enhanced renal COX-2 expression, increased uric acid levels both in plasma and urine and tendentially elevated plasma urea, urinary H<sub>2</sub>O<sub>2</sub> and isoprostanes levels in SHR.

**Conclusions:** The combination of low-dose ASA and losartan improves the antihypertensive efficacy of losartan in SHR. This protective action of ASA might be related to the attenuation of adverse counterregulatory effects induced by losartan in hypertensive animals.

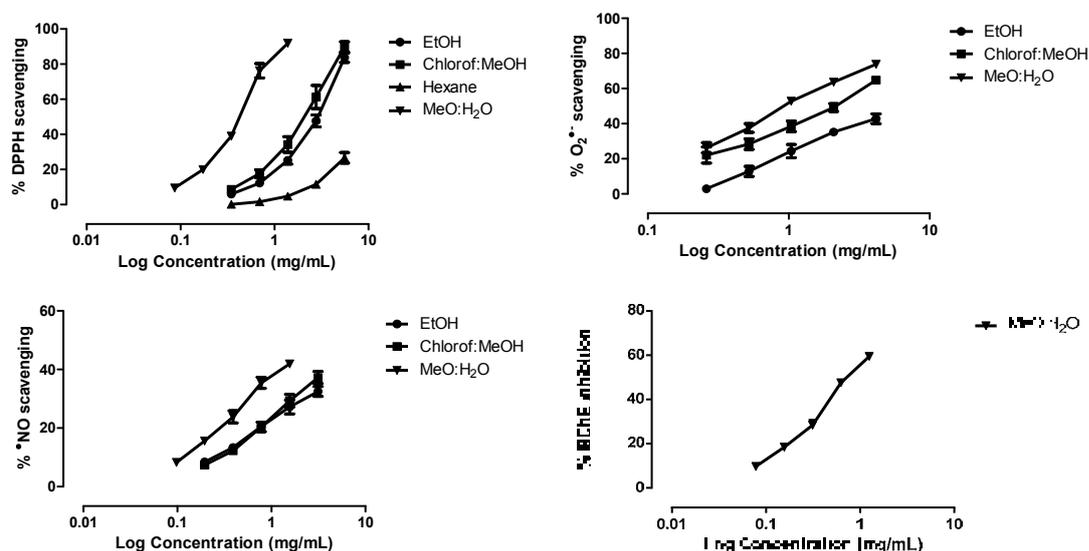
## *Lycopersicon esculentum* Mill. leaves: from waste to bioactivity

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*Lycopersicon esculentum* Mill. (tomato) fruit is a widely studied matrix. However, only few works focus their attention on its leaves, which constitute a major byproduct of the tomato processing industry. Industrial byproducts usually are promising sources of compounds with interest for their nutritional properties and biological potential. One alternative route could be their use as dietary supplements and in food fortification, since it is generally assumed that consumers prefer natural functional ingredients. In this study the anticholinergic effect (acetyl and butyrylcholinesterase inhibition) and antioxidant potential against DPPH, superoxide ( $O_2^{\bullet-}$ ) and nitric oxide ( $\bullet NO$ ) radicals of four different tomato leaves extracts were evaluated (**Fig. 1**).



**Fig. 1** – Antioxidant and anticholinergic potential of *L. esculentum* var. *cerasiforme* leaves. EtOH: ethanol; Chlorof: chloroform; MeOH: methanol; BChE: butyrylcholinesterase.

The chemical composition of the different extracts was also determined. The phenolics and steroidal alkaloids were identified by HPLC-DAD and fatty acids, sterols and triterpenes by GC-MS. The most promising results were presented by the hydroalcoholic extract, which seems to be associated to the higher content of phenolic compounds. Thus, tomato leaves extracts can be used as food supplement, source of bioactive compounds or an alternative to synthetic preservatives in the food industry. As so, a profitable use can be given to this discarded material.

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# Microarrays cross-platform analysis and genetic algorithm optimization for gene prioritization in preeclampsia

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Preeclampsia has been studied for many years but the underlying mechanisms as well as an early predictive marker remain unknown. Increments in the number of genomic/proteomic studies in preeclampsia, as well as free access to microarray experiments, opened an incredible opportunity to explore simultaneously thousands of genes by bioinformatics procedures. The objective of this study is to identify groups of genes responsible for differential expression between normal (N) and preeclampsia (PRE) combining microarrays cross-platform analysis and several genetic algorithms procedures.

We selected five projects from GEO database comprising 123 and 104 microarrays from PRE and N pregnant women, respectively, corresponding to different platforms. The microarray results correspond with placenta tissue analysis obtained at delivery. We mapped the probe arrays to the respective entrez gene ID by manual observation and also using the updated manufactures annotation information for all the platforms. Only those genes common to all platforms were used in the subsequent steps (6816 genes). Genes with more than one probe were combined by averaging the intensity values using *collapseRows* and *intersect* functions available in WGCNA package. The second normalization was performed to re-scale the intensity and also remove cross-platform batch effects using *Combat* function in SVA package. For the identification of statistically significant gene differentiated between N and PRE groups we used the *lmFit* from Limma R-Package and only genes with p-value  $\leq 0.05$  (n=1146 genes). The selected genes were used in a “variable selection problem”, using several approaches. In this context, we apply a combination of genetic algorithm (GA) optimization with different classification methods: Nearest Neighbor (GANN) and Discriminant Analysis (GADA). We also applied an algorithm recently published GA modified algorithm (GA(M)E) for feature selection that also combine ensembles based methods.

A reduced number of genes have been identified, more than 90% correct classification, opening a possibility for new diagnostic/therapeutic targets. Some well know PRE related genes were identified as TPBG, MMP1, INHBA, while several others were also relevant in the GA models but remain little explored or unknown during pregnancy opening new branches for future experimental designs.

# Multidimensional optimization of xanthenes with potential antitumor activity: plasma protein binding

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Xanthenes are compounds from natural or synthetic origin that have revealed a wide range of biological activities, namely antitumor activity. The xanthone nucleus can be considered a “privileged scaffold” in drug discovery, since it can be an excellent starting point for the search new pharmacological active compounds [1].

In the drug discovery pipeline, a “hit compound” is structurally modified in order to achieve a drug candidate. This optimization procedure must follow a multidimensional approach, which devotes special attention to both pharmacodynamics and pharmacokinetics [2].

XC10 is a promising antitumor prenylated xanthone that was synthesized by our group and is being optimized using a multidimensional approach. So, several XC10 analogues were obtained and a number of bio-physico-chemical parameters are being evaluated. Among the several factors that affect pharmacokinetics, the binding to plasma proteins is one of the most important [3].

Therefore, the interaction of XC10 and the synthesized analogues with human serum albumin (HSA) was studied. HSA is the most abundant and important human plasma protein. The binding to HSA was evaluated by fluorescence quenching technique and UV–Vis absorption spectroscopy [4]. Dissociation constants and the number of binding sites for all compounds were determined.

The obtained results allowed the establishment of structure-albumin binding relationship. Accordingly, they will help to propose new xanthenes in order to achieve more potent and with a better pharmacokinetic profile compounds.

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## Acknowledgments:

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## NaCl variability in canned sardines

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An adequate ingestion of canned sardines in a balanced diet is important, being associated with positive health outcomes, particularly in the prevention of cardiovascular diseases. However, these products also contain significant amounts of salt (NaCl), used for sensorial enhancement rather than as a preservation strategy. This salt is present in the brine where the sardines are conditioned for a predetermined period before canning, and in the sauce, particularly when using tomato sauces or brine.

In order to evaluate the variability in the salt content and the determining factors, 35 samples were analyzed for NaCl content. Sampling included sardines canned in olive oil, vegetable oil, tomato sauce, brine and water. All samples were from the same producer and prepared by equivalent processing times/temperatures. The only uncontrolled variable was the sardine chemical composition and size, known to be highly variable [1]. The sardines were drained for 5 minutes, homogenized; and the chloride was quantified by the classical Charpentier-Volhard method, being directly used to estimate the total NaCl amounts.

The results were highly variable, ranging from 0.1% to 1.0% on a fresh basis. When grouped by the sauce type, the variability is similar, highlighting that the sauce composition is not the main factor contributing to the dispersion observed. The results obtained are discussed against the possible cause of variability.

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## Neuregulin-1 decreases the passive force of cardiomyocytes from the right ventricle in pulmonary arterial hypertension

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Neuregulin (NRG)-1 is implicated in the maintenance and structural integrity of the cardiovascular system. Recent studies showed the involvement of NRG-1 in the preservation of left ventricular performance in pathophysiological conditions [1]. Nevertheless, the role of NRG-1 in pulmonary arterial hypertension (PAH) and right ventricular (RV) failure is still unknown. Therefore, the goal of this study was to evaluate the effects of a NRG-1 chronic treatment on intrinsic myocardial properties, namely on the modulation of active and passive force of cardiomyocytes isolated from the right ventricle of animals with PAH.

Male Wistar rats (180-200g) randomly received monocrotaline (MCT, 60mg/Kg, sc) or vehicle. After 14 days, animals from these groups were randomly assigned to receive treatment with either NRG-1 (4ug/Kg/day, ip) or vehicle. The study resulted in 4 experimental groups: control (CTRL, n=9); CTRL+NRG (n=12); MCT (n=12); MCT+NRG (n=18). Between 21 and 24 days after MCT administration, samples were collected for functional studies. Right ventricular samples were mechanically disrupted and incubated in relaxing solution supplemented with Triton (0.2%). Single cardiomyocytes were subsequently attached with silicone adhesive between a force transducer and a piezoelectric motor and active and passive forces were measured. Only significant results ( $p < 0.05$ ) are given.

MCT-group isolated cardiomyocytes developed higher passive force when compared to CTRL-group cells at the sarcomere lengths of 2.0 (MCT vs. CTRL:  $1.76 \pm 0.26$  vs.  $1.43 \pm 0.29$  N/m<sup>2</sup>), 2.2 (MCT vs. CTRL:  $3.74 \pm 0.71$  vs.  $2.68 \pm 0.24$  N/m<sup>2</sup>), and 2.3  $\mu$ m (MCT vs. CTRL:  $5.73 \pm 1.22$  vs.  $3.86 \pm 0.87$  N/m<sup>2</sup>). Treatment with NRG-1 was able to restore passive force development to levels similar to the CTRL-group cardiomyocytes, at 2.0, 2.2, and 2.3  $\mu$ m (MCT+NRG:  $1.28 \pm 0.25$ ,  $3.04 \pm 0.55$ , and  $3.63 \pm 0.89$  N/m<sup>2</sup>, respectively). CTRL+NRG-group cardiomyocytes developed significantly less passive force when compared to CTRL-group cells (CTRL+NRG:  $1.19 \pm 0.25$ ,  $2.32 \pm 0.55$ , and  $3.16 \pm 0.54$  N/m<sup>2</sup>, at 2.0, 2.2, and 2.3  $\mu$ m respectively). The analysis of the active force showed that in the MCT+NRG-group cardiomyocytes active force development was decreased when compared to MCT-group cells (MCT+NRG:  $9.67 \pm 2.83$  N/m<sup>2</sup>).

NRG-1 chronic treatment is able to reverse the changes in both active and passive myocardial forces that occur in the presence of PAH. Interestingly, NRG-1 chronic treatment also decreases the passive force of cardiomyocytes isolated from the right ventricle of healthy animals. These findings suggest that the NRG-1 pathway has a relevant role in the regulation of diastolic function and in pathophysiology of PAH by decreasing passive force and thus myocardial stiffness, pointing to its potential role as a therapeutic target.

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## Oral yeast colonization in patients with chronic kidney disease on peritoneal dialysis

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Peritoneal dialysis (PD) is a home-based and widely used therapy of renal replacement for patients with chronic kidney disease (CKD). Fungal peritonitis is more rare but its treatment is usually very difficult [1]. Some authors have highlighted the importance of the oral cavity infection as a starting point for dissemination of pathogenic organisms to distant body sites, mainly through bacteremia [2]. The present study aims to evaluate the oral and peritoneal catheter exit-site yeast colonization of peritoneal dialysis (PD) patients and identify possible similarities between fungal peritonitis agents and normal microbiota. Also, the prevalence of oral yeast colonization in a CKD population undergoing PD was compared with a healthy population.

Twenty-seven PD patients and 18 family members without CKD were included. A non-invasive intra-oral examination was performed in order to evaluate the following parameters: decayed, missing, and filled teeth (DMF) as well as oral hygiene indexes. Samples of non-stimulated and stimulated saliva were collected for the microbial analysis and pH evaluation. Samples from exit-site catheter were also collected from PD patients. Identification of *Candida albicans* was possible using CHROMagar Candida™<sup>®</sup>. The other non-identified isolates were identified using a sequencing approach.

The median residual renal function in the PD group was 12ml/min, reflecting the compromised renal function. Both groups had low levels of education. Most participants had a poor oral hygiene and a high DMF index, although the prevalence of decayed teeth was lower in PD patients. The pH of the non-stimulated and stimulated saliva was significantly higher in PD patients compared to the control group. PD patients had a prevalence of yeast colonization in saliva and exit-site catheter of 11% and 7.4%, respectively. Saliva showed greater diversity of *Candida species* in relation to the exit-site catheter. No differences were observed between DP patients and control group concerning the prevalence and quantification of yeasts in saliva.

The study demonstrated that *Candida species*, namely *C. albicans*, is a normal colonizer of oral cavity and exit-site catheter mucosa in PD patients. This yeast was present in the oral cavity of both PD patients as well as controls, but was less prevalent in the first group.

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# Definition of Flood Prone Areas Based on Hydraulic Modeling in the Leça Watershed (Matosinhos Municipality)

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Flood events can be reported almost worldwide and cause every year huge material losses, as well of human lives. Taking this into account we delineated flood prone areas in the *Leça* watershed, specifically, for a terminal sector included in the *Matosinhos* Municipality (fig.1A).

For the definition of flood prone areas, it was used the hydraulic method, supported by HEC-RAS software, that enables the creation of geometric data as well the flow data calculated with the *Giandotti* cinematic formula [1]. The geometric and flow data were used as inputs [2] to generate the results displayed on fig.1B.

The fig.1B, presents the results obtained with the application of these methodology. These results were validated through field work and inquiries to locals, and was concluded that the model is highly influenced by the quality of the altimetry data used and also that it has a great accuracy based on the past flood marks obtained in the fieldwork.

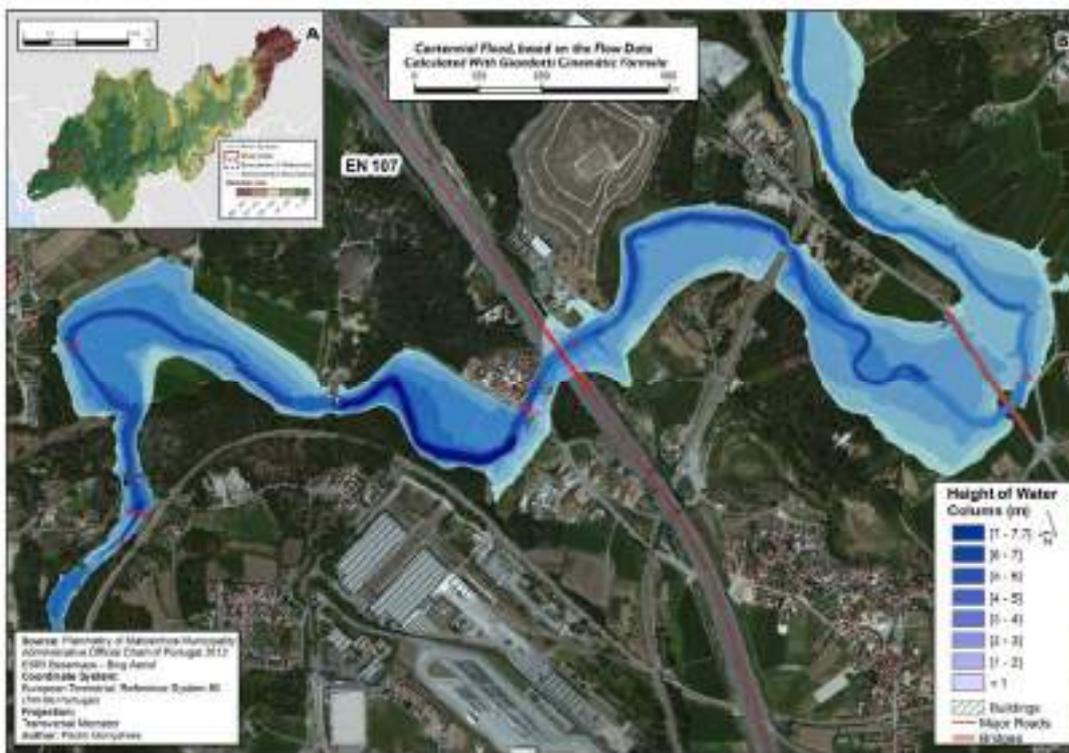


Fig. 1- A: *Leça* watershed and location of the study area; B – Results calculated for the study area considering a centennial flood event.

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# Land Use Changes in *Leça* Watershed (Portugal): a Prospective Approach

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Currently, we live in a human-dominated planet where the changes in the environment are mainly driven by anthropogenic activity. One of the most pernicious effects of this activity on Earth is the quick alterations of land use in order to meet human needs [1]. The knowledge about the space-time dynamics of the land-use is a key feature in the study of hazard events such as floods, once the water circulation, the infiltration rate and the public safety are conditioned by this [2].

The *Leça* watershed, located in the north of Portugal, covers an area of 189.5km<sup>2</sup> and is characterized by a great demographic and industrial pressure, since the beginning of the twentieth century. Using cellular automata/Markov chain analysis coupled with multi-layer perceptron neural networks we addressed the following objectives: (i) to describe the land use transitions in the *Leça* basin among two time periods (1990 – 2007) and, (ii) to model land cover changes for 2030, in order to forecast the representativeness of three main different land use classes (urban, agriculture and forest areas). In this preliminary effort it was used three variables, known as key variables affecting the land use transition in an urban-dominated context: distance from buildings, distance from roads and slope.

Our first results (Fig. 1) showed that be expected a continued growth of urban areas until 2030, registering the same trend observed in previous years. Related to the forest and agricultural use, between 2007 and 2030, it's predicted a cover decrease of 8.24% and 6.58%, respectively. The urban growth should remain in the near future, which can be a problem in a flood risk point of view, since the reduction of permeable surface can increase the risk of flood occurrences. This fact highlights the importance of establishing long-term mitigation measures in order to prevent hazard events.

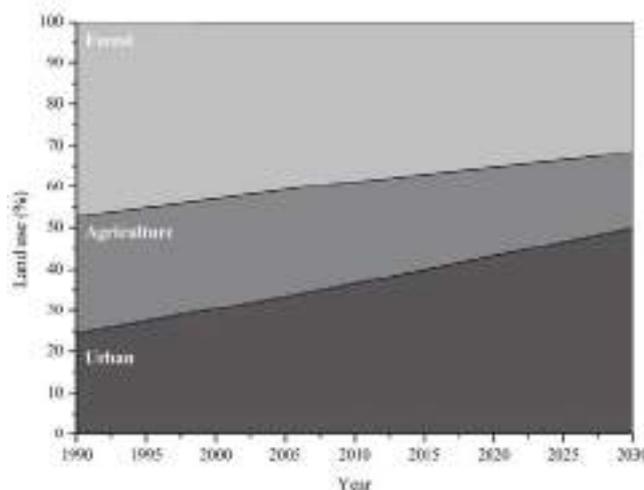


Fig. 2 – Forecast land use changes across a time period of 40 years.

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# The Places of Space

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This research on Architecture Theory, reflects upon the production of architectural space and upon the latter as product and producer of social, physical and symbolic relations between the subject and the space that surrounds him. It is then fundamental to the development of this speech, to understand Architecture as indivisible from the personal, social and cultural reality of the inhabitant and thus, expression of the global, yet fragmentary and volatile world we inhabit.

The intent of this thesis, is to understand the processes upon which the inhabitant – as an individual and as a collectivity – relates with himself and with the other, with the world and, particularly, with space in Architecture. On the other hand, and considering space not only as a product of social practice, but also as a producer of the latter, this thesis intends to understand the way these relations evolve on space and how does space reflect and inflect them.

The research will then be draw upon the understanding of architectural space as a perceptual field in which we can clarify three different levels: Represented space, Representational space and Spatial practice. Through the understanding of these moments in space production, as well as in space experience, the aim is to clarify the way space, as an abstract and mathematical product, acts upon the inhabitants lives and how can architectural space be considered so that lived experience is not vanquished by what is conceived.

The first approach to this thesis will then be, the understanding of the physical, mental and social levels of space, so as to clarify the distinction between the concepts of space and place, i.e., of Architecture beyond matter.

Therefore, and considering the production of space as an intrinsically linked level of the construction of the Being in the world and of the latter by the first, this research intends to understand the motion in abstract space production. After decoding it, the intent will be to understand the construction of space as place, that is, of space as a process of free appropriation, open to the unpredictable: “space as directly lived through its associated images and symbols, and hence the space of ‘inhabitants’ and ‘users’ (...). This is the dominated – and hence passively experienced – space which the imagination seeks to change and appropriate.” [1]

In short, due to the theoretical nature of this research, it’s unsafe to draw a conclusion of how must architectural space be. In fact, the intent of this investigation is, precisely to clarify the impossibility of concluding a method for Architecture production, so it can be comprehended as a process that reflects the indeterminate and interminable construction of the Being in the world through space.

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# Bionics and architecture. Narrative on biological analogy

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Gonçalo Furtado (dissertation advisor).

Bionic architecture has been discussed in different fields such as theory and history, but the best way to explain someone the basics of bionic concepts is by giving them examples of architecture projects or constructions that were thought according to a bionic or biological concept – case studies. Bionic architecture is the evolution of biological analogies and it has become part of its history. It can be explained by the use of nature, organisms, biological analogies or science in the development of a project. It can be either the imitation of the form, organism structure or material applied in the project; or the imitation of some specific skills or abilities that can be transferred to architecture. Two basic examples are Frank Guerry's "Fish Dance Restaurant" in Kobe (Japan) – where the fish form creates a unique space phenomenon – and Teutonic Holst rotating house – which imitates the sunflower that turns during the day searching for the sun light.

The work methodology can be summarized by reading books or articles about the theme, and the resume of scattered ideas. This critical overlook will help in the construction of solid opinions, the analysis of case studies that embody organic or biological analogies and specific study of bionic architecture projects.

*"The most expert Artists among the Ancients were of the opinion that an Edifice was like an Animal, so that in the Formation of it we ought to imitate Nature."*[1]

It is not unusual to use Nature as an inspiration in art or architecture. Using Nature and, therefore, biological analogy to describe a work of ours it's not as it may seems contemporary or modern, it's a old and natural process that can't be traced in history. The concepts of correlation or integration used to express the harmony in the biological process or anatomy of an organism, can be compared to the terms used to describe a project in architecture. That's why bionic architecture should be explored and understood, not by an utopian vision, but with an innovative eye that can acknowledge the benefits in bionics.

The main objective is not only to study the development of the concepts and their case studies, but to distinguish in these analogies or bionic projects what's useful and valuable; and what's dangerous and harmful. In the end we can speculate about future developments. Is it still a utopian and childish concept, or is it an innovative and possible idea?

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## CITY IMAGE

### An approach to the impact of Metro do Porto in the image of the city

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*"Every citizen has had long associations with some part of the city, and his image is soaked in memories and meanings."* [1]

This study focus on the image of the urban realm, taking into consideration the aspects that relate to the communicative character of the city, while issuer of a multitude of messages and meanings to those who move through it; as well as the aspects related with the case of the specific implementation of *Metro do Porto*, that shows, consequently, impacts on the transforming image of the consolidated city and in the way that the social protagonists experience, perceive and appropriate the urban realm.

As a changing organism, the city develops itself mainly as a product of the relationships between people, and through their interaction with the urban elements and its referents on the public space context, such as the consolidation of new infrastructures – as it is the case of the Metro do Porto – which contributes to the creation and transformation of its identity and its historical, cultural, economic, social and imagery memory. Among its built forms and its intricate visual fabric, the cities nowadays are an active and inevitable part of our urban daily life pursuits and, probably, they are the most powerful symbol, artefact and stage of our complex society. Within this scenario, its visual development, manifested through the media, through the architecture itself and through other forms that give expression to the city, can acquire a strong and expressive meaning to the individuals concerning their perception and spatial representation.

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# Dwelling Modes: "Contemporary Architecture face the Rural"

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Dwelling modes in architecture it's a variable concept due the multiples characteristics that influence them. The evolution of science, technology or the ways of organize us in the city contributed to a constant transformation of swelling in the pass of the 20<sup>th</sup> century. These changings required also a change of those who conceive the environment that we live in, affecting our houses, our homes.

“UM HABITANTE É-O QUANDO INSERIDO NO TEMPO E QUANDO INSERIDO NO ESPAÇO.”<sup>1</sup>

During the practice of architecture it became matter of interest the role-played by the architect as definer of the space and of the social relations in these created. Starting from a theoretical study the conclusion of this investigation it's done with the confrontation of the process of projecting a house. In the 20<sup>th</sup> century, the subject of dwelling modes started to be discussed in the main congresses of architecture (CIAM) defending a new role of the architect as a definer of people lives. That's why nowadays it is necessary to us, students of architecture, be conscientious of the importance of our acts and how they can define how people behave in their day lives or even how they relate with people.

Considering the subject in mater, it cannot be presented results at the moment. It is a work that has as final product a reflection on what's supposed to be done, to understand where it finishes the fine line where the architect stops to be a creator and starts to be product of the client who's working for. And that only can be verified with the practice itself that right now doesn't exist. The work is then distributed into three main phases: a study of existing theoretical reflections and confrontation with the design of a real project, to be executed, creating a design family house in the village of Mourisca do Vouga and adjacent to its theoretical execution, and finally the confrontation of the project carried out with the veracity of the study previously developed. For this reflection and execution of the project, the development and study of this subject is simultaneously the analysis of the space in which it operates and the creation of conducting indicators of project and design such as plans, sections, elevations, models, etc.. a process requiring both practical and theoretical work to justify the choices made.

In conclusion, this work investigates how contemporary architecture in rural struggles with the primary thought, or alters the specificity of single-family housing in the context of low density. The principles of integration in rural areas pose other problems outside the urban area, such as integration into the landscape and prioritization of nature as characterization of the new created spaces of the house

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# Optimization of a pharmacy's medication stock using the theory of constraints: preliminary study of sales ruptures

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The pharmacy sector is under pressure in Portugal. Due to the financial crisis, the Portuguese government is reducing public expenditure on medicines [1]. The distribution companies and pharmacies have been forced to reduce their profit margin into a regressive mark-up and a flat fee [2] which may lead to an increase of stock ruptures. The traditional Supply Chain Management (SCM) is a methodology where the strategy replenishment model is based on forecasts to push products into retailers (pharmacies), and the replenish order is only triggered when the inventory reaches a minimum level of a product [3]. The Theory of Constraints (TOC) replenishment adopts a policy where the replenishment lead time (RLT) is based on sales [4]. This change implies daily delivers or more, to restore the stock according to the sales in the previous period. To better assess the need for such a change of replenishment paradigm, pharmacies' sales ruptures should be studied. The aim of this work is to observe and analyze the amount and type of sales ruptures (stock or non-stock related) currently happening in pharmacies.

An online questionnaire was developed to register prescription-required medications sales ruptures in pharmacies. For a preliminary study, a single pharmacy was inquired, and the data was collected during 27 days. In that period, a total of 76 sales ruptures were registered. In 90% of the cases only one medication was missing, and in 94% of these only one package was missing. The reason for the sales rupture was, in 81% of the cases, not enough stock in the pharmacy. There were some days when the number of ruptures is significant, between 7 and 10.

This analysis permits the hypothesis that, with a stock adjustment to the demand, pharmacy could be able to reduce its ruptures in around 90%. Moreover, there are some possible explanations for those days with higher amount of ruptures which should be highlighted (e.g. higher demand in these days; delay in stock reposition; wrong order quantities; or wrong medications stock policy). Next steps will include the simulation of the TOC replenishment model applied to medications' supply chain, analyze the TOC replenishment model concerning financial and operations measures, and compare it with the current model.

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# ***The Tales of Beedle, the Bard & Os Contos de Beedle, o Bardo of J.K. Rowling:*** **Analysing the wizarding terms into Portuguese**

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Constituted by seven books, the series *Harry Potter* written by J. K. Rowling was firstly published in 1997, and immediately draws the readers' interest of all ages, reaching the rank of one of the most famous work of children literature not only in Brazil but also abroad. After publishing the seventh book *Harry Potter and the Deathly Hallows*, J.K. Rowling affirmed it would be the last one. However, the publication of *The Tales of Beedle, the Bard*, in 2008, translated in Brazil by the same translator of the whole work, Lia Wyler, entitled *Os Contos de Beedle, o Bardo*, Rowling once more addresses the reader to the world of the boy wizard.

In this sense, it seems to be interesting to analyse the work *The Tales of Beedle, the Bard*, and its translation into Brazilian Portuguese, not only to foster the debate about meaning production which constitutes the translation process and its impact in the result of the target text and culture, but also to show some particular aspects within the pair of languages English-Portuguese that would serve as instruments for the translator's education and/or professionals and scholars interested in children literature.

The nature of this research is comparative, whose purpose is to list and analyse wizarding terms of the witchcraft world presented in the work *The Tales of Beedle, the Bard* and their translation into Brazilian Portuguese in *Os Contos de Beedle, o Bardo*. The terms will be analysed according to Aurbert's approach (1998), who classifies translation procedures as omission, transcription, loan, calque, literal translation, transposition, explicitation/implicitation, modulation, adaptation, intersemiotic translation, error, correction and addition. The Aubertian model proposes a descriptive and quantitative approach, which leads the researcher to identify existing patterns and regularities in translation, and verify the degree of linguistic differentiation between the source and target text.

Up to this stage of the research, it is possible to affirm that 38,1% of the wizarding terms in the original text were adapted into Brazilian Portuguese, showing the translator's attempt of cultural assimilation, setting a partial equivalence within the 42 wizarding terms here collected.

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## **follow.up: University of Porto News Aggregator**

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The goal of the “follow.up” project is the development of a web-based information system for the aggregation, consolidation and organization of news published by the multiple organic units of the University of Porto (UP). UP is a large academic organization, with dozens of organic units, each one publishing several articles a day. Our vision is to create a centralized access point to all the published content to improve information consumption within the university.

We started collecting data on June 2012. Since then we have stored more than 5,200 articles published through the RSS channels available on the SIGARRA platform of 15 organic units. We also implemented a web-based system to perform a statistical analysis on the collected data. We classified the organic units as sources; the RSS feeds as channels and the feeds' names as topics. Each source has one or more channels and each channel has one topic and one or more articles published. Next, we summarize some of the main findings for the period between June 1<sup>st</sup> and October 31<sup>st</sup> of 2012.

The number of channels that each source has varies greatly, going from only 2 channels (FADEUP) to 12 (FCUP, FEUP, FMUP and FPCEUP). There is a total of 108 channels. FEUP was the source with the most articles published in the analyzed period (498 articles published through 12 channels) and FMDUP the one that published the least (52 through 3 channels). We identified 56 distinct topics. The topic “Geral”, shared by 9 sources, lead with 554 publications, while the topics “Divulgação” and “Serviços Administrativos”, both belonging to just one source, published a single article. The topic from a single source with the highest number of published articles was “Notícias FEUP”, with 229.

September was the month with the most articles published (900) and August the one with the least (431). As for the hourly data, the main publishing period is between 06h00 and 21h00, with some occasional publication out of that period. There's a peak in the morning, at 10h00, and another, higher, in the afternoon, at 15h00.

This initial study was important to understand the volume and dynamics of the data published by the several organic units. Based on this information, we are currently working on the design of the interface and already have an initial prototype available online, aiming to aggregate in a user-friendly and joyful manner all this information oriented to the UP academic staff.

## Asteroseismic Modelling of 16 Cyg A & B

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The NASA Kepler satellite has provided exquisite asteroseismic data for hundreds of main-sequence stars, and accurate individual oscillation frequencies have been determined for some of them, from time-series that span several months [1]. These frequencies allow for the detailed modelling of the stellar interiors, and many different stellar evolution codes and fitting methods have recently been used for selected targets.

The 16 Cyg binary system is one of such targets, and has been subject to a detailed study using the first three months of Kepler data [2]. We present here a fit to these same frequencies, done using the MESA stellar evolution code. We focus on the details of the fitting process while trying to reproduce previous results.

*Acknowledgments* This work was supported in part by project PTDC/CTE-AST/098754/2008 from FCT (Portugal).

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# Analysis of alternative transportation of natural gas regions for missed in gas pipelines: the case of running low-Pecém Jaguaribe, in Ceará

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The natural gas industry (GN) in Brazil exposes significant levels of growth, presenting this input as an important alternative energy. Since 2008, Petrobrás operates terminals Liquefied Natural Gas (LNG) terminals in the Porto do Pecém/ CE and the Bahia de Guanabara/ RJ. These terminals store LNG regasification capacity and have respectively seven million and fourteen million cubic meters per day. However, according to the CEGÁS (2011), there is contention four million cubic feet of gas per day.

Given this scenario, it is timely to develop policies and projects that assist in facilitating the expansion of gas distribution in the region, from the supply available in the Pecém LNG terminal. Therefore, it is necessary to analyze the logistics alternatives that allow the gas flow to regions with potential demand by energy input.

To this, one should take into account the economic viability and aspects related to environmental conservation and regional development of each alternative. In this context, it is important to check what forms to enable the transfer of the product.

Given the above, it is assumed that the best alternative transport gas to regions not yet served by that input, will give through virtual pipelines (GSV). Second Praça (2003), apply virtual pipelines where pipeline nonexistent distribution network and aims to ensure access to natural gas.

This study analyzes distribution models in the states compressed gas and liquefied to urban areas remote from catchment areas of major pipelines, using the tool Full Cost Assessment (CCA) and exposes the means of transport used for the transfer of product. In the approach used in the paper, we use a case study for the gas transportation in the corridor that extends from the Port of Pecém micro Lower Jaguaribe.

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# ANALYSIS OF THE STABILITY AND DESIGN OF OVERHEAD SIGN FRAMES IN HIGHWAYS

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This work intends to contribute to a better understanding of overhead signs structures in Highways.

The main objective of the present work consists in the analysis of the stability and design of three signs steel frames in Highways (Fig.1) with different structural behavior. The frames studied tend to emphasize the more common structures used on Highways (cantilever, frame and frame with a lattice girder). It is highlighted the specific properties of hollow sections that materialized the sections of the structures [1, 2].



Fig.1 Types of structures analyzed

The assumptions used for the type of structural analysis at a material and geometrical level are exposed. At the end, a design of all components of the structures is completed, i.e., sections, end plates, column bases and concrete block foundations.

After the designing of the three structures [3] presented at the beginning of this work, one can make a few final remarks. As seen in the chapter 5 [4] the sections of the structures were always conditioned by the Spanish standard UNE and not by EC3 [5, 6].

A final consideration for the structure type 3 is made, once it was shown an excellent performance in the field of deformations, i.e., very small deformations in terms of permanent actions.

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# Application of oxidation with Fenton's reagent and direct photolysis in the degradation of PBDEs for water treatment

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Polybrominated diphenyl ethers (PBDEs), belonging to the brominated flame retardants group, are synthetic organic compounds that have high persistence, bioaccumulation and toxicity. PBDEs are one of the classes of compounds studied in the framework of the European project *SecurEau*<sup>1</sup>, a project in which this work is integrated, which aims to respond rapidly and effectively to a deliberate contamination of water supply systems. It also aims the treatment of contaminated water, having been chosen the Fenton's process and other advanced oxidation processes (AOPs) for water decontamination. In short, this process makes use of an oxidant (hydrogen peroxide) and a catalyst (iron salt), leading to the production of highly active species, like the hydroxyl radicals, that will attack and degrade the organic compounds present in the water matrix.

Thus, in this work, it was intended to evaluate the degradation of these compounds in water by oxidation with Fenton's reagent; BDE-100 was used as a model PBDE compound. It was also evaluated the BDE-100 degradation in water by direct photolysis. The methodology for identification and quantification of BDE-100 in aqueous matrices during the degradation studies was the dispersive liquid-liquid microextraction (DLLME) followed by gas chromatography with mass detector (GC-MS).

In what concerns the analysis method of BDE-100 in water, it was carried out an optimization of the experimental conditions which involved, among other issues, the selection of the best extraction and dispersion solvents. Thereafter it was proceeded to evaluate the analytical methodology, which presents as main characteristics a linearity range from 0.01 µg/L to 50 µg/L and an average precision of 11%.

As for the degradation of BDE-100 in water by oxidation with Fenton's reagent, the process was studied under different concentrations of the species (catalyst and oxidant doses) and at different temperatures; it was verified that the compound is effectively degraded after 4 hours under the following conditions:  $[H_2O_2]_0 = 4.35 \times 10^{-2}$  M,  $[Fe^{2+}]_0 = 1.36 \times 10^{-3}$  M,  $[BDE-100]_0 = 50$  µg/L,  $T = 30.0$  °C,  $pH_0 = 3.0$ . The direct photolysis, performed with a TQ Heraeus lamp (150 W), has shown to be a very effective method for BDE-100 degradation in water (complete degradation was reached after 1 h of exposition to radiation) and has the advantage of not requiring the use of chemicals.

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# Energy assessment of radiative cooling systems in Mediterranean climatic weather conditions

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The main goal of this work is an energy performance analysis of the thermal behavior of a system that use a cool storage (inertia tank) and radiative panels to release the energy absorbed inside a building during the day. The idea is to evaluate the thermal performance in a Mediterranean climatic condition.

This paper present two different solutions of radiative panels, one is radiative panels coupled in the backside of the photovoltaic (PV) panels and the second a simple modulation of a panel used only to release energy.

Initially, will be modeled a radiative cooling system in order to obtain the maximum energy absorbed inside the building (TRNSYS). The inertia tank volume will be studied in order to obtain the volume that minimizes the auxiliary energy needs. Secondly, the system will be modeled on EES in order to simulate a simple plate with small copper pipes attached. With this model is possible to eliminate the negative influence of glass and improve the radiation to the sky.

For the transient simulation was considered 64 m<sup>2</sup> of PV-panels and a volume of 4 m<sup>3</sup> for the inertia tank (water,  $\rho = 1000 \text{ kg/m}^3$ ). With this conditions the energy removed from de building was 6835 kWh/year, and the necessary auxiliary energy was only 891 kWh/year, representing 12 % of the total cooling demand. In brief, the conclusion of this sensibility analysis is that the energy removed from the building is highly influenced by the tank volume, and this value needs to be studied and optimized.

Increasing the tank volume, the energy passively removed from the building increases and, in consequence, the need of auxiliary energy decreases. However, these values have tendency to stagnate for very high tank volumes, which indicates a balance in the system due to the high thermal mass.

The total energy removed from the inertia tank by the radiant panels was 7177 kWh in a year that corresponds to 36 W/m<sup>2</sup>. This value can be majored with some changes in the panels, like removing the glass and using materials with highest emissivity.

The model in EES was running for an instant, and obtained 161 W/m<sup>2</sup> of heat reject. At the same instant with the transient simulation, the heat rejected was 92,24 W/m<sup>2</sup>. The main difference is the nonexistence of the cover glass, and the higher copper emissivity.

# Establishment of a cell culture system to obtain homogeneous neurospheres of embryonic stem cell (ESC)-derived neural progenitors

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**Introduction:** Traumatic spinal cord injury (SCI) is a pathology frequently leading to persistent neurological dysfunction that is characterized by a loss of neurons and oligodendrocytes, axonal injury, and demyelination of spared axons. To compensate for injury-induced cell loss, transplantation of neural progenitors is currently being explored. With the rapid progress in stem-cell biology of the central nervous system (CNS) and the establishment of protocols for efficient derivation of neural progenitors from embryonic stem cells (ESCs), the prospect of using ESC-derived neural progenitors to attain regeneration in the CNS holds much promise. Nevertheless, the inhibitory microenvironment established following SCI may strongly compromise cell survival and differentiation of donor cells following transplantation. In this sense, attempts are being made to combine neural progenitors with biodegradable vehicles such as hydrogels. In the framework of project *NeurON*, a fibrin-based hydrogel is being developed for the delivery of ESC-derived neural progenitors into the SCI. Neural progenitors are suspended in fibrin as a single cell suspension or in the form of spherical aggregates (neurospheres), usually obtained growing the progenitors under suspension culture. Still, the use of static suspension culture often results in neurospheres with heterogeneous size/morphology and in neurosphere aggregation. Therefore, the present work was aimed at establishing a controlled hydrodynamic culture system to obtain floating aggregates of ESC-derived neural progenitors with homogeneous and reproducible size/morphology. The hydrodynamic culture system should not affect the viability of neural progenitors neither their ability to differentiate along the neuronal and oligodendrocytic lineages.

**Methods:** A modified mouse ES cell line (46C) expressing GFP under the promoter of the neural-specific *sox1* gene, was used. Neural progenitors were derived under adherent monoculture and in serum-free RHB-A medium, and the % of neural progenitors (Sox1-GFP<sup>+</sup>) determined by flow cytometry. To induce the formation of neurospheres, neural progenitors were seeded in non-adhesive substrates and cultured in RHB-A medium supplemented with bFGF and EGF for 4 days. To culture neural progenitors under hydrodynamic conditions, rotary orbital suspension cultures were established using orbital speeds ranging from 40 to 70 rpm. Neurosphere size and morphology were followed daily by phase contrast microscopy followed by image analysis. Neural progenitors cultured under static conditions were used as control.

**Results and discussion:** Preliminary studies revealed that rotary orbital suspension culture can be successfully used to form floating aggregates with spherical morphology and homogeneous size, for orbital speeds higher than 50 rpm. Moreover, the neurospheres formed using this hydrodynamic culture system presented a narrower size distribution as compared to those obtained under static conditions. The effect of higher orbital speeds on neurosphere size, morphology, yield, as well as on cell viability and differentiation is presently being assessed.

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# On the ventilation of bicycle helmets

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A bicycle helmet is essential for the rider's safety, however, the lack of thermal comfort may be an obstacle for helmet acceptance. The heat transfer in a helmet occurs through various mechanisms which include conduction, convection and radiation (dry heat transfer) and evaporation of sweat. To maximize heat losses, the air must flow as freely as possible, between the helmet and the head, thus making ventilation an important factor.

The thermal performance of a helmet can be evaluated with a thermal manikin headform positioned at the exit of a wind tunnel in a climate chamber, and measuring the temperature changes, or the power needed to main the temperature stable, in various points of the headform [1-2]. The cooling effect of sweat evaporation may also be studied if the thermal head can simulate sweating (pouring of water at the surface of the head). For the study of the effects of radiant heat transfer, a heat lamp may be mounted above and directed downward onto the headform [3].

As an alternative to experimental methods, which can be expensive and time consuming, one can use (CFD-based) numerical approaches to compute the temperature, pressure and velocity distributions across the domain [4]. It also has the advantage of allowing the assessment of prototypes potential before actually building the first prototypes.

Adequate ventilation beneath a helmet favours the removal of heat and sweat by forced convection. The inclusion of helmet openings promotes ventilation, but it may be a disadvantage for protection against impacts - which is the primary function of the helmet - and can also promote heat gain by radiation, which is undesirable [1-3]. Thus, helmets must be optimized considering the balance between these effects. The goal should be to include as few openings as possible, so as to maintain the structural integrity of the helmet, and still allow its efficient ventilation.

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# Optimal Sizing of Hybrid Power Source for Electric Vehicle using Li-ion Battery and Supercapacitors

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The electric vehicle (EV) has the main advantage of being an environment-friendly transportation mean, with non-polluting characteristics (locally only). Yet, it has some drawbacks, which the energy storage system (ESS) is one of the biggest. The success of the EV will only be achieved if the ESS offers a long life-cycle, reduced costs and charge time, high power and energy densities. Unfortunately, the current technology doesn't possess those characteristics in a single source and, from an energy density point of view, it is still distant from the internal combustion engine vehicles. As using only a single source it's difficult to fulfil the requirements above mentioned, in this work we will consider an hybrid ESS composed by two different sources, with complementary characteristics: batteries and supercapacitors (SCs) [1]. The lithium battery is used as energy tank and SCs as power source which ensures high power needed for the propulsion and regenerative braking.

As the costs are one of the major concerns of the automotive market, we will investigate how to reduce them. To tackle this problem we need to derivate the model for the storage system [2], which will then be used to formulate the optimization problem for the hybrid ESS sizing [3]. After that, the goal is to choose the optimal amount of batteries and SCs cells that allows us to complete the driving cycle with the requested performance. For that it was used a linear optimization algorithm to minimize the installation cost of the ESS, subjected to the total energy required and the instantaneous power that the vehicle needs to fulfil throughout the standard driving cycles [1].

With this preliminary work we concluded that the installation costs can be reduced compared with the use of only one power source. However, there are more advantages. Usually the hybrid power source becomes more compact, i.e., smaller mass and volume. The use of the SCs allows energy loss reduction, benefiting the fact that the SCs present smaller internal resistance. So, there is an increasing of energy efficiency, which permits higher autonomies [4]. There is also a better use of the battery, which is important to reduce the aging problems, promoting long term cost reduction.

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# Project of Lattice Wind Towers and Comparison with the Typical Self-supported Tubular Towers

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The rising demand of wind energy has led to a huge development of the related technologies. The following work aims to model and dimension a lattice tower heighten 150 meters, and afterwards establish a comparison with the typical self-supported tubular towers.

The taken actions in this kind of design were the wind action, the action of the ice combined with the seismic action, taking into account all dynamic effects. To the design process it was developed an Excel program, using a Visual Basic program which calculates the wind action according to the disposals established by the Eurocodes (EN 1991-1-4 and EN 1993-3-1).

It was modulated a lattice tower with the geometrics defined by the author and with the needed characteristics to support a wind turbine. The metallic calculation was achieved with the Autodesk Robot Structural Analysis Professional 2012, iteratively with the calculation program of the wind action developed by the author. As expected to this kind of towers the actions that conditioned the design were the action of the wind and also combined with ice.

The final results were the expected ones; in the body of the tubular towers it is possible to save steel and concrete in its foundations. The concrete amount used in a lattice tower foundation represents only 35% of the concrete used in a tubular tower with the same height. In relation to the tower's weight it is possible to save almost 40%, when compared to a tubular tower with the same height (150 m).

In conclusion, the initial cost of a wind lattice tower is cheaper and the capacity to elevate the rotors is higher, which allows them to produce more energy, in relation to the self-supported tubular towers. start writing your short abstract, it would be convenient to write first a very short introduction, to better orientate the reader to the matter covered/discussed below.

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# Reengineering of a Prototype for Autonomous Driving

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**Abstract:** In this paper the concept of a completely autonomous vehicle is presented. The prototype is intended to take part in the autonomous driving competition that will be held at the National Robotics Festival (FNR) hosted at Lisbon, Portugal. The prototype has already participated in previous editions of the FNR and this project objective is to present a redesign of the previous version, featuring a new, simpler and cheaper approach. The prototype features a vision system for lane tracking, signal detection and identification and obstacle detection as well as a control system for mapping, navigation and world state data management.

**Introduction:** The FNR is an annual competition with its first edition in 2001. Several teams compete in different types of events, such as robotic soccer or autonomous driving. The autonomous driving competition features an 8-shaped track with two lanes. A crosswalk exists in the center of the track to indicate the starting and finishing position. Several obstacles, a tunnel, a construction site and several traffic signs are placed in the track. All participant prototypes must avoid all obstacles and obey all traffic rules and limitations stated by the signs.

**Project Description:** The developed prototype has a distributed and modular architecture. The software is composed by two modules: the Control Module (which is subdivided into world state update, navigation, mapping and decision making modules) and the Vision System module (which is subdivided into obstacle detection, signal identification, lane tracking and crosswalk detection modules). The hardware features a single circuit board which integrates and connects the motors and corresponding drivers, a data acquisition platform, security mechanisms and distributes power to all the components. The main components for the control system are a laptop and the data acquisition board, whereas the main components for the vision systems are the laptop and a RGB-D sensor.

**Conclusion:** It is expected from this project that a simple, modular and low cost prototype will be able to perform complex real-time image processing and real-time decision making tasks, leading to the development of a complete autonomous road navigation system. An in-depth study will also be performed to investigate the relevance and accuracy of RGB-D sensors.

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# Study on the Impact of Using PCM to Enhance the Energy Efficiency of a Radiant Ceiling System

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Phase Change Materials (PCM) present interesting properties conditioning a building due to its latent heat storage capability within the range of comfort temperatures [1-3]. In this project, a model of a cooling system with PCM inside pipes, where cool water flows around the PCM's capsule, is studied. During the day, water at ambient temperature circulates in closed loop in the pipes arrangement, decreasing the room's air temperature due to the PCM capability to absorb heat. During the post occupancy schedule, a backup cooling unit will restore the PCM initial conditions to its initial state by cooling the circulating water. The increase in thermal inertia generated by the addition of PCM in the building envelope allows for the system to have a passive behaviour, without need for conventional cooling systems. By allowing this, it creates the opportunity to bridge the gap between energy demand and supply profiles.

A parametric and transient study is conducted to evaluate the advantages of using an intermediate working fluid and to quantify the heat absorbed by the PCM system. Based on early studies, the parameters tested are: water flow velocity, the room's air velocity, the phase change temperature and three different chemical compositions of PCM. Two different configurations of the PCM unit are also tested: plate and cylinders. To make this study, a model using basic heat transfer correlations was created. The energy balance of this system is as follows: heat convection flux between the room air and the water flow, heat convection transfer flux from the water to the PCM container and heat conduction through the PCM container with energy storage in the PCM.

Results show that cylinders configuration have an average heat transfer per surface area 50% higher when compared to plate configurations for the period studied. It was concluded that the thickness and conductivity of the PCM container plays an important role in the heat transferred, because the PCM had not completed its phase change after the 10h period of simulation. For the three PCMs tested with phase change temperatures of 21 °C, 22 °C and 23°C, the average heat transfer rates per surface square meter were 22 W/m<sup>2</sup>, 17 W/m<sup>2</sup> and 13 W/m<sup>2</sup>, respectively. When an air velocity of 0.5 m/s was set, the average heat transfer rate was 37% higher than for natural convection and when an air velocity of 2 m/s was set, the average heat transfer rate was 100% higher than with natural convection. This indicates that there are benefits in the use of a fan in this system.

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# The influence of resolution when extracting color features for an image classification task

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Image classification and retrieval tasks can be computationally demanding on large databases of high resolution images. Those databases are growing in number of images, and are often acquired through sensors with high sampling capacity. For this reason it is important to investigate alternatives to process such images so that retrieval and classification are not hampered.

The feature extraction can be very time consuming with high resolution images, specially when local and contextual information is used to compute the features. In this study we propose fast methods to image resolution and color reduction in order to speed-up the feature extraction step and investigate how this reduction affects the performance of the classification task. The results are analyzed in terms of the relation between accuracy and resolution reduction.

One of the important questions we tried to answer was if is possible to significantly reduce the image size while maintaining accuracy.

The features were extracted from images of different resolutions, starting with the original resolution of the database and afterwards with versions of 75, 50 and 25% of the original size (Fig. 1). Besides, two different distance functions were applied to compare the images from a database of natural images. Then, the Optimum-Path Forest (OPF) classifier was trained to obtain the results. This classifier was chosen because it has no parameters to tune, so it removes the possibility of misinterpretation of the results by a wrong setup. Also, three different hold-out configurations were used.

As a main result we found that, in general, the use of low resolution images maintains the quality of classification. The exception is one of the descriptors. The results indicate that reducing the size of images is an alternative for applications with large number of objects since it reduces the computational effort and maintain the effectiveness of classification.



Figure 1 - Images of different resolutions

# TiO<sub>2</sub>-mediated heterogeneous photocatalytic degradation of antibiotics: influence of inorganic ions

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The overall efficiency of the TiO<sub>2</sub> photocatalytic process can be affected by the presence of inorganic ions, which can compete for incident photons [1], for oxidizing radicals and either block or promote an increase in the surface density of TiO<sub>2</sub> adsorption sites, given the pH [2]. These mechanisms can promote or inhibit the rates of photocatalytic oxidation of organic pollutants depending on the nature and concentration of ions and on the pH of the solution. Therefore, this work aims to assess the effect of the presence of different inorganic ions typically found in wastewaters in the TiO<sub>2</sub> photocatalysis of three antibiotics, oxytetracycline (OTC), amoxicillin (AMX) and oxolinic acid (OXA). Experiments at lab-scale photoreactor were carried out in order to investigate the individual effect of Cl<sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, PO<sub>4</sub><sup>3-</sup>, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup> (1 g L<sup>-1</sup>) and HCO<sub>3</sub><sup>-</sup> (0.1 g L<sup>-1</sup>) in the degradation of 20 mg L<sup>-1</sup> of individual aqueous solutions of OTC, AMX and OXA with 0.5 g L<sup>-1</sup> TiO<sub>2</sub>. The photoreactor comprises a solar radiation simulator; a compound parabolic collector with 0.023 m<sup>2</sup> of illuminated area with aluminium reflectors and borosilicate tube; a 1 L glass vessel with a cooling jacket coupled to a refrigerated thermostatic bath to ensure a constant temperature during the experiment; a magnetic stirrer to ensure complete homogenization of the solution inside the glass vessel; one peristaltic pump to promote the water recirculation between the CPC and the glass vessel, and pH and temperature meters. The antibiotics degradation was followed by High Performance Liquid Chromatography (Diode Array Detector) and the experimental data obtained from the kinetic studies were fitted to a pseudo-first-order mathematical model by a non-linear regression method. The fitting of the results obtained have shown that all the reactions approximately followed pseudo-first order kinetics. The rate constants for the individual degradation of OTC, OXA and AMX (in the absence of ions) were found to be 4.3 ± 0.4 L kJUV<sup>-1</sup>, 1.09 ± 0.07 L kJUV<sup>-1</sup> and 0.47 ± 0.01 L kJUV<sup>-1</sup>, respectively. The outcome of the screening experiments for individual inorganic ions effects on the kinetic rate constants are as follows: for OTC negative effects were in the order PO<sub>4</sub><sup>3-</sup> > SO<sub>4</sub><sup>2-</sup> > NO<sub>3</sub><sup>-</sup> > Cl<sup>-</sup> > NH<sub>4</sub><sup>+</sup> > HCO<sub>3</sub><sup>-</sup>, for OXA negative effects were found as PO<sub>4</sub><sup>3-</sup> > SO<sub>4</sub><sup>2-</sup>, while positive effects were found as NH<sub>4</sub><sup>+</sup> > Cl<sup>-</sup> > NO<sub>3</sub><sup>-</sup> > HCO<sub>3</sub><sup>-</sup>. Finally, for AMX positive effects were found in the order PO<sub>4</sub><sup>3-</sup> > NO<sub>3</sub><sup>-</sup> > NH<sub>4</sub><sup>+</sup> > SO<sub>4</sub><sup>2-</sup> > Cl<sup>-</sup>, HCO<sub>3</sub><sup>-</sup>.

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# Update of demonstrator for autonomous driving using ROS

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The ROS (Robotic Operation System) was developed to facilitate the integration of various functionalities of the robot (locomotion, vision, navigation, positioning, sensors). This robotic operation system provides several libraries, services and tools for robotic applications.

This project uses the demonstrator autonomous driving developed in another project for the *Festival Nacional de Robótica* and presented in *IJUP'11* (Autonomous Driving Demonstrator – CondeDois). The objective of the study is to perform the migration of the systems of vision, navigation, control and locomotion, developed across platforms and apply the migration to a single platform, ROS.

In the vision system is used the infrared sensor (Kinect) for detection of obstacles and the pre-defined library of ROS, PCL (Point Cloud Library) to obtain the identification signals, semaphores and identification of the track.

The differential locomotion of the robot consists in two brushless motors (EC 45 flat 50 W) controlled by Advance Motion Controls drivers (dzralte-012L080). In the system developed previously for controlling the motors, the command signal and odometry communication is performed between the driver and the microprocessor - Arduino Mega 2560. In this study, the motor data communication implemented in ROS is replaced by serial port, which eliminates the need to use microprocessor and facilitates, in the future, the sharing of information of odometry system.

The relative location of the robot is based on the odometer pulses from the hall sensor of the motor. The robot also uses an absolute location by markers located using real time vision system.

The control system will be developed in C++ IDE (Integrated Development Environment) with help of the 3D simulator (rviz) of ROS, thus replacing the previous control software, based FPC/Lazarus platform. This simulator can be used as a tool test and debugging purposes, while the software will control path planning and decisions to guide the robot.

The conclusion of this study is intended that the robot is able to perform autonomous driving competition with full ROS operation. In migration to ROS, it is expected that data sharing and features of robot perform in less time, less computational processing and improved facility of interacting with other future systems.

# Which parameters have dominant effect in the thermal performance of concrete-based materials?

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Concrete is one of the most important construction materials. It is used in a wide range of applications and, therefore, it is crucial to investigate the performance of various concrete-based materials. One of the relevant factors concerning the applicability of concrete structures is its thermal performance, whether applied to residence buildings or industrial facilities. [1,2]

In this study, we used a FEM-based approach to study the thermal performance of concrete under different constant and transient conditions. Firstly, starting from a representative domain of a concrete wall, we studied the effect of parameters such as the particles diameter, spacing and disposition inside a concrete wall. In a second phase, it was evaluated the effect of external transient conditions, such as the ambient temperature, radiation heat losses and solar incidence (Fig 1-a)).

It was possible to observe that the effective thermal conductivity of a concrete wall is widely influenced by the aggregate fraction, as well as the particle disposition inside the wall. It was also observed that, not considering variables like the radiation heat losses and solar incidence causes considerable deviations on the calculation of thermal fluxes through a wall (Fig. 1-b)).

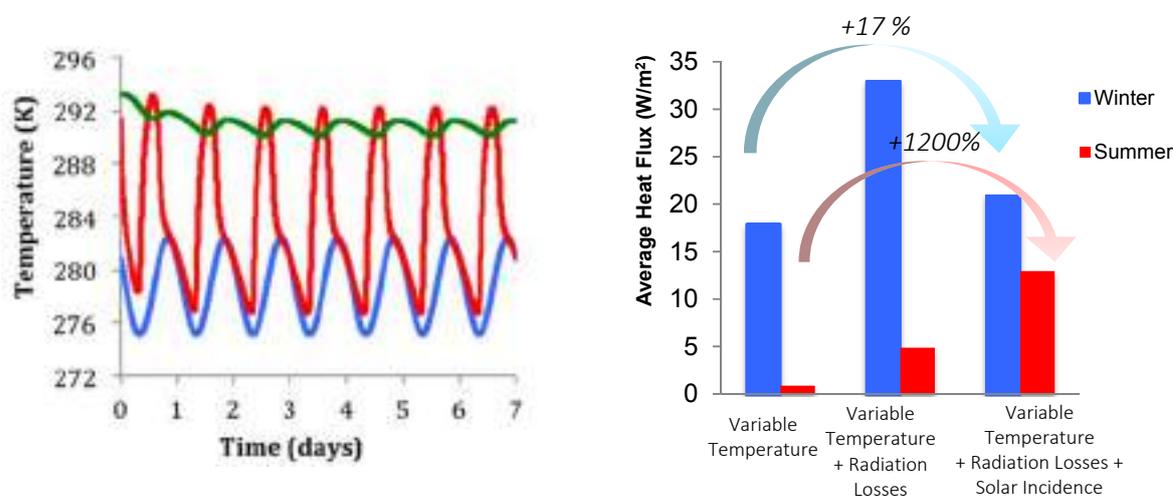


Figure 1 – a) Evolution of the ambient temperature (blue), exterior surface temperature (red) and interior surface temperature (green) during a week in winter time; b) Average thermal flux for the 3 different study steps, considering different boundary conditions.

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# Wireless Sensor Network for Refrigerated Vehicles and/or the Food Industry

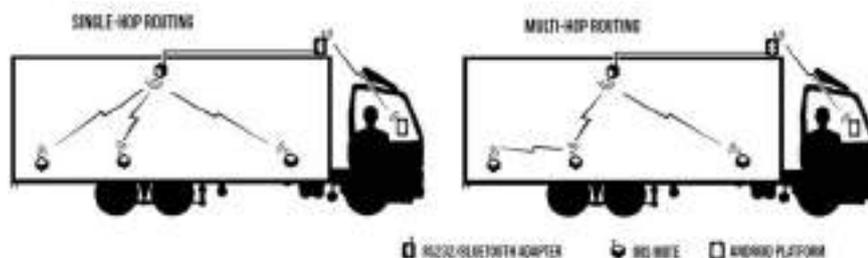
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The transport of perishable foods in refrigerated vehicles requires control of ambient conditions, particularly relative humidity and room temperature. Fluctuations of these values beyond specific tolerance windows can deteriorate the quality of the transported food, potentially leading to economic loss and or public health issues.

Wireless Sensor Networks (WSN) are a sensing technology particularly appropriate for this application [1], due to its flexibility on sensors placement and adaptability to different environments as those found in the containers used in the supply chain of perishable food [2], with multiple types of load, with different sizes and characteristics.

The focus of this work is on the energy efficiency of the WSN, especially regarding the routing topology. Two strategies were implemented (Fig. 1), a single hop topology with each mote communicating directly with the gateway, and a multi-hop topology where a mote forwards the information of other motes that are farther away from the gateway [3]. In both cases, the receivers feedback the Received Signal Strength Indicator (RSSI) of each packet to the respective sender so that it can adjust its own transmit power to the minimum needed for good communication, thereby implementing transmit power adaptation to the actual RF medium propagation characteristics [4].



*Figure 1. Two topologies under test, single-hop (left) and multi-hop (right).*

A test bed is currently set up with a number of IRIS motes. Experimental results now being generated will allow discriminating the best option for maximizing WSN lifetime.

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## A preliminary SEM study of an iron meteorite

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Iron meteorites are thought to originate from impact disrupted bodies of 1000 km or more in size that accreted before chondrites, possibly at 1-2 AU, rather than in the asteroid belt, during the formation of our Solar System [1].

Most iron meteorites exhibit Widmanstätten patterns [2], which develop as a two-phase intergrowth of kamacite and taenite and can provide valuable information on the cooling rate of the parent body.

In this work, we present a preliminary SEM/EDS characterization of an iron meteorite collected at Campo del Cielo. The meteorite evidences Neumann lines [3] superposed on a microscopic Widmanstätten-like structure and several inclusions of schreibersite and rhabdite. EDX chemical analysis revealed an unusually high Ni-content and an approximate bulk composition of Ni-60 wt% Fe-40 wt% P-7 wt%.

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## Magnetic susceptibility of meteorites

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In this project, we intend to perform a basic structural and magnetic characterization of collections of meteorites with non-destructive and non-invasive techniques. Since meteorites are rare material and in some situations are difficult to bring to the laboratory to perform scientific studies (example: meteorites in museums), we plan to use a mobile facility to measure magnetic susceptibility and bulk density. The study will include meteorites from collections of Porto, Coimbra, Lisboa and other cities's museums. The results of this study will be added to existing planetology research databases, in order to allow a reliable interpretation of magnetic information carried in extraterrestrial materials. Such information provides constraints on ancient magnetic field intensities and on the evolution of minor bodies in our Solar System.

We expect to establish and promote intra and interuniversity scientific collaborations in the study of meteorites and asteroids. This is a research area of growing interest, as highlighted by the NASA 2011 Dawn space mission to asteroid Vesta, with great potential for attracting new students and young scientists.

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# From phonemes to mythemes: structural correspondences in two tragedies

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The myth, being a kind of speech with an entirely contingent content, encrusted by a number of contradictory findings. We wonder, which produces a facet of similarity between two compositions from one extreme to another of Earth? Lévi-Strauss, proclaiming the uniqueness of myth against other linguistic incursions, emphasizes the place of mythical thought as opposed to poetry through the translation process. While poetry, being translated into a language non-vernacular raises misshapen semantics, the myth reaches the level of intelligibility even in the worst translation (Lévi-Strauss, 1985) <sup>[1]</sup>. This panorama sheds fruitful light for the structural interpretation of a short story and a play. What's that approaches "The Black Cat" by Edgar Allan Poe (2010) <sup>[2]</sup> to the mythical part of Nelson Rodrigues, "Dark Angel" (2005) <sup>[3]</sup>?

This, that's said before, this textual composition analyzes structurally, at first, two distinct narrative genres that, despite belonging to different historical junctures, provide, in general, potential elements of broader perception of the structure of myths. Therefore, the axis of examination focuses on the comparative study of both analyses, searching similes of differential removals and significant correlations in the process of paradoxical formation of myth, continuous and discontinuous.

The initial conclusions of the research show that, even having been produced by different intellectual impulses, the story and a play present a founding membership in the antinomy of mythical thought, working on developing a clad structure that relates to earlier times, the current panoramas, and at the same time allows predict future guidelines. Both compositions of Poe and Rodrigues not only create dualisms, slightly distinct from each other, as also repeat the historic tessitura established in each of the narratives, in a movement similar to a spiral. It is also noted universalizing and universal features of works in so far as it doesn't exist a defined spatial and temporal delimitation, "the action is happening at anytime, anywhere" (Aguiar, 2005) <sup>[3]</sup> as well as the myth in Lévi-Straussian perspective.

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# Interfaces between the teaching of sociology and cognitive psychology: metacognition

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This work has as main orientation interfaces between learning strategies, management and evaluation in teaching Sociology in High School. Learning to learn, metacognition or learning strategies, as some authors call, designates the procedures that the individual uses to plan, monitor and regulate the flow of thought in their own learning.

In this sense, there is an evaluation process in the institutional management of metacognition: record and rubric. According Davis, Nunes and Nunes (2005) <sup>[1]</sup> record is supported in the accompanying of teachers and students in the functioning of the group, through a report in which all participation, demonstration of the students are registered. The team dynamics in the evaluation process consists of a definition of roles that alternate every class, ie, there is alternation in the activities developed by the students, while one is the mediator of the discussion and the other is the timekeeper of exposure time and the indicator is responsible for record the group's decisions in the minutes of a meeting. The rubric, in turn, is characterized in the terminology of Davis, Nunes and Nunes as objective criteria of assessment that are exposed to students at first time.

The process of learning founded by these metacognitive practice was applied in teaching Durkheimian concept of *anomie*. So rubric, guided in objective criteria of evaluation, such as interpretation and expression of theorists covered in the subject, mastery of concepts and application in other socio-cultural contexts, social relationships with teachers and peers, beyond the scrutiny of punctuality.

Partial results of such an enterprise analytics show that students can anticipate the details of characterization conceptual through logical unfoldings of definition of anomie for Durkheim. This intellectual operation also points to effective reflective exercises in so far as students provided with the conceptual tools apply to other contexts and anthropo-sociological realities. Performed gauging until then signalize the formation of a holistic array beyond the curriculum components.

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# EFFECT OF DIRECTION AND CONSISTENCY OF HANDEDNESS ON MANUAL PROFICIENCY OF CHILDREN WITH AND WITHOUT DOWN SYNDROME

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It has been proposed that left-handedness is linked to pathology. However, recent studies on developmental disorders tend to show that lack of asymmetry, rather than left-handedness, is associated with pathology (Carlier et al., 2011). Hand preference (HP) and its relation with manual proficiency were studied in Down Syndrome (DS) and typically developing (TD) children.

The participants were pre-schoolers (age range: 3-5 years) divided in groups according to the HP direction (DS: 16 right-handers and 8 left-handers; TD: 32 right-handers and 4 left-handers) and consistency (DS: 13 strongly lateralized and 11 weakly lateralized; TD: 29 strongly lateralized and 7 weakly lateralized). Laterality was assessed using a preference task (Gabbard & Rabb, 2000; Harris & Carlson, 1993) and it was applied a manual dexterity test adapted to children.

Although the two groups did not differ for the frequency of left-handers ( $\chi^2 = 1.333$ ,  $p = 0.248$ ), the occurrence of left-handedness was higher in children with DS ( $n=8$ ) than in TD ( $n=4$ ) children. Moreover, inconsistent left-handers were higher in the group of DS ( $n=7$ ) compared to TD ( $n=2$ ), although differences did not reach statistical significance ( $\chi^2 = 1.000$ ,  $p = 0.317$ ).

Concerning performance, the interaction between group and consistency of handedness ( $F(1,56) = 5.632$ ;  $p = 0.021$ ) revealed that in the TD group, the more strongly lateralized subjects exhibited better performance, while the opposite was found for the DS children. These results somewhat support other studies indicating that less rightward preference and weakly handedness are associated with intellectual deficiency in children.

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# Exercise training prevents left ventricle maladaptive remodeling induced by pulmonary arterial hypertension

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Despite pulmonary arterial hypertension (PAH) specifically overloads the right ventricle (RV), it is known that left ventricle (LV) can also become dysfunctional. Exercise training provides a cardioprotective phenotype against several deleterious insults. We addressed the role of exercise training in the modulation of LV structural and functional changes secondary to PAH induced by monocrotaline (MCT). Male Wister rats (n=50), were randomly divided into 2 groups: 1) treated with vehicle solution (VEI; n=20) and 2) with MCT (MCT; 60mg/kg, sc; n=30). After administration of VEI or MCT, half of the animals from each group were submitted to an exercise training protocol during 4 weeks (5 days/week, 60 min/day, 25m/min; EX+VEI and EX+MCT, respectively), while the other half remained sedentary (SED+VEI and SED+MCT, respectively). One day after ending their protocols, all animals were prepared for LV hemodynamic evaluation with conductance catheter. The following data were collected in baseline conditions and after isovolumic conditions: peak systolic pressure (LVP<sub>max</sub>), rate of pressure rise and fall (dP/dt<sub>max</sub> and dP/dt<sub>min</sub>) and relaxation velocity evaluated by the time-constant *tau*. Next, all animals were sacrificed and LV samples were collected for histological analysis. Results are summarized in the table and are expressed as mean± standard deviation.

	SED+VEI	EX+VEI	SED+MCT	EX+MCT
<b>Morphometric Parameters</b>				
LV (g)	3.877±0.057	3.823±0.072	3.760±0.168*	3.879±0.164 <sup>#</sup>
<b>Histological Parameters</b>				
CSA (μm <sup>2</sup> )	0.3433±0.013	0.3237±0.022*	0.2756±0.025*	0.3209±0.020 <sup>#+</sup>
<b>Hemodynamic Parameters in Baseline Conditions</b>				
HR (bpm)	399.6±26.51	389.0±32.23	330.1±54.19*	376.2±50.71 <sup>#</sup>
LVP <sub>max</sub> (mmHg)	114.2±15.38	121.4±18.11	92.74±19.19*	101.7±13.36 <sup>+</sup>
dP/dt <sub>max</sub> (mmHg/s)	7765±1678	7228±1233	5633±2078*	6204±1162
dP/dt <sub>min</sub> (mmHg/s)	-8925±2069	-8613±940,2	-5315±2692*	-7059±1800
tau (ms)	8.75±0.86	10.59±2.32	12.33±3.08*	10.22±1.41 <sup>#</sup>
<b>Hemodynamic Parameters in Isovolumic Conditions</b>				
LVP <sub>max</sub> (mmHg)	169.7±9.351	164.7±10.39	151.0±22.77	188.7±17.47 <sup>#</sup>
dP/dt <sub>max</sub> (mmHg/s)	7260±946,6	7971±3079	5364±1769	7043±1198
dP/dt <sub>min</sub> (mmHg/s)	-7002±2465	-6718±2600	-4425±1852*	-5654±1047
tau (ms)	9.540±0.9315	13.14±1.761	16.98±4.326 <sup>#</sup>	11.35±1.811 <sup>#</sup>

LV: Left ventricular weight; CSA: Cardiomyocytes cross sectional area; HR: Heart rate; LVP<sub>max</sub>: Peak systolic pressure; dP/dt<sub>max</sub>: Rate of pressure rise; dP/dt<sub>min</sub>: Rate of pressure fall; tau: Relaxation velocity;

\*P<0.05 vs SED+VEI; #P<0.05 vs SED+MCT; +P<0.05 vs EX+VEI.

**Conclusion:** Exercise training performed during the development of PAH prevented LV dysfunction and cardiomyocyte atrophy.

# **Ginástica de Trampolins: Efeito do Sexo e do Treino no Equilíbrio Estático Unipodal**

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Trampoline Gymnastics is characterized by the capability of performing jumps and combined movements using techniques that allow performing complex trajectories in the air, with rotations in the body axes in sequence and without stops. By the fact that the balance is a coordinative motor ability which translates itself into the ability to maintain a desired position of the body on its support base, its importance seems clear regarding this modality.

In this sense, the main purpose of this study is to ascertain what influence has both training and gender on static balance, within a given group of young athletes of Gymnastics on Trampoline.

To perform this procedure, we used a sample of 12 athletes, 6 males and 6 females, between ages 10 to 12 years-old. These athletes fit into the echelon of the category Kids Trampolines and belong to Vilacondense Gym Club, based in Vila do Conde.

To evaluate the study subjects, we used the one-leg static balance of the preferred member before and after training, through the Flamingo Balance Test, Eurofit [1].

In processing the data we used descriptive statistics, the Mann-Whitney and Wilcoxon tests, in which the value of significance was set at  $p \leq 0.05$ .

The general conclusions to be drawn from this study are: i) the male and female athletes obtained better results in the static one-leg balance test, after the training; ii) the male athletes presented more positive results in what concerns to the female sex, before training; iii) in the static one-leg balance test, after training, we have found that the male individuals, obtained a better performance than the female individuals.

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# Immediate effect on blood pressure in normotensive individuals when executing eccentric-only resistance exercises with various intensity levels.

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**Introduction:** The importance of resistance exercise for the reducing and maintenance of blood pressure levels is well-known. However, people suffering from hypertension and cardiopathy should be more careful doing this type of exercise due to the inevitable increase in level of cardiovascular activity [1]. Therefore, it's necessary to study various methods of training, which would permit to decrease the risk of cardiovascular injury. Researches proposed to use the eccentric-only method as its application provided lower levels of cardiovascular stress than the ones obtained with the concentric-only method [2]. Nevertheless, the exercise intensity used in the study was lower than the one recommended for gaining force and hypertrophy. **Objectives:** To check the immediate response to the resistance exercise performed using the eccentric-only method and to verify the blood pressure changes in the normotensive individuals. **Methodology:** During the evaluation the auscultatory method of measuring blood pressure was applied. The participants were thirteen young people who had practised musculature for more than six months. The task was to perform a one-arm biceps curl, eccentric-only, in three series with three repetitions each. In order to analyse the pressure levels the results obtained during the periods of exercise, rest and recovery were considered (three intensity levels with 85%, 100% and 115 % of one *repetition maximum*). **Results:** systolic pressure and diastolic pressure were compared in the following conditions: rest vs. exercise, rest vs. recovery as well as between the intensity levels within each series. As a result, no significant differences were verified, except for the comparison between the systolic and diastolic pressure while exercise and rest during the second and third series at 85% of RM and the first series at 115% ( $p < 0,05$ ). **Conclusions:** the eccentric-only method proved to provide lower levels of cardiovascular stress. It's considered to be a viable alternative to individuals for whom intense resistance training is inadvisable. This notwithstanding, further research is necessary.

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# Initial training contributions to professional preparation: a study with students' teachers of Physical Education

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Training should be focused more on practice and practice analysis [1]. Teacher education tend to be overly theoretical with a deficit of practice, of practice reflection and of knowing how to do. Taking account this ideas it is important to know the perception of the student about their educational process. How the novice teachers' views their teaching skills and identity as teachers, constructed during the initial training.

The main purpose of this study is to analyze the students' teachers perceptions about the contribution of the initial training to their professional life. The sample was composed of thirty one students' teachers, of both genders, attending the masters degree in Teaching of Physical Education in Primary and Secondary Education, from the Institute Superior da Maia, during the academic year of 2011/2012. The data was gathered in the final stage of the pedagogical practicum using a Portuguese preliminary version of a questionnaire adapted for Ezer, Gilat and Sagge [2]. The questionnaire comprise seven components: personal data; agents; dimensions of the teacher training, teachers' roles; motivation to teach; teaching/learning conceptions; and an open question about the most significant change that the student teachers experienced during their initial training.

Regarding the novice teachers, it was highlighted that the *role of the cooperating teacher*; in terms of the dimension of the teachers' training, it was emphasized the *importance of the practical component* (practicum). The questions regarding the development of the students' abilities, and *continuing training* were also pointed out in the conclusions of the study. Another relevant aspect it was the participants' low-level of expectation on the questions about the *employability, career and promotions*, as well as the *financial stability*.

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## Perception of students' teachers about the contribution of initial training for their professional life

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The purpose of this study was to analyze the novice teachers' perceptions about the contribution of initial training for their professional life. The sample includes forty one physical education novice teachers that are attending the second year of master degree on Physical Education, Faculty of Sport of University of Porto, of the academic year 2011/2012.

The instrument used was an adaptation of the questionnaire elaborated by Ezer, Gilat & Rachel Sagee [1], which includes a part with structured questions concerning to five aspects of the teaching profession: i) agents of training; ii) components of teachers' training; iii) roles of teachers; iv) motivation for teaching; v) and conceptions of teaching and learning process. The other part includes an open question about the most significant changes that novice teachers experienced on their initial training. For data analysis statistical descriptive and inferential analysis procedures were applied. The content's analysis was also used for gathering more relevant information.

The results show that the *passage from theory to practice*, the *supervised teaching practice* and the *cooperation within teachers* were the dimensions that they perceived as more important in their initial training. They also state that the *teacher's role* is a contribute to the development of individual skills of each student and transmit universal values to them. The results highlight that the *interactions between students and teachers* lead to self-realization and that the *teaching profession requires constant development and learning*. However, they refer that the teaching profession does not ensure employability both financial stability and the pre-service teachers prefer a traditional approach of teaching.

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# Prior exercise prevents left ventricle dysfunction and maladaptive remodelling secondary to pulmonary arterial hypertension in rats

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**INTRODUCTION:** Exercise training provides a cardioprotective phenotype against several cardiac insults such as ischemia, infarction and toxic compounds. The impact of exercise training on left ventricular dysfunction (LVD) induced by pulmonary arterial hypertension (PAH) is unknown. The present study analyzed the preventive effect of exercise preconditioning on LVD in a rat model of PAH induced by monocrotaline (MCT).

**METHODS:** Sixty male Wistar rats were randomly separated into two experimental groups: sedentary animals (SED, n=35) and trained animals (EX, n=25). Exercise training consisted of running sessions of 60 min/day, 5 days/week, with a treadmill speed of 25 m/min. Four weeks after the beginning of experimental protocol, animals from each group were injected with 60mg/kg of MCT (SED+MCT, n=25; EX+MCT, n=15) or vehicle (SED+V, n=10; EX+V, n=10). After this procedure, all animals remained sedentary for an additional period of 4 weeks. Next, the animals were submitted to a LV hemodynamic evaluation, sacrificed and samples from LV were collected for histological analysis.

**RESULTS:** Main results are presented in Table 1 as mean  $\pm$  standard deviation.

**CONCLUSION:** Exercise preconditioning attenuated the left ventricular dysfunction induced by pulmonary arterial hypertension, being this improvement associated with a reduced level of cardiomyocytes atrophy and tissue fibrosis.

	SED+V	EX+V	SED+MCT	EX+MCT
<b>Morphometry</b>				
LV+S (g)	0.6166 $\pm$ 0.049	0.6663 $\pm$ 0.072	0.5588 $\pm$ 0.036*	0.6440 $\pm$ 0.050 <sup>†</sup>
<b>Hemodynamic evaluation</b>				
HR (bpm)	401.5 $\pm$ 26.23	366.3 $\pm$ 27.64	330.3 $\pm$ 54.13*	371.1 $\pm$ 33.45 <sup>†</sup>
P <sub>max</sub>	115.8 $\pm$ 14.58	119.7 $\pm$ 13.11	86.74 $\pm$ 24.81*	102.6 $\pm$ 14.31
dP/dt <sub>max</sub> (mmHg/s)	7742 $\pm$ 1495	8678 $\pm$ 1407	5174 $\pm$ 2403*	6530 $\pm$ 172 <sup>‡</sup>
dP/dt <sub>min</sub> (mmHg/s)	-9483 $\pm$ 2110	-8727 $\pm$ 1595	-3967 $\pm$ 1885*	-6486 $\pm$ 159* <sup>†‡</sup>
Tau (ms)	8.742 $\pm$ 0.814	9.379 $\pm$ 0.766	13.17 $\pm$ 3.879*	10.28 $\pm$ 0.990 <sup>†</sup>
<b>Histology</b>				
CCSA (m <sup>2</sup> )	239.0 $\pm$ 79.83	254.4 $\pm$ 95.02*	184.1 $\pm$ 57.32*	235.7 $\pm$ 75.93 <sup>†‡</sup>
Fibrosis (% from total area)	3.017 $\pm$ 1.384	2.241 $\pm$ 0.9138*	3.819 $\pm$ 1.364*	2.841 $\pm$ 1.382 <sup>†</sup>

LV+S: left ventricle+septum weight; HR: heart rate; P<sub>max</sub>: ventricular maximum pressure; dP/dt<sub>max</sub>: peak rate of pressure rise; dP/dt<sub>min</sub>: peak rate of pressure fall; Tau: time constant of ventricular pressure decay; CCSA: cardiomyocytes cross sectional area. \*P<0.05 vs SED+V, <sup>†</sup>P<0.05 vs SED+MCT, <sup>‡</sup>P<0.05 vs EX+MCT.

## Selection and detection of female talents at the Classical Ballet:

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**Introduction:** Detecting sport talents regards to search for ideal people for a basic formation process, and during the selection process people prepared to reach high levels of performance are desired [1,2]. To ensure good results in the process of detecting and selecting talents technical-scientific instruments and structural measures are needed [3]. The Classical Ballet, as per its perform requirements, has criteria that establish how to identify talents. However, some literature doesn't consider clarifying and deepening the criteria assessed by schools during contests.

**Objective:** Regarding the gap discussed above, this study purposes elucidate the processes that are used to select and detect new female talents at the Classical Dance.

**Methodology:** Besides the literature review, it was conducted an interview with fourteen professionals (professors and physiotherapists) from "Escola de teatro Bolshoi do Brasil" in Joinville, at Santa Catarina and at the "Escola Estadual Maria Olinewa" from "Teatro Municipal do Rio de Janeiro", both located in Brazil.

**Results:** The interviews, still in analysis, show that the detection of new talents occurs in different levels of requirements, according to the experience of the candidates, through medical-physiotherapy exams and observations made by a committee on traditional or free classes. Children between eight and ten years, without experience in the modality, are evaluated by their physical characteristics, searching for body proper to the technical of this dance, such as: joint flexibility, with emphasis on the feet/ankles, hips (en dehors) and backbone (hyperextension); limbs proportionality, body composition and posture. In a second analysis, musicality, physical coordination and "intelligence to the dance" are assessed. Individuals with more than 10 years, besides the characteristics above, others are being evaluated in a class: technical knowledge; power of jumps; general muscular strength and personality. At the selection, all the skills above are considered but the most important are the artistic qualities.

**Conclusion:** The studies about these processes are going to be a support for professionals who deal with the preparation, selection, and detection of dancers. Given the peculiar demands of dancer's body, appear complex issues about teaching/learning, performance, training and subjectivities of the body that are going to be describe at the study.

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## Strength profiles of football players in different age groups

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Isokinetic strength tests provide important information on muscle functional. This study analysed the strength profile of youth football players in different age groups.

Sixty-eight youth male football players (U14, n=27, age=13.5±0.4 yrs; U15, n= 26, age=14.4±0.2 yrs; U16, n=14, age=15.6±0.3 yrs; U17, n=26, age=16.4±0.4 yrs) were tested. Isokinetic tests were performed on the hamstrings and quadriceps, consisting on concentric (CON) exertions of the quadriceps and hamstrings, and eccentric (ECC) exertions of the hamstrings, at 60°/s. Maturational status was self-reported. Differences by age levels were tested with ANCOVA, with maturation as covariate. Bonferroni *post hoc* tests were used to assess between-group differences. Significance was set at P<0.05.

Overall, U17 players presented higher PT values than U15 and U14 in all muscle groups, both in concentric and eccentric modes (p<0.001; table 1). There were also differences between U16 and U14 players in all muscle groups, both in concentric and eccentric modes (p<0.001; table 1). The U16 players also differed from U15 in most tests (p<0.001; table 1). No age group differences were observed in the conventional and dynamic H/Q ratios (p>0.05; table 1).

Table 1. Isokinetic strength profiles of youth football players in different age groups, with maturation as a covariate. Values are mean±SD.

	U17	U16	U15	U14	P	<i>Post hoc</i>
Q <sub>CON</sub> Dom 60°/s	214±40	187±42	157±31	139±37	<0.001	U17>U15,U14 U16>U14
Q <sub>CON</sub> Ndom 60°/s	212±37	179±50	140±27	132±28	<0.001	U17>U15,U14 U16>U15,U14
H <sub>CON</sub> Dom 60°/s	115±22	109±30	80±20	72±22	<0.001	U17>U15,U14 U16>U15,U14
H <sub>CON</sub> Ndom 60°/s	103±21	97±26	76±15	66±18	<0.001	U17>U15,U14 U16>U15,U14
H/Q <sub>CON</sub> Dom 60°/s	55±10	58±10	51±7	52±7	0.450	
H/Q <sub>CON</sub> Ndom 60°/s	48±8	54±5	55±9	50±8	0.470	
H <sub>ECC</sub> Dom 60°/s	195±46	191±44	150±35	142±28	<0.001	U17>U15,U14 U16>U14
H <sub>ECC</sub> Ndom 60°/s	176±46	170±40	139±34	141±34	0.013	U17>U15,U14
H <sub>ECC</sub> /Q <sub>CON</sub> Dom	104±24	105±15	95±19	91±19	0.289	
H <sub>ECC</sub> /Q <sub>CON</sub> Ndom	90±25	106±21	99±25	93±15	0.478	

Q, quadriceps; H, hamstrings; CON, concentric; ECC, eccentric; Dom, dominant limb; ND, non-dominant limb.

The results of the present study suggest that football players increase muscle strength due to maturational development and age. Notwithstanding, youth football players appear to present normal values of conventional and functional H/Q ratios.

# Explosion of differentiability for tridimensional suspension flows

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We show the following result for two diffeomorphisms defined on surfaces with basic sets: if a topological conjugacy between them exists and is differentiable at a point in the basic set, then the conjugacy has a differentiable extension to the surface.

Moreover, we extend this result to three dimensional suspension flows. More specifically, given two topologically conjugated diffeomorphisms on their basic sets, contained on surfaces, and two ceiling functions defined in these basic sets, the associated suspension flows are equivalently conjugated. Furthermore, if this equivalence is differentiable at a point, the differentiability extends throughout the suspension manifold.

# ***Gelsemium sempervirens* L.: Phenolic profile and *in vitro* antidepressant activity**

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Monoamine oxidase - A (MAO-A) catalyzes the oxidative deamination of serotonin and norepinephrine, this reaction leading to the production of aldehydes and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). A high MAO-A activity has been implicated in the etiology of depression, a psychiatric disorder that affects 150 million people worldwide. Furthermore, end products of this enzymatic reaction can also cause oxidative stress. Therefore, compounds with ability to inhibit MAO-A activity and/or with H<sub>2</sub>O<sub>2</sub> scavenging potential are seen as useful for the treatment of this disorder.

Taking into consideration that *Gelsemium sempervirens* L. (Loganiaceae) is a medicinal plant traditionally used as sedative, anti-spasmodic, anti-anxiety, anti-seizure and analgesic, and therefore able to alleviate symptoms of central nervous system disorders, we investigated the possible beneficial effects of the hydromethanolic extract of this species against depression. This extract displayed moderate *in vitro* inhibition of MAO-A (IC<sub>50</sub> = 611 µg/mL) and H<sub>2</sub>O<sub>2</sub> scavenging activity (IC<sub>50</sub> = 504 µg/mL), showing its ability to prevent oxidative deamination, but not to directly prevent oxidative stress. In order to correlate the bioactivity with the chemical composition an HPLC-DAD-ESI/MS<sup>n</sup> analysis of this extract was performed, revealing that it is mainly composed by quercetin-3-*O*-(2,6-di-rhamnosyl)glucoside, besides other flavonoids and three phenolic acids (Fig. 1).

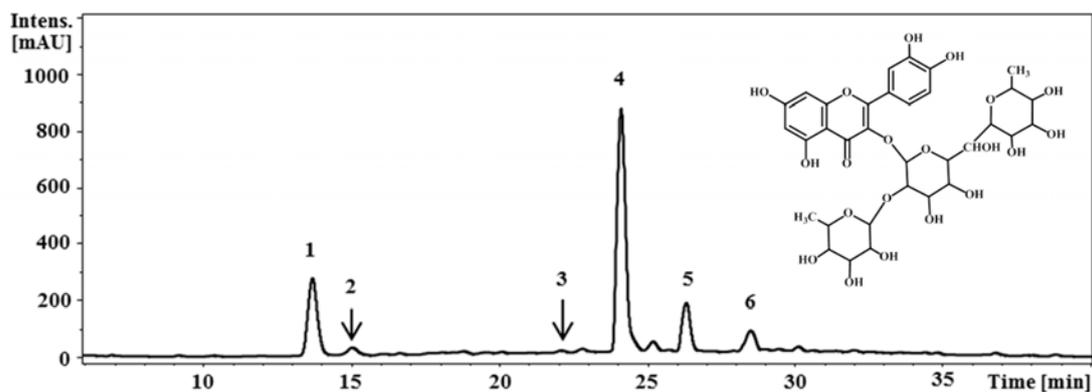


Fig. 1 – HPLC-DAD chromatogram of the hydromethanolic extract of *G. sempervirens* obtained at 320 nm and structure of the main compound. 1 - 5-dihydrocaffeoylquinic acid; 2 - 5-dihydromethoxycaffeoylquinic acid; 3 - 4-*p*-coumaroylquinic acid; 4 - quercetin-3-*O*-(2,6-di-rhamnosyl)glucoside; 5 - kaempferol-3-*O*-(2,6-di-rhamnosyl)glucoside; 6 - quercetin-3-*O*-(6-rhamnosyl)glucoside.

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## ***Glandora diffusa*: advances in bioactive compounds**

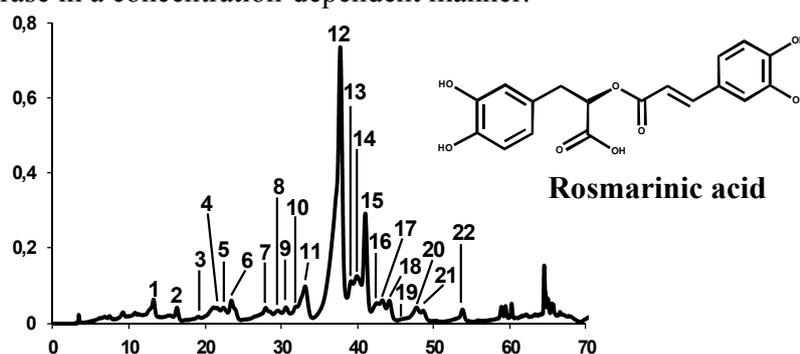
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*Glandora diffusa* (Lag.) D. C. Thomas (Boraginaceae) is one of the 6 species of the *Glandora* genus. Commonly known in Portugal as “sargacinha” or “erva-das-sete-sangrias”, *G. diffusa* is traditionally consumed as an infusion and this extract revealed antiradical and antidiabetic potential [1]. In the present work, ethanolic extracts of *G. diffusa* purchased in the local market, from three different medicinal plants distributors, were studied. Their phenolic profile was assessed by HPLC-DAD and their sugars, fatty acids, sterols and triterpenes composition was determined by GC-MS.

Polymers of caffeic acid are the most abundant phenolics found, represented mainly by rosmarinic and salvianolic acids (Fig. 1). Additionally, the studied extracts are rich in sugars, fatty acids, sterols and triterpenes. *G. diffusa* ethanolic extracts were able to inhibit acetylcholinesterase and butyrylcholinesterase in a concentration-dependent manner.



**Fig. 1.** HPLC-DAD phenolic profile of ethanolic extract from *G. diffusa* aerial parts. (1) Caffeic acid; (2) Salvianolic acid H; (3) *p*-Coumaric acid; (4) Quercetin-3-*O*-(2,6-di-rhamnosyl)galactoside; (5) Quercetin-3-*O*-(2,6-di-rhamnosyl)glucoside; (6) Salvianolic acid E isomer; (7) Salvianolic acid I; (8) Kaempferol-3-*O*-(2,6-di-rhamnosyl)hexoside; (9) Kaempferol-3-*O*-(2-rhamnosyl)galactoside; (10) Kaempferol-3-*O*-(2-rhamnosyl)glucoside; (12) Rosmarinic acid; (15) Salvianolic acid B; (16) Kaempferol-3-*O*-(6-rhamnosyl)hexoside; (18) Isorhamnetin-3-*O*-(6-rhamnosyl)hexoside; (20) Methyl rosmarinic acid; (21) Salvianolic acid E isomer; (22) Salvianolic acid C isomer. Compounds 11, 13, 14, 17 and 19 are unknowns caffeic acid derivatives.

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### Acknowledgements:

The authors are grateful to Fundação para a Ciência e a Tecnologia (FCT) for Grant No. PEst-C/EQB/LA0006/2011.

# Glutathione and the antioxidant potential of binary mixtures with phenolic compounds

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Glutathione (GSH) is a water-soluble tripeptide composed of the amino acids glutamine, cysteine, and glycine. The thiol group is a potent reducing agent, rendering GSH the most abundant intracellular small molecule thiol, reaching millimolar concentrations in some tissues.

Polyphenols have the ability to trap free radicals, which contributes for their known antioxidant activity. In plant extracts, these secondary metabolites may act in concert, in such a way that their combined activities will be superior to their individual effects (synergistic interaction). Different types of polyphenols exist in a wide variety of foods. As so, the intake of these compounds at certain concentrations in the diet may potentiate the activity of GSH and thus fight better the oxidative stress.

The aim of this work was to investigate the DPPH<sup>\*</sup> scavenging activity of different classes of polyphenols: hydroxycinnamic acids; benzoic acids; flavonols, flavones, flavanones, 3-OH-flavanones. In addition, to evaluate the influence of methoxylation and glycosylation, isorhamnetin and luteolin-7-*O*-glucoside were also tested respectively. The mixtures with glutathione (GSH) were prepared with three different concentrations of compound in order to see if the synergistic or antagonistic behavior was concentration dependent (Fig.1).

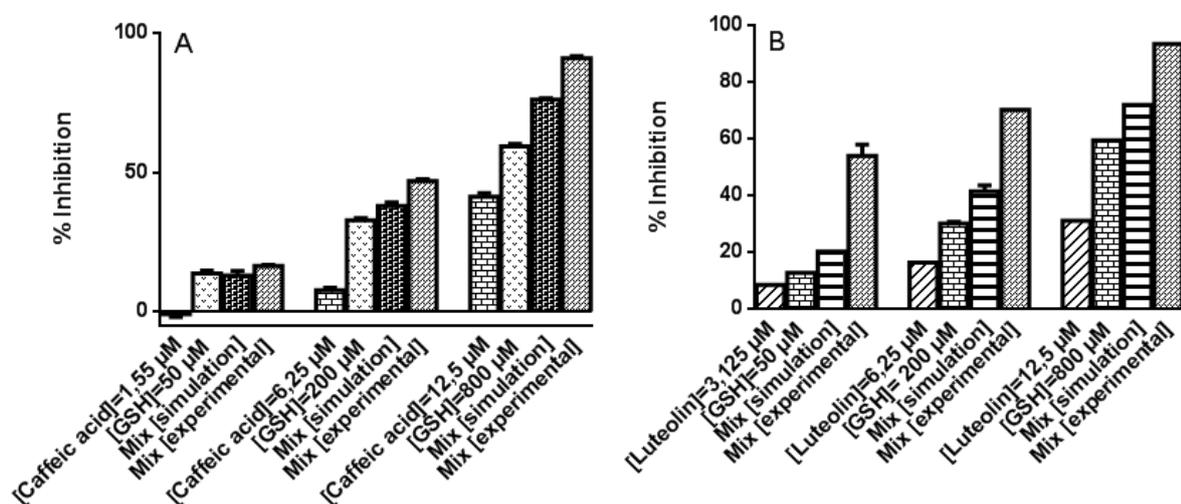


Figure 1 - DPPH<sup>\*</sup> scavenging by phenolic compounds alone or in mixture (Mix) with GSH. A, Caffeic acid; B, Luteolin.

The results revealed that phenolic compounds have synergistic effects with GSH.

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# Immunolocalization of arabinogalactan proteins and pectins, in the cork Oak female flower

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The cork oak is a dominant tree in the forests of southern Portugal. This tree has a socio-economic, a cultural and an environmental extreme importance for our country. Studies on the sexual reproduction of this tree are essential to understand the molecular mechanisms of fertilization and identify the difficulties associated with seed production.

Arabinogalactan proteins (AGPs) and pectins belong to a large family of plant glycoproteins, whose members have hydroxiproline-rich protein backbones which are highly glycosylated, and are generally associated with the cell membrane and wall, although they can also be found on the extracellular matrix. Previous studies in other flowering plants have shown that cell wall glycoproteins such as pectins and arabinogalactan proteins are of special relevance for the reproductive processes. However the localization of pectins and AGPs during cork oak floral development was never done before.

Plant cell walls give physical strength to the plant body and provide a barrier against the outside environment. Pectins, together with other polymers, participate in these functions..

AGPs are ubiquitously expressed in plants, and many evidences suggest their involvement in all major cell wall associated processes such as cell growth and development, reproduction and seed formation; our own previous work has shown a specific AGP expression pattern during plant gametogenesis in *Arabidopsis thaliana* and in *Trithuria submersa*.

In the present work, we performed immune citochemical localization of AGP and pectin epitopes, using specific monoclonal antibodies, in female flowers at different stages of flower development. The following monoclonal antibodies (MABs) for AGPs JIM 8, JIM 13, JIM 16 and JIM 17 were used LM7 and LM5 were used to discriminate between methyl esterified and unesterified pectins.

Our preliminary results revealed the presence of AGPs and pectins during cork oak female flower development with a specific pattern, suggesting that some AGPs can be important for the stages of sexual reproduction in cork Oak.

The authors are grateful for the financial support through FCT for the Project PTDC/AGR-GPL/118508/2010



# **Influence of the change of habitat in a group of lions ( *Panthera leo* ), in captivity**

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Nowadays there is a bigger concern from Zoo's to improve their facilities in order to provide a better quality of life to its animals. That's what happened in Maia's Zoo.

This work consists in studying the behaviour of a group of lions before and after their change of habitat. In the beginning of this work, the group consisted in two females (Mara and Nala) and one male (King). Unfortunately, in the past 25th of January, euthanasia was applied to one of the lionesses, Mara, due to health problems that kept her of having a pain free life. Since that moment, the observations of Mara's behavior were ignored and the study continued with King and Nala observations. It is important to note that Mara was an old lioness and in wild she would probably be dead by now.

Nala and King are about 17 and 22 years old, respectively, so it's unlikely that they're still fertile.

To study the behaviour of this group, *Ad libitum* and focal sampling were used. The observations started in August of 2011 and after a month of *Ad libitum* sampling, it was already understandable some behaviour of this particularly group. An ethogram was elaborated and then it was possible to do focal sampling in a better way. From September 2011 to November, focal sampling was done about 3 times per week, since 2 p.m. to 5 p.m.

Some environmental enrichment was also done once, using recorded roars of others lions to see how the group would react. Nothing happened.

Because of external reasons, like the delay of the constructions of the new habitat and bad weather conditions, the second part of the job was only possible to do from April to May 2012.

The behaviour of this group of lions is likely to be different of others because they're an old group and they don't interact so much as a younger group or a group with cubs would react.

However, the change of habitat showed an improvement of their behaviour.

## **Influence of water environmental factors on physiological parameters in *Lipophrys* sp**

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*Lipophrys* sp., (shanny) is a common intertidal fish, abundantly in the Portuguese coast. Given its importance in the dynamics of rocky intertidal communities, this species' ecology and behaviour have been intensively studied. Shanny was recently adopted as a sentinel species in biomonitoring toxicological studies, since it promptly responds to environmental alterations, without the occurrence of significant population structure alterations. The main goal of this study was to evaluate the influence of the seasonal variation of some environmental parameters (namely, water characteristics of tide pools) in physiological oxidative stress parameters of *Lipophrys* sp.

To attain this objective, juveniles are captured monthly along one year, in two beaches, in the north of the Portuguese coast, Cabo do Mundo (n=5) and Agudela (n=5). Immediately upon capture, individuals were weighed, measured, and different organs were collected for posterior biomarkers quantification (muscle - TBARS, eyes - AChE and liver - GST). Additionally, relevant environmental parameters were assessed in three different pools to characterize the water in terms of pH, conductivity, dissolved O<sub>2</sub> concentration, salinity, and oxidation-reduction potential.

Based in the obtained results (so far, only winter months have been assessed) we can conclude that individuals in both beaches were similar in terms of morphologic parameters. Data obtained after biomarkers quantifications showed slight variations between beaches; however a consistent pattern of variations among the different sampling intervals, was more evident. Environmental parameters did not varied significantly between the two sampling stations and along the sampled periods.

## Inside the sea urchin *Paracentrotus lividus* Lamarck

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The great structural diversity, associated to the high degree of complexity of some marine organisms and to the possible pharmacological effects of their constituents, confer to the sea an inestimable value as source of new molecules. *Paracentrotus lividus* Lamarck is a sea urchin, which gonads are considered a delicacy in several countries. The metabolic composition and the bioactivity of this macro invertebrate were studied by chromatographic means and microassays.

An ethanolic extract was prepared and its analysis by HPLC-DAD revealed the presence of fucoxanthinol, lutein and zeaxanthin. In addition, eight fatty acids and three sterols were characterized by GC-MS (Fig. 1).

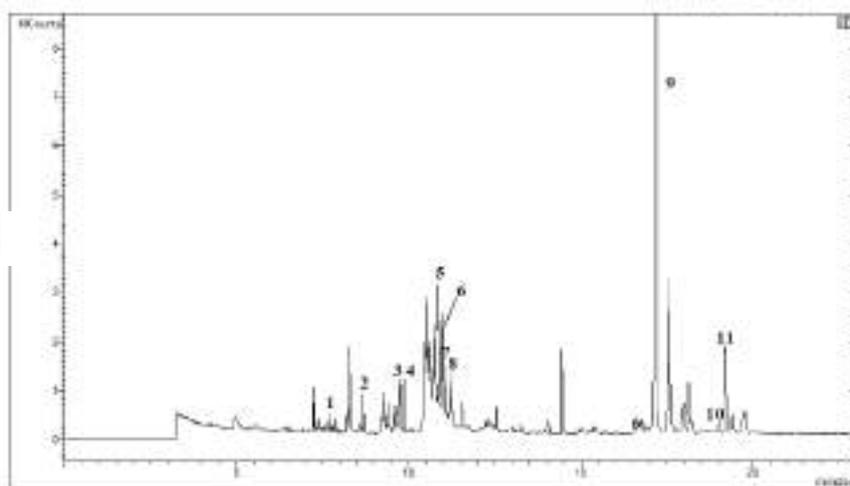


Fig. 1. GC-MS analysis of the ethanolic extract of *P. lividus*. Compounds: (1) myristic acid; (2) palmitic acid; (3) oleic acid; (4) stearic acid; (5) arachidonic acid; (6) eicosapentaenoic acid; (7) *cis* 11,14-eicosadienoic acid; (8) *cis* 11-eicosanoic acid; (9) cholesterol; (10) stigmasterol; (11)  $\beta$ -sitosterol.

The antioxidant capacity was checked against 1,1-diphenyl-2-picrylhydrazyl (DPPH<sup>•</sup>) and nitric oxide (<sup>•</sup>NO), but only a small effect was observed. No activity was found against selected Gram positive and Gram negative bacteria.

Further studies involving other biological assays may put some light on the biological potential of this species.

This work was developed within the optional curricular unit “**Bioactivity of Natural Matrices**” of the **5<sup>th</sup> year of the Master Degree in Pharmaceutical Sciences of the Faculty of Pharmacy**, University of Porto, under the responsibility of Paula Andrade (Head), Patrícia Valentão and Carla Sousa.

# Investigation of the Nitrogen Cycle and the Anaerobic Lifestyles of *Planctomycetes*

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*Planctomycetes*, a quite unexplored phylum of ubiquitous bacteria, have some unique features that make them stand out from the other groups. From their intracellular compartmentalization and lack of peptidoglycan in the cell walls to their budding reproduction they are a group worth of studying<sup>1</sup>. However, despite the knowledge already acquired there are still many things to discover about them, namely their nitrogen cycle metabolism.

The goals of this study were to investigate the anaerobic survival of 19 different strains of *Planctomycetes* (FC9.2, FC18, FC19, FC25, FF15, FF4, LF1, LF2, MsF5, Pd1, Sm4, UC8, UC9, UC17, UF6, *Blastopirellula marina*, *Planctomyces brasiliensis*, *Planctomyces maris*, and *Rhodopirellula baltica*) and their intervention in the inorganic nitrogen cycle.

Of the 19 strains studied only FC18, FF15, LF1, *R. baltica* and *P. brasiliensis* do not have an anaerobic metabolism when cultured in modified M13 medium<sup>2</sup> in anaerobic chambers (GENbox anaer; bioMérieux).

Regarding the nitrogen cycle and when comparing to the organic-nitrogen complete medium (modified M13 medium), 10 strains had a substantial growth (50–130%) in ammonia medium, nitrate only supported to a certain extent (20-80%) the growth of five strains and in the nitrite medium, only eight strains presented a residual growth (6-23%) showing thus resistance to this toxicant. Strains LF2 and MsF5 showed a preference towards ammonia.

Our results show that *Planctomycetes* and more precisely the *Planctomycetales* present a wide metabolic diversity.

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# Involvement of Peripheral Protein Quality Control (PPQC) in the regulation of E-cadherin stability at the Plasma Membrane

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E-cadherin (Ecad) is a transmembrane cell surface molecule with a key function in epithelial cell adhesion, through the establishment of calcium-dependent homophilic interactions at sites of cell-to-cell contact. Disruption of Ecad expression at the Plasma Membrane (PM) induces cell scattering and increased cell invasiveness, both *in vitro* and *in vivo*, representing a hallmark of invasive carcinomas [1]. Moreover, mutations in the CDH1 (gene encoding Ecad) are the only known genetic cause of Hereditary Diffuse Gastric Cancer (HDGC) [2]. In the majority of invasive carcinomas, the mechanisms leading to Ecad loss are poorly understood.

Peripheral Protein Quality Control (PPQC) has recently been proposed to be a specialized pathway for the regulation of unfolded proteins at the PM. It involves the recruitment of Hsc70 and CHIP E3 ubiquitin ligase by unfolded substrates to the PM, resulting in substrate internalization to the early endosome and subsequent lysosome-dependent degradation [3].

In this work, we have used one HDGC-associated unfolded mutant of Ecad (E757K), and a denaturing extracellular stimuli ( $\text{Ca}^{2+}$  removal), to analyse if unfolded PM Ecad is a substrate of PPQC. Our results show that E757K accumulates in endosomes and CHIP overexpression induces Ecad destabilization at the PM. The distinct domains of the ligase were found to differently influence WT and mutant Ecad, suggesting different mechanisms of regulation by CHIP. Furthermore, silencing of Hsc70 leads to increased PM expression of WT Ecad, indicating a role for Hsc70 in the regulation of Ecad expression at the PM. Additionally, we show that  $\text{Ca}^{2+}$  depletion is enough to destabilize WT and mutant Ecad at the PM, resulting in lysosome-dependent degradation of the unfolded protein. Inhibition of the lysosome in denaturing conditions results in Ecad retention at the PM, suggesting that unfolded Ecad follows the PPQC route.

Herein, we present the first group of evidences suggesting that unfolded Ecad might be a substrate of PPQC.

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# Isolation and characterization of codifying cDNAs for potentially allergenic proteins of *Acer negundo* pollen

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In the last years the increase of pollen allergies in urban areas has been attributed to atmospheric pollution. In urban public gardens and streets, *Acer negundo* specimens are among the most commonly used trees, being also known for their wide production of pollen.

Previous studies demonstrated that *Acer negundo* pollen contains multiple potentially allergenic proteins and the immunodetection assays indicated IgE recognition with all sera of polysensitized patients [1]. Proteomic analysis of the most prominent bands allowed identifying several proteins namely calreticulin a protein with recognized allergenicity with a molecular weight between 48-60 kDa.

A molecular approach was undertaken to isolate, clone and characterize the cDNA codifying for calreticulin with the aims of producing calreticulin recombinant protein and determine variations in calreticulin gene expression in pollen samples exposed to various air pollutants by RT-PCR as air pollution has an important influence in pollen allergenicity.

Since no known sequence of *Acer negundo* calreticulin cDNA has been described, a bioinformatics approach [2] was undertaken to design conserved degenerated oligonucleotide primers based on sequence alignments to known sequences of calreticulin from related species. Total RNA was extracted from *Acer negundo* pollen previously collected and stored at -20 °C and partial cDNAs of calreticulin and other potentially allergenic proteins were obtained by RT-PCR techniques, cloned and sequenced.

The extension of the cDNAs is being undertaken by 3'RACE and 5'RACE. Complete cDNAs will be characterized, sequenced and cloned into appropriate vector and bacterial strains for recombinant protein production for future allergenic tests.

Acknowledgments: This work was supported by FEDER funds through the COMPETE and National funds through FCT (PTDC/AAC-AMB/102796/2008).

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## Jellyfish *Pelagia noctiluca* Forsskal: more than a threat

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In the last few years, the continuous search for new bioactive molecules from Nature has shifted its target ecosystems from terrestrial sources to marine environments.

*Pelagia noctiluca* Forsskal is a jellyfish that emits a characteristic luminescence when threatened. Additionally, it releases a toxin that is responsible for erythema and edema. The metabolic profile of the ethanol extract of this species was analyzed by HPLC-DAD and GC-MS and revealed the presence of cholesterol and derivatives,  $\beta$ -sitosterol and one ergosterol derivative. No phenolic compound was noted, but the presence of saponins was confirmed.

The antioxidant capacity was checked against the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH<sup>•</sup>) and nitric oxide. A concentration-dependent activity was found, albeit maximum activity was set around 15% (Fig. 1).

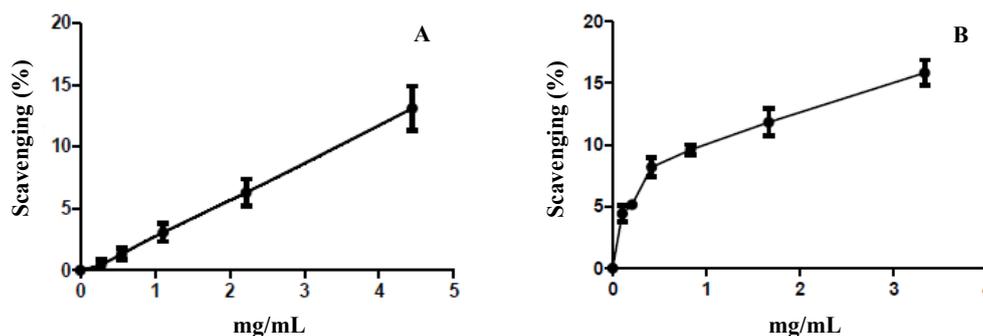


Fig. 1. Anti-radical capacity of *P. noctiluca* ethanol extract against (A) DPPH<sup>•</sup> and (B) nitric oxide. Results correspond to mean  $\pm$  standard error of three assays, performed in triplicate.

Antibacterial activity was assessed against selected Gram positive and Gram negative species, but all of them were resistant to the tested extract's concentration. In fact, it favored the growth of *Bacillus cereus*.

The results suggest that *P. noctiluca* can be an interesting source of saponins for pharmaceutical and food industry.

This work was developed within the optional curricular unit “**Bioactivity of Natural Matrices**” of the **5<sup>th</sup> year of the Master Degree in Pharmaceutical Sciences of the Faculty of Pharmacy**, University of Porto, under the responsibility of Paula Andrade (Head), Patrícia Valentão and Carla Sousa.

# Lactic acid bacteria isolated from Andean fermented foods with antibacterial activity against pathogenic bacteria

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Food fermentation is one of the oldest food processing and preservation methods, this process add safety, nutritional value and different flavors. Food which are fermented have been subjected to the actions of microorganisms or enzymes, so that desirable biochemical changes have occurred. Although the microbial and enzymatic processes responsible for the food manufacture have not been fully elucidated until now, it is generally accepted that lactic acid bacteria (LAB) contributes significantly to the properties of many fermented foods [1].

LAB are the most commonly used microorganisms in food processing industry and includes the genera *Lactococcus*, *Streptococcus*, *Lactobacillus* and *Pediococcus*, among others. Lactic acid and other end products of LAB metabolism, including hydrogen peroxide, diacetyl, acetoin, organic acids and bacteriocins, act as bio-preservatives by inhibition of the food spoilage microorganisms [2]. While the role of these metabolic end products has long been appreciated, the contribution of LAB-derived bacteriocins may frequently have been overlooked.

The objective of this work was to isolate the LAB from fermented Andean food products (meat, vegetables and goat milk) and tested their antibacterial activity against several pathogenic bacteria. The antibacterial substances produced by isolated bacteria were tested against *Listeria monocytogens*, *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis*, *Salmonella typhimurium* and *Escherichia coli* by agar well diffusion test [3].

A total of 44 colonies were isolated from MRS and all strains were characterized as Gram positive and catalase negative. Concerning antibacterial activity, the results obtained show a higher antibacterial activity against *Listeria monocytogens* and *Enterococcus faecalis*.

These results that confirm the antibacterial activities of autochthonous strains isolated from Andean fermented foods could be used as natural food preservatives.

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# LOCALIZATION AND ENZYMATIC ACTIVITY OF ECTO-NTPDASES AND ECTO-5'-NUCLEOTIDASE IN THE RAT ILEUM

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The regulation of contractile activity of the gastrointestinal tract is mainly controlled by the myenteric plexus (MP), the outer part of the enteric nervous system localized between the circular (CM) and the longitudinal (LM) smooth muscle layers [1]. Acetylcholine (ACh) is the primary excitatory neurotransmitter released from myenteric motor neurons, which release is controlled by purines. Previous results from our group demonstrated that ATP transiently increases basal ACh release from myenteric motoneurons through the activation of P2X2 receptors. The extracellular hydrolysis of ATP into ADP via ecto-NTPDases favors the activation of P2Y<sub>1</sub> receptors leading to inhibition of neuromuscular transmission in LM and CM layers [2,3]. Adenosine generated from the hydrolysis of released adenine nucleotides plays a dual role on evoked ACh release from myenteric motoneurons via the activation of inhibitory A<sub>1</sub> and facilitatory A<sub>2A</sub> receptors localized on ganglion cell bodies and axon terminals of myenteric neurons, respectively. Considering the relevance of the 'purinergic cascade' regulating cholinergic neurotransmission in the enteric nervous system, we investigated the regional distribution and activity of ectonucleotidases responsible for the extracellular catabolism of adenine nucleotides and adenosine formation in the isolated rat ileum. Ecto-NTPDase1 is relatively absent from smooth muscle layers and nerve plexuses of the rat ileum. The MP expresses high levels of NTPDase2 and NTPDase3 immunoreactivity, which is also extended to the underlying LM. The CM also stained positively with the anti-NTPDase3, but not with anti-NTPDase2, antibodies. NTPDase 2 immunolabeling was also found in the submucous plexus (SP). The selective NTPDase3 inhibitor, PSB 06126 (10 μM), prevented tissue deposition of lead phosphates (Wachstein-Meisel histochemistry reaction) [3] in the expected layers of the ileum upon incubation with 100 μM of ATP or ADP. The Wachstein-Meisel histochemical reaction almost completely prevented by POM-1, when this compound was applied in a concentration (100 μM) high enough to inhibit both NTPDase2 and NTPDase3 activities. Immunolabeling against ecto-5'-nucleotidase was predominant in muscle layers. Incubation of ileum sections with AMP (100 μM) in the presence of the ecto-5'-nucleotidase inhibitor, α,β-methyleneADP (200 μM), blocked the deposition of lead phosphate in both LM and CM layers. Data suggest that released ATP may be sequentially hydrolysed into ADP by ecto-NTPDase 2 (an enzyme that preferentially hydrolyses nucleoside triphosphates), as well as by ecto-NTPDase 3, which converts equally well ATP and ADP into AMP. Localization of ecto-NTPDase2 at the MP-LM facilitates ADP accumulation and, thereby, inhibition of evoked ACh release by P2Y<sub>1</sub> receptors activation [2]. NTPDase3 seems to be the key enzyme in the catabolism of ATP, without much ADP accumulation, at the MP-CM level. Adenosine formation from AMP, via ecto-5'-nucleotidase, occurs preferentially at the smooth muscle level indicating that the nucleoside might be a key regulator of the neuromuscular transmission through tonic activation of facilitatory A<sub>2A</sub> receptors [5].

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## Marines do it better: the case of *Cystoseira usneoides* (Linnaeus) M. Roberts (Phaeophyta)

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Marine ecosystems are considered to present the greatest phylogenetic biodiversity. Marine organisms have been attracting the researchers' interest in the last decade due to their bioactive metabolites, which arise from several biosynthetic pathways. Among them, macro algae and their derived products have been used for both dietary and therapeutic purposes. For the present study *Cystoseira usneoides* (Linnaeus) M. Roberts was selected. This Phaeophyta species has a restrict distribution, being found in the northeast and east Atlantic ocean and west Mediterranean sea. The purpose was to establish some relationship between the metabolic composition of an ethanolic extract and its antioxidant and antimicrobial potential.

The HPLC-DAD analysis allowed the detection of four carotenoids, while one sterol and two fatty acids were determined by GC-MS. The occurrence of phenolic compounds in the extract was confirmed by the reaction with alkaline hydroxide.

The extract revealed a concentration-dependent antioxidant capacity against 1,1-diphenyl-2-picrylhydrazyl (DPPH•) and nitric oxide (•NO) radicals (Fig. 1) and activity against two Gram positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*).

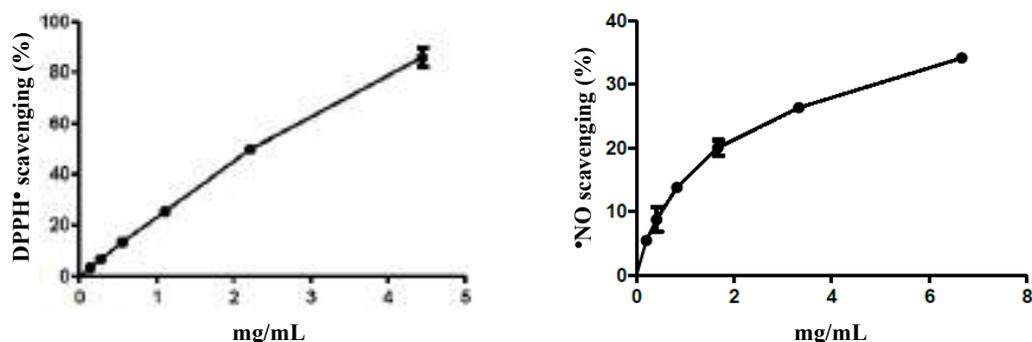


Fig. 1. Scavenging activity of *C. usneoides* ethanol extract against DPPH and nitric oxide radicals. Results correspond to mean  $\pm$  standard error of three assays, performed in triplicate.

The observed activities can be, at least partially, explained by the determined compounds, particularly phenolic compounds and carotenoids, and by the interactions between them. The results point to the interest of exploring this macro alga for pharmaceutical applications.

This work was developed within the optional curricular unit “**Bioactivity of Natural Matrices**” of the **5<sup>th</sup> year of the Master Degree in Pharmaceutical Sciences of the Faculty of Pharmacy**, University of Porto, under the responsibility of Paula Andrade (Head), Patrícia Valentão and Carla Sousa.

# Metabolites from brown seaweeds with promising biological activities

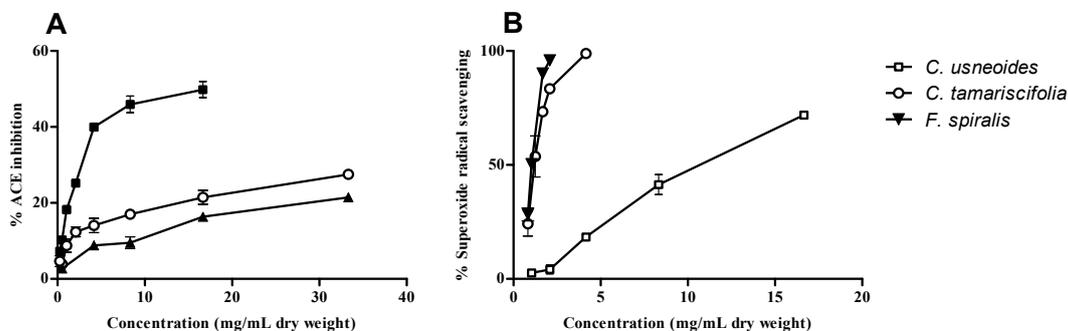
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Marine macroalgae constitute a rich source of bioactive compounds, being used in food, textile, and pharmaceutical industries. Due to the competitive environment where these organisms live, they are promising matrices in the search for new bioactive compounds. Brown algae (Phaeophyta) are particularly interesting, as they are the only organisms on earth producing phlorotannins, and have a great variety of carotenoids. These classes of compounds exhibit important chemical properties and physiological roles in algae organisms, being also promising for the development of new drugs, due to their antioxidant properties and their capacity to inhibit target enzymes related with several neurological diseases.

Reactive oxygen species (ROS) formed during aerobic life, such as superoxide radical ( $O_2^{\bullet-}$ ), hydroxyl radical ( $HO^{\bullet}$ ) and hydrogen peroxide ( $H_2O_2$ ), are recognized for being associated not only to initiation, but also to promotion and progression of multiple diseases. Alzheimer's disease is one of the most common forms of dementia, for which there is still no cure. The presence of free radicals can exacerbate disease progression.

In the present study interest is focused in the determination of antioxidant activity of phlorotannin purified extracts and carotenoids extracts from three species of Phaeophyta (*Cystoseira tamariscifolia* (Hudson) Papenfuss, *Cystoseira usneoides* (Linnaeus) M. Roberts and *Fucus spiralis* Linnaeus) collected along the Portuguese west coast.



**Fig. 1.** Acetylcholinesterase inhibitory capacity of carotenoid extracts (A) and superoxide radical scavenging activity of purified phlorotannins extracts (B) of three Phaeophyta species.

In addition to their antioxidant potential, Phaeophyta extracts are capable of inhibiting acetylcholinesterase and butyrylcholinesterase, which are involved in Alzheimer's disease (Fig. 1).

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## MIPs recovery methodology for red wines attacked by *Dekkera bruxellensis*

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Unpleasant odors may appear in wine in different stages, although organoleptic alteration usually occurs during ageing, prior to bottling, especially when wines are kept in old barrels [1,2]. Contaminating yeasts of the genus *Dekkera/Brettanomyces*, especially *Dekkera bruxellensis*, are able to produce large amounts of unwanted volatile phenols (VP), leading to wine deterioration and high economic losses associated to the presence of unpleasant aromas often described as “phenolic”, “leather”, “horse sweat”, “stable” or “varnish” [1,2].

For these reasons, the development of new methods for easy and cheap recovery of wines affected by the off-flavours produced by *Dekkera* spp. becomes an important target. In this study we propose the use of molecular imprinted polymers (MIPs) for wine recovery. MIPs are synthetic polymers with recognition sites, able to specifically bind to a target molecule (template).

The aims of this work were to define the MIPs conditions to recovery wines affected by VP and to evaluate their effect on wine chemical composition. For this purpose, a wine with VP was treated with two MIPs synthesized with different templates, namely 4-ethylphenol (MIP1) and 4-ethylguaiacol (MIP2). As a control, non-imprinted polymers (NIP) were prepared for each assay.

The recovery of VP obtained with both MIPs was not significantly different ( $\approx 55\%$ ), MIP1 revealing the highest retention capacity. In a general way, in the wine sample treated with MIPs was observed a decrease of the amounts of other volatile compounds. Regarding phenolic composition, some qualitative and quantitative differences were noticed among the different treatments, particularly the decrease of colored phenolic compounds with the MIP treatment.

Nevertheless, this methodology may contribute to the recovery of spoiled wines, although it needs to be improved to reduce the modifications of other organoleptic characteristics, namely color.

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# Multibiomarker responses of *Carcinus maenas* exposed to moderate heavy metal contamination

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Estuarine pollution is a major environmental issue of concern. The input of contaminants can induce chemical stress to estuarine organisms. Additionally, estuaries are transitional zones with high variation of many abiotic factors able to influence distribution and physiology of these organisms<sup>[1]</sup>. In the last decades, efforts have been applied to mitigate pollution effects by limiting discharges and emissions of priority substances, among others, and attaining a good environmental status of water bodies<sup>[2]</sup>. This raises the need to establish parameters providing good baseline data and useful to monitor the effects of moderate to low contamination levels. Specially because moderate levels elicit subtle biological responses difficult to discriminate from responses to confounding variables<sup>[3]</sup> like abiotic factors<sup>[4]</sup>. This study thus aims at investigating biological responses of *Carcinus maenas* to moderate heavy metal contamination using a set of biochemical biomarkers.

*C. maenas* is a key species of estuarine food webs with wide geographical distribution, easy to identify and collect, and used as bioindicator<sup>[4]</sup>. Male crabs were captured in the winter and summer of 2012 in two NW Portuguese estuaries: the Lima estuary is moderately polluted by heavy metals <sup>[5]</sup>, the Minho estuary is low impacted and used as reference site. Water samples were collected at the same time to determine abiotic parameters (pH, salinity, conductivity, concentration of nutrients). In the laboratory crab tissues were isolated and used to determine several biomarkers according to established methods<sup>[4]</sup>: the activities of lactate dehydrogenase (LDH) and acetylcholinesterase (AChE) in the muscle, the activities of glutathione *S*-transferases (GST) and glutathione peroxidase (GPx), and the levels of total glutathiones (TG) and lipid peroxidation (LPO) in the digestive gland.

Preliminary results point out that biomarkers related to detoxification (GST) and anti-oxidant defences (TG and GPx) appear to be the most useful to discriminate different levels of estuarine contamination using *C. maenas*.

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## Neurotoxin accumulation in *Gibbula umbilicalis* fed with BMAA-producing cyanobacteria

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The  $\beta$ -methylamino-L-alanine (BMAA) is a potential neurotoxin, related to neurodegenerative diseases (ALS/PDC and Alzheimer). Hypothesis that BMAA can be accumulated by some marine invertebrates has been formulated [1,2]. It was recently discovered that in Portuguese estuaries various strains of cyanobacteria can produce BMAA [3]. Thus, the objective of this work is to obtain more data about the potential transfer of the neurotoxin from cyanobacteria to marine invertebrates, which are part of the human trophic chain.

Several strains of cyanobacteria were grown in BG11 and MN growth media for two weeks, at 25°C of temperature and under cycles of 14 hours of light followed by 10 hours of obscurity.

BMAA was extracted from the cyanobacteria through a microwave assisted extraction, and its detection and quantification were obtained by LC-MS/MS. BMAA-producing strains were selected to feed the snail *Gibbula umbilicalis* that had been collected from Memória beach (Matosinhos). The snails were placed in aquariums in BOGA (Biotério de Organismos Aquáticos) at CIIMAR. The aquariums had the capacity of 20 L and had aeration. These were filled with 3 L of marine water and every two days the water was changed and the organisms were fed.

The specimens were collected after 5, 10, 15 days of feeding with a BMAA-producing strain and 5 days of depuration. The analyses for the presence of BMAA will be performed during 2013.

This project presents a very relevant topic because of the wide geographic distribution of cyanobacteria, and the possible implications of the neurotoxic properties of BMAA to public health.

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## Oxidative metabolism of the endocannabinoid anandamide in decidual cells

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Endocannabinoids (eCBs) are endogenous signalling mediators that bind to cannabinoid receptors, which are molecular targets of  $\Delta^9$ -tetrahydrocannabinol, the main active component of *Cannabis sativa*. These molecules along with receptors, transporters and respective metabolic enzymes form the endocannabinoid system (ECS). Several eCBs have been discovered, with emphasis on N-arachidonylethanolamine, also called anandamide (AEA). Anandamide can be degraded through different pathways, either by fatty acid amide hydrolase (FAAH), the main pathway, which produces arachidonic acid (AA), or by cyclooxygenase-2 (COX-2), with the production of prostaglandin ethanolamides (PG-EAs) or prostamides whose roles in health and disease remain to be elucidated. Endocannabinoids are involved in several basic biological processes like neurotransmission, immune response, fertility and pregnancy [1]. However, the mechanisms by which they exert their effects at maternal interface are not clearly understood. We have previously demonstrated that ECS machinery operates on decidual cells [2] and that AEA has the ability to induce apoptosis [3], although it is not clear if this effect is mediated by AEA or its metabolites. In this study we investigated in rat primary decidual cell cultures the implications of the modulation of AEA metabolic pathways on cell death. Decidual cells were cultured for 24h in the presence of AEA (10  $\mu$ M) and COX-2 and FAAH inhibitors, the Celecoxib (0,1-10 $\mu$ M) and the URB597 (0,1 $\mu$ M; 1 $\mu$ M), respectively. The effects on cell viability were analysed by MTT assay whereas COX-2 expression was analyzed by Western blot. We found that 10 $\mu$ M of AEA induced a statistical significant reduction of cell viability. This effect was partially reversed by Celecoxib at 0,1 $\mu$ M, while no alteration was detected with FAAH inhibitor. No differences in the levels of COX-2 expression were observed with AEA treatment. These results suggest that the effects of AEA in reducing decidual cells viability are achieved through the oxidative metabolism by COX-2, and not by FAAH. The data about AEA metabolism in decidua is yet insufficient, thus it is important to study the significance of oxidative metabolism of eCBs in decidual cells to discover the underlying effects. Therefore, the use of COX-2 inhibitors may provide information about the implications of the modulation of this metabolic pathway, whose alterations may be involved in cases of infertility and pregnancy disorders.

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# Phenolics profile: a tool in the distinction of grape varieties

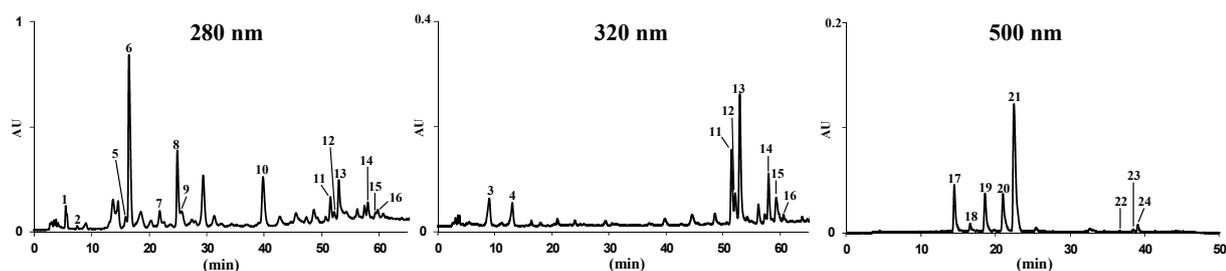
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Characterization of phenolic compounds in grapes has attracted much interest. These phytochemicals have demonstrated potential beneficial effects for health. They also contribute strongly to the organoleptic characteristics of grapes and therefore of the wine obtained from them and they have showed to be good chemical markers to characterize different varieties of grapes [1].

In this work, phenolic profile of 14 different varieties in the same maturity stage of red and white grapes collected in Northeast Portugal were studied. Several flavonols, phenolic acids, flavan-3-ols, one stilben and anthocyanins were determined. In a general way, all grape varieties present the same kind of compounds, but quantitative differences were found. The most abundant compounds were malvidin-3-*O*-glucoside for anthocyanins, the flavan-3-ols catechin, epigallocatechin and epicatechin or the flavonols quercetin-3-*O*-galactoside/quercetin-3-*O*-glucuronide and quercetin-3-*O*-glucoside for non-coloured phenolics.



**Fig. 1.** HPLC-DAD phenolic profile of *Vitis vinifera* cv Moscatel Galego Tinto grapes methanolic extract. (1) Gallic acid; (2) HMF; (3) Caftaric acid; (4) Coumaric acid; (5) Catechin; (6) Epigallocatechin (7) Syringic acid; (8) Epicatechin; (9) Epigallocatechin gallate; (10) Epicatechin gallate; (11) Quercetin-3-*O*-galactoside/quercetin-3-*O*-glucuronide; (12) Quercetin-3-*O*-rutinoside; (13) Quercetin-3-*O*-glucoside; (14) Kaempferol-3-*O*-glucoside; (15) Isorhamnetin-3-*O*-glucoside; (16) Syringetin-3-*O*-glucoside; (17) Delphinidin-3-*O*-glucoside; (18) Cyanidin-3-*O*-glucoside; (19) Petunidin-3-*O*-glucoside; (20) Peonidin-3-*O*-glucoside; (21) Malvidin-3-*O*-glucoside; (22) Petunidin-3-*O*- $\rho$ -coumaroylglucoside; (23) Peonidin-3-*O*- $\rho$ -coumaroylglucoside; (24) Malvidin-3-*O*- $\rho$ -coumaroylglucoside.

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Posters

**Friday, 15<sup>th</sup>**



# Polyacrylic acid and naked iron oxide nanoparticles modulate neutrophils' oxidative burst and apoptosis

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Iron oxide nanoparticles, with different sizes and coating, are extensively used in biomedicine, namely in drug delivery, cancer therapy and as contrast agents in imagiologic techniques, such as magnetic resonance imaging. The different structural and size features may influence their efficacy and toxicity to the human health. However, there are few studies evaluating the toxicity of these nanoparticles, particularly on cellular defenses that are recruited for their elimination [1].

Neutrophils constitute the first line of innate defense of the organism, and are of great importance in the defense of the organism against pro-inflammatory foreign bodies [2]. Nevertheless, and despite the importance of these cells on immune system, the effect of iron oxide nanoparticles on human neutrophils has not been reported so far. For this reason, the aim of this study was to evaluate the effect of two different types of superparamagnetic iron oxide nanoparticles (polyacrylic acid coated and naked) on human neutrophil' activation, through the measurement of oxidative burst, and lifespan, through the measurement of apoptosis signaling. In order to fulfill this aim, the modulation of the neutrophils' oxidative burst was studied using the probe dihydrorhodamine 123, and apoptosis was evaluated by morphologic analysis, determination of p53 activation, and measurement of caspases 3, 8 and 9 activities.. The obtained results demonstrated that iron oxide nanoparticles have the ability to activate neutrophils, inducing oxidative burst, and that polyacrylic acid coated nanoparticles accelerate apoptotic death of human neutrophils, while naked nanoparticles exert the opposite effect. The obtained results suggest that the studied iron oxide nanoparticles may have significant modulating effects in the innate defense of the organism, during the above mentioned applications of iron oxide nanoparticles in biomedicine.

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# Portuguese autochthonous *Laurus* sp.: nutritional and phytochemical composition of leaves

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Medicinal plants have been used for centuries as remedies for human diseases due to their chemical components of therapeutic value. *Laurus* sp. belongs to the family Lauraceae, which grows in the Mediterranean region. Their leaves have been used to treat neurological disorders, while the essential oil is useful in pain relief and presents antibacterial activity [1]. In Portugal, there are only three species of *Laurus*: *L. nobilis*, native to mainland Portugal, *L. azorica* (Seub.) Franco and *L. novocanriensis*, two autochthonous species from Azores and Madeira archipelagos, respectively. Scarce information exist about autochthonous *Laurus* sp. collected in Portugal, especially those usually consumed in the Portuguese diet [3,4]. Therefore, in this work, we studied the nutritional value and some phytochemicals content of fresh and dried plants collected in the North of Portugal (*L. nobilis*), Azores (*L. azorica*), and Madeira archipelagos (*L. novocanriensis*), where people frequently use them for traditional medicine and gastronomy.

Moisture, ash, protein and fat contents were evaluated according to AOAC methodologies [5]. Total carbohydrates were determined by difference. Fatty acid profiles were obtained by GC-FID analyzing fatty acid methyl esters which were prepared by transmethylation using BF<sub>3</sub>. Total phenolics, flavonoids, and tannins were also isolated and their contents determined by spectrophotometric measurements.

Qualitative and quantitative chemical differences were observed between *Laurus* sp. collected from the three different regions of Portugal. Samples also showed different morphologic properties. The results suggest that species discrimination could be possible based on their leaves characteristics.

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## Portuguese cookies: a “fat” concern

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For the last years a general discussion related with *trans* fatty acid (TFA) reduction in the diet and potential benefits with respect to health outcomes is taking place. It is well known that TFA from partially hydrogenated fats are harmful, affecting adversely multiple cardiovascular risk factors and contributing significantly to an increased risk for coronary heart disease events [1]. A number of approaches have been initiated in different countries to reduce TFA intakes. In the European Union (EU) there are no mandatory guidelines dealing with their amounts, only a voluntary commitment to reduce industrially produced *trans* fatty acids. It is therefore important to study the lipid composition of industrially processed foods, particularly those that are potentially major sources of TFA in diet. In Portugal, biscuits and cookies are widely consumed and regarded as potential TFA sources due to the use of margarines and shortenings. Following a previous study took in 2006 [2], a survey was performed in order to follow this market evolution regarding fat quality and potential concerns.

For the purpose, a total of 50 samples of biscuits sold in Portugal were analysed. Total fat was extracted with organic solvents (cyclohexane/2-propanol) and analysed by gas chromatography as fatty acid methyl esters.

The TFA amounts varied from 0.1% up to 27.4% of the total fat content, with only 5 samples presenting more than 1.0% of TFA, the worst case being a wafer type cookie with 5,7g/100g, from outside EU. Although not being an ideal position regarding TFA ingestion, these results are not of major concern from a global perspective and represent an improvement regarding the results achieved on previous survey in 2006, where 10% of the samples had TFA > 1%. Unfortunately, the saturated fat amounts are very high, ranging from 9.7% up to 92.4% of the total fat content, with only 5 biscuits having less than 40% of saturated fat. A generalized substitution of hydrogenated fat with palm fat, highly saturated, is observed, representing an additional problem from the health point of view. Indeed, the nutritional recommendations highlight the importance of reducing saturated fat ingestion due to its atherogenic potential, among other health concerns. There is, however, a growing market segment of healthier biscuits that provide a lower TFA and SFA content simultaneously, representing in the present study 6% of the samples analysed.

Acknowledgements to FCT, for the financial support through PEst-C/EQB/ LA0006/2011.

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## Preparation and physical characterization of a moisturizing hand cream containing a coffee silverskin extract

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**Introduction:** The skin of the hands is daily subjected to various types of damage caused by exposure to chemicals, microorganisms, low environmental temperature, and low humidity. In addition, normal aging have been reported to influence skin barrier homeostasis. Acute and chronic perturbations of barrier function may lead to the development of irritant hand eczema [1]. A daily moisturizing routine is an essential part of the management of patients with atopic dermatitis and dry skin conditions [2].

In this study, a hand cream containing coffee silverskin extract with moisturizing and antioxidant properties was prepared and characterized.

**Methodology:** The extract was prepared by grinding the coffee silverskin, then the extraction solvent (ethanol:water, 50:50) was added, and the mixture was stirred at 40°C for 30 minutes and filtered through a Whatman No. 1 filter paper. The coffee silverskin extract was then incorporated in oil-in-water creams consisting of dimethicone, stearic acid, cetyl alcohol, mineral oil, triethanolamine, glycerine, methylparaben and water. The physical stability of the hand creams stored at room temperature during 4 weeks was studied by the analysis of texture (firmness and adhesiveness) (Texturometer *Stable Micro Systems TA-XT2i*), rheological behavior (Viscosimeter *Brookfield DV-E*), color (Colorimeter *Minolta CR-400*) and accelerated stability by centrifugation (Centrifuge *Eppendorf 5804*).

**Results:** Accelerated stability studies showed that the developed formulation remained stable after being subjected to 2 cycles of centrifugation at 3000 rpm during 30 min. The hand cream presented a shear thinning behavior without tixotropy and this rheological behavior remained unchanged after storage. The values of firmness showed a slight decrease, but the values of adhesiveness didn't present significant differences after 4 weeks of storage at room temperature. The color assessment showed a slight decrease in brightness after 4 weeks storage.

**Conclusions:** The developed cream containing coffee silverskin extract showed good stability after 4 weeks of storage at room temperature and physical characteristics suitable for application to the hands.

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# Prevalence and Characterization of Elderly Patients with Polypharmacy in the North of Portugal

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Nowadays, many patients (mainly elderly) use a great number of medications simultaneously, a situation often referred to as polypharmacy, which is widely reported to increase the risk associated to the use of medicines [1]. Studies of medicines use are the primary tool for detecting bad medicine use, identify the factors responsible for this misuse and develop interventions to improve the benefits of pharmacotherapy [2]. In this aspect, pharmaceutical care through counseling and other pharmaceutical services has proved to be the most efficient way of improving therapeutical outcomes [3].

**Aim:** The first aim of the present study is to characterize the profile of the elderly population of Northern Portugal regarding to polypharmacy and problems associated with the use of medicines.

**Methodology:** Individuals elected to this study will be those that satisfy two independent variables: being 65 years old, or more, and use 5, or more, medicines each day [4]. The first phase of the study will rely on data collection of the target population and will be carried out by 5th year students of the Masters Degree in Pharmaceutical Sciences of the FFUP, during their final traineeship in community pharmacy, using a structured questionnaire, developed by our group. This instrument includes questions to characterize: i) demographic data; ii) diseases and use of medicines iii) assessment of patient compliance to treatment using the Measure Treatment Adherence (MTA) Scale validated by Delgado and Lima [5].

**Outcomes:** After data analysis from this first phase it is intended to establish pharmacotherapeutical protocols with pharmaceutical interventions to improve health outcomes, leading to direct health gains for patients, and ultimately to positive economic outcomes for the overall budget of the Portuguese national health system. The third phase will lead to a prospective study to evaluate the impact of the pharmaceutical interventions in this population.

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# **Production of innovative trojan horses for the treatment of inflammatory chronic conditions**

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Rheumatic diseases are painful conditions that affect millions. These diseases cause inflammation, swelling, and pain in the joints or muscles and have increased prevalence in developed countries. In fact, rheumatoid arthritis (RA) is the 31st leading cause of years lived with disability at global level, accounting for 0.8% of total global YLDs [1]. RA is a systemic inflammatory disease characterized by chronic, progressive inflammation and gradual joint destruction. The primary target of the inflammatory process is the synovial tissue. Currently, there is no cure for RA. The goal of treatment is to alleviate the burden on the patient and to minimize joint damage which is achieved with different classes of drugs among which non-steroidal and steroidal anti-inflammatory substances are the most frequently used agents. However current therapies have several drawbacks once most of the drugs used for RA treatment have low bioavailability, high clearance rates and limited selectivity. In this context, controlled drug delivery systems, which deliver drugs at predetermined rates for predefined periods of time, can overcome the shortcomings of conventional drug formulations.

The aim of the current project is to incorporate anti-inflammatory drugs in pH sensitive long-circulating liposomes for the treatment of rheumatic diseases. The proposed nanoparticles will passively and actively be targeted towards the sites of inflammation where they will selectively deliver the drugs, enhancing thereby the drug concentration at the sites of inflammation and reducing the number of dosages. The long circulation will be guaranteed by the surface modification with a poly ethylene glycol (PEG), molecules capable to reduce non-specific interactions between the liposomes and opsonins and the active targeting by the surface incorporation of folic acid, once inflamed cells have overexpressed folate receptors which normal cells do not. The lipid composition, proportions and relative molar percentage compared with the PEG and folic acid and drug concentration will be optimized performing adequate characterization of the nanoparticles. Also in vitro drug release studies will be performed. Although pH sensitive liposomes are already known, no optimization for the use of such nanosystems was ever performed for anti-inflammatory loading or for the treatment of rheumatic diseases. The high selectivity of the proposed system will increase the concentration of the anti-inflammatory drugs at the site of inflammation, reducing thereby the numbers of dosages and leading to a more efficient treatment and pain relief.

Preliminary results show good stability of the liposomes at pH 7.4 and destabilization at pH 5.

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# **Prolonged food restriction do not prevent age-related neuronal loss in hippocampal dentate hilus**

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Growing evidence shows that prolonged dietary caloric restriction have several beneficial effects, including increasing of the mean and maximum lifespan, retarding of the age-associated development of the metabolic, structural, and physiological alterations and neuronal protection after metabolic and ischemic insults. However, food restriction has also some deleterious effects, particularly in the dendritic trees of the hippocampal dentate gyrus granule cells, but with no neuronal loss. Most of the caloric restriction studies are realized in young animals and the information of the effects of the food restriction in aged animals is very scarce. In this way, we intended to analyze the effects of the prolonged food restriction on the total number of neurons and volume of the hippocampal dentate hilus of aged rats.

Male Wistar rats were individually housed and maintained throughout the experiment under room temperature and a daily photoperiod of 12 h. Young control rats (2-month old) were fed for 6 months ad libitum with standard rodent laboratory chow. Aged rats (18-month old) were randomly assigned to aged control group and to a aged food-restricted group. Aged control rats were fed for 6 months ad libitum with standard rodent laboratory chow and food-restricted rats were fed 60% of the mean amount of food consumed by control animals every day for 6 months. All rats had free access to water throughout the experimental period and were weighted at 2 week intervals. After treatments the animals were perfused and processed for glycolmethacrylate embedding. The total number of neurons and the volume of hilus were estimated using stereological methods. The handling and care of the animals followed the Principles of Laboratory Animal Care (NIH Publication No. 86-23, revised 1985) and the European Communities Council Guidelines in Animal Research (86/609/UE). All efforts were made to minimize the number of animals used and their suffering.

It was found that the total number of neurons in the hilus of the dentate gyrus was dramatically decreased in old rats when compared with young rats. Interestingly, it was also found that there were no differences in total number of hilar neurons when aged rats subjected to food restriction and control old rats were compared.

Our results corroborate previous works demonstrating that aging induces dramatic reduction of the dentate hilus total number of neurons. Furthermore, the present results clearly demonstrate that moderate food restriction during aging did not prevent the age-related neuronal hilar loss.

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# Quality Assessment of Snacks in a Population with Special Needs

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**Introduction:** People with deficiency or incapacity, particularly in Western countries, have more often an inadequate nutritional intake, when compared with general population [1, 2]. Prevalence of obesity in this population [2-4] has been pointed out as an important factor to life expectancy reduction and to increased needs for care [2]. This study assessed quality of mid-morning and midafternoon snacks in a population with special needs attending to a social solidarity institution.

**Methods:** The sample comprised 58 clients, aged 17 to 61 years. It was estimated the nutritional composition of 621 mid-morning snacks and 733 midafternoon snacks, through direct observation and recording of each type of food, as well as trademarks at the time of consumption. Total energy content and the percentage of macronutrients of total mid-morning snacks and midafternoon snacks were compared to recommendations for adults. Anthropometric, demographic, family and socio-economic issues, obtained through a survey applied to caregivers.

**Results:** Most mid-morning and midafternoon snacks consumed at were brought from home. The median sugar content of mid-morning and midafternoon snacks was significantly higher than recommendations (three and four times higher, respectively), while the protein and total energy content of midafternoon snacks were lower than recommended. Boys consumed more caloric snacks than girls. 87.0% of caregivers considered mid-morning and midafternoon snacks prepared by themselves as healthy choices. Nevertheless, parents were the caregivers who prepared the most energetic snacks.

**Conclusion:** It seems appropriate to consider educational policies for caregivers about the quality of prepared snacks, emphasizing the importance of choosing few refined and low sugar items to include in snacks.

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## Regular physical exercise attenuates the cardiac alterations induced by age in female Wistar rats.

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**Introduction:** Aging is associated with functional and structural cardiac alterations, which compromise the organ ability to sustain stressful stimuli. Regular physical activity is known to provide a cardioprotective phenotype, preventing or delaying the deterioration of cardiac function in response to several cardiac insults. Our aim was to determine if long-term voluntary wheel running is capable to attenuate the age-induced impairment of cardiac function in laboratory animal. **Material and Methods:** Female Wistar rats ( $n = 18$ ) with 5 months of age were randomly divided into three groups: 1- Sedentary Young (SED-Y,  $n = 6$ ; sacrificed one week after acclimation); 2- Sedentary Old (SED-O;  $n = 6$ ; movements confined to the cage's space for 9 months) and 3- Active Old (ACTIVE-O;  $n = 6$ ; housed in cages with a running wheel for 9 months). After ending their respective protocols, all rats were prepared for hemodynamic evaluation of the left ventricle (LV) with conductance catheters. After that, animals were sacrificed and LV samples collected for histological analyses. **Results:** Results are shown in Table 1 as mean $\pm$ standard deviation.

**Conclusion:** Regular voluntary running attenuates the impairment of cardiac function induced by chronological age.

**Table 1:** Results from histological and hemodynamic analysis.

	SED-Y	SED-O	ACTIVE-O
<b>Histological Evaluation</b>			
CSA ( $\mu\text{m}^2$ )	262.5 $\pm$ 98	278.9 $\pm$ 130	308.4 $\pm$ 114* <sup>#</sup>
<b>Hemodynamic Evaluation</b>			
HR (bpm)	430 $\pm$ 18*	358 $\pm$ 50	416 $\pm$ 27*
$P_{\text{max}}$ (mmHg)	112 $\pm$ 6	135 $\pm$ 11 <sup>#</sup>	143 $\pm$ 7 <sup>#</sup>
EDP (mmHg)	4.8 $\pm$ 1.7	6.2 $\pm$ 4.9	3.9 $\pm$ 1.1
$dP/dt_{\text{max}}$ (mmHg/s)	8157 $\pm$ 900	7341 $\pm$ 1439	9659 $\pm$ 997*
$dP/dt_{\text{min}}$ (mmHg/s)	-10608.9 $\pm$ 1268	-9942 $\pm$ 1789	-12633 $\pm$ 635*
$\tau$ (ms)	8.1 $\pm$ 0.6*	10.74 $\pm$ 2.2	8.13 $\pm$ 0.3*

CSA: Cardiomyocytes Cross-sectional Area; HR: Heart Rate;  $P_{\text{max}}$ : Ventricular Peak Systolic Pressure; EDP: End-diastolic Pressure  $dP/dt_{\text{max}}$ : Peak rate of pressure rise;  $dP/dt_{\text{min}}$ : Peak rate of pressure fall;  $\tau$ : Relaxation velocity. \* $P < 0.05$  vs. SED-0; <sup>#</sup> $P < 0.05$  vs. SED-Y.

# Rheological properties of anti-psoriatic topical formulations with vitamin D derivatives

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Psoriasis is a chronic, non-contagious inflammatory skin disease, characterized by scaliness, thickened and erythematous lesions. This dermatosis requires prolonged treatment and there are currently several therapeutic options available, including topical formulations based on vitamin D derivatives. These drugs have shown good tolerability and efficacy for long term use, with minimal side effects. They are also available in different dosage forms which can contribute to improve treatment adherence by taking into account patients' preference.

The objective of this study was to evaluate the rheological properties (flow behavior) of anti-psoriatic topical commercial formulations with vitamin D derivatives. Four semi-solid formulations (F1, F2, F3 - ointments and F4 - gel) were analyzed at 20°C and 32°C. Measurements were performed in a viscometer (Viscotester VT 550, Thermo Haake, Germany) fitted with concentric coaxial cylinder geometry. The samples were evaluated in the shear rate range from 0.1 to 500 s<sup>-1</sup>, with a 60 s delay time between the measurements. Measurements were performed in triplicate and the results were fitted to the Power Law model.

All the tested formulations presented a shear-thinning behavior. Only the ointments presented a hysteresis area (characteristic of thixotropic behavior). Table 1 presents the rheological parameters obtained with the Power law model. Power law indexes (n) were similar among ointments while the gel presented a less shear-thinning behavior (higher n). Temperature influenced the mechanical properties and at 32°C there was a decrease of the formulations' consistency coefficient (K), which was more relevant for the ointments. The ointments are petrolatum based and therefore this decrease is not surprising considering the dropping point of this excipient (35-70°C). Although containing different drugs the three ointments presented similar behavior which can be due to the dominant role played by petrolatum regarding mechanical properties.

**Table 1- Rheological parameters of the formulations obtained with Power Law model (mean ± SD)**

	20°C		32°C	
	K	n	K	n
F1	148.21 ± 4.67	0.25 ± 0.01	33.48 ± 2.38	0.30 ± 0.01
F2	263.97 ± 35.01	0.24 ± 0.01	68.23 ± 9.42	0.29 ± 0.01
F3	164.83 ± 19.44	0.26 ± 0.01	63.54 ± 1.98	0.26 ± 0.00
F4	2.45 ± 0.09	0.71 ± 0.01	2.01 ± 0.53	0.63 ± 0.05

The rheological properties of topical formulations have been recognized to influence patients' adherence<sup>(1)</sup>. The differences observed between the rheological properties of the dosage forms studied could be relevant for patients' perception and adherence to treatment.

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# Strategies for promoting healthy eating habits at the workplace

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## **Introduction**

An increasing number of consumers have at least one meal out of home. The World Health Organization considers that this is an interesting and friendly environment for the implementation of projects to promote healthy eating habits.

The implementation of these projects triggered improvements in productivity and income of companies. The quality of workers' meals can be improved through simple economic actions, which in addition may be transferred by workers to their families and society.

In this thematic review various strategies to promote healthier eating habits of workers will be presented, which can be applied in the workplace.

## **Discussion**

In Portugal, some private companies are already distributing fruit at the workplace. Similarly distribution of dairy products, soups, snacks of vegetables and/or vegetarian sandwiches should also be encouraged. Moreover is important restrict competitive foods available at snack bars, cafeterias and vending machines. Another option for cheaper and nutritionally richer meals is to provide facilities to bring lunchboxes from home.

Nutritionists should influence catering contract leasing including specifications concerning food available at the workplace. Moreover, the integration of a nutritionist in occupational safety companies would allow nutritional evaluation of employees, conducting screenings, awareness and nutrition education <sup>[1]</sup>.

The Government should act as a model for private entities, implementing programs that promote a healthier lifestyle of its workers <sup>[1]</sup>.

## **Conclusions**

Portugal has insufficient culture in strategies to promote healthy eating habits at the workplace.

It is proposed that official entities, employers and employees to act together in this area, developing strategies, tools and actions that will improve healthy eating at the workplace, with repercussions on public health and life quality. This increases corporate profits and therefore improves the country's economy through increased motivation and profitability.

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# Structural stability of proteins encapsulated into PLGA nanoparticles

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Proteins are able to suffer enzymatic degradation, denaturation or hydrolyze process in harsh environments such tract gastrointestinal. To overcome these problems, their encapsulation into nanoparticles has been pointed as a good alternative, particularly into PLGA nanoparticles which have several properties suitable for proteins delivery such biodegradability, safety, good tolerability and biocompatibility [1]. This work pretends to study the secondary structure of proteins-loaded *poly(lactic-co-glycolic acid)* (PLGA) nanoparticles in solution, during three months, in different storage conditions (4,25 and 40° C), using insulin as a model protein.

After production and characterization of insulin-loaded PLGA nanoparticles, the secondary structure of insulin was assessed through Fourier Transform Infrared Spectroscopy (FT-IR) by the absorption of amide I at 1600–1700 cm<sup>-1</sup> [2], at different shelf-life conditions and compared with native insulin in order to achieve the area overlap and correlation coefficient.

Immediately after insulin encapsulation it was achieved an area overlap of 86,99%, which was very similar after 3-months of storage at 40° C (86,73%). Furthermore, the area overlap of insulin-loaded PLGA after 3 months of shelf-life at 4°C was even higher than the one achieved immediately after encapsulation (87,46%). Although PLGA is able to maintain the insulin structure, more prolonged stability studies should be performed, as well long-term stability studies of insulin encapsulated at solid state, as proteins are mostly storage in that physical state.

## Acknowledgments

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# Study and Development of a Model for Mimicking the Stomach Mucosa

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The high mortality rates of gastric cancer (GC) urge the need for tumour diagnosis at an earlier stage [1]. Current diagnostic means (gastroscopy) fail to identify neoplastic cells usually located in the tissues underneath the stomach epithelium [2]. The association of the cell-surface hyaluronate receptor - CD44v6 - with the early transformation of GC motivates the search for a new means for diagnosis [3]. Chitosan nanoparticles arise as a promising vehicle for an anti-CD44v6 moiety aiming for diagnosis or even treatment of GC [4].

This project's fundamental aim was to develop a specialised in vitro model for mimicking the gastric wall for pharmaceutical permeation studies. Such models are invaluable tools as they tend to substitute the use of animal models, circumventing serious ethical and financial considerations of the pharmaceutical industries [5].

Two gastric epithelial cell lines, AGS and NCI-N87, were cultured upon transwell membranes, either coated or not with BD Matrigel, to mimic both the mucosa's epithelium and *lamina propria*. Two sets of Fluorescently-labelled Chitosan Nanoparticles (FLCNPs) (low molecular weight (LMW) and glycol chitosan) were used to test the model's integrity in permeation studies. Expression of E-cadherin was assessed in both cell lines by Western Blotting, as an important premise to create a functional epithelial barrier [6].

NCI-N87 (E-Cad<sup>(+)</sup>) proved to be the most promising candidate to mimic a functional epithelial barrier when juxtaposed to AGS cells (E-Cad<sup>(-)</sup>). The model returned sustainable results, with the NCI-N87 model showing higher trans-epithelial electrical resistance (TEER) values, an indication of epithelial integrity, and functioning as a more efficient barrier to CNP permeation. CNPs presented no toxicity towards cells and LMW CNPs presented higher permeation-enhancing properties in comparison to the modified glycol variant.

This work served as a proof-of-concept for further studies setting important grounds on the field of epithelial permeation models since, to our knowledge, no such work is being developed regarding the stomach mucosa.

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# Synthesis of prodrugs of polysulfated diosmin as potential orally-active antithrombotic agents

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Unfractionated heparin (UFH) and low-molecular weight heparin (LMWH) are the most commonly used antithrombotic and thromboprophylactic agents in hospital setting. Extended out-of-hospital treatment is both inconvenient and costly since these agents must be administered parenterally [1]. So the long-term outpatient management of thrombotic disorders is mainly limited to oral vitamin K antagonists (VKA). Although effective, VKA possess an extensive list of drug and food interactions, metabolic polymorphisms, and a delayed onset and offset of effect, which implies the association of heparins during the initial phase of the treatment [2]. Therefore, the search for new orally-active anticoagulant agents is a major challenge to medicinal chemists. In CEQUIMED-UP a new class of antithrombotic agents, namely polysulfated diosmin, with dual anticoagulant/antiplatelet activity, was identified [3-4]. However, by oral administration in mice, they were not active [3-4]. Important strategies such as the use of oral absorption enhancers, the development of lipid conjugates of UFH, and the incorporation of UFH in polymeric matrix systems have been carried out for the development of heparin as oral anticoagulant [5]. These strategies can facilitate its enteric absorption reaching adequate levels for prevention and/or treatment of venous thromboembolic disease in humans. Considering this, the aim of the present work was to combine the polysulfated derivative of diosmin with several types of carriers to obtain a new orally-active antithrombotic agent. Future work will consist in the investigation of the permeability of the prodrugs derivatives across mouse small intestine by the Ussing chamber technique.

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## Synthetic strategy to obtain chiral derivatives of xanthenes

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It is well known that many biologically and pharmacologically interesting compounds are chiral and their physiological properties, such as pharmacodynamics, pharmacokinetics and toxicity, are often different between enantiomers [1]. As a result, the preparation of optically active compounds has become crucial in the fields of pharmaceuticals, agrochemicals, foods, fragrances, among others [2]. Additionally, the chiral compounds can be prepared with other purposes, such as being chiral selectors for chromatographic tools in HPLC [3].

The development of new chiral derivatives of xanthenes (CDXs), that will potentially reveal interesting biological activities and that could also be interesting selectors of chiral stationary phases (CSPs) for HPLC, is in progress in CEQUIMED-UP. In this context, a strategy based on the coupling of the carboxyl group of a xanthonic scaffold with adequate chiral building blocks was developed in order to obtain new CDXs.

Firstly, the total synthesis of a carboxymethoxyxanthone was carried out by a multi-step pathway, *via* Ullmann reaction, with the formation of diaryl ether intermediate. The synthesized xanthone is suitable functionalized, been an interesting chemical substrate for the synthesis of other compounds such as CDXs. Then, by coupling the carboxyl group of the xanthone to a chiral building block and demethylation of the methoxyl group, a new CDX was obtained.

The new CDX will be evaluated for growth inhibition of human cancer cell lines and for anti-inflammatory activity by inhibition of enzymes involved in the inflammatory process, namely phospholipase A2 (PLA2) and cyclooxygenases (COX-1 and COX-2). Additionally, after been covalently bonded to silica to yielding the corresponding CSP, its performance will be evaluated for chromatographic separation of mixtures of enantiomeric compounds.

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# The antioxidant potential of coffee substitute beverages

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The word “antioxidant” is increasingly popular in modern society as it gains publicity through mass media coverage of its health benefits. Antioxidant activity in foods and beverages is one of subjects that have generated much interest within the scientific community in recent years [1]. Among them, coffee stands as an important dietary source of antioxidants, with increasing evidences associating moderate coffee consumption with potential health benefits in healthy adults. Still, to avoid caffeine and/or due to economical restraints, several consumers use decaffeinated coffee or coffee substitute beverages, based on roasted cereals with reduced or absent coffee. The aim of the present work was to compare the antioxidant activity of commercial water-soluble extracts used for the preparation of coffee (including decaffeinated) or substitute coffee beverages. Among this last designation are included soluble powders with reduced coffee amounts (20% to 40%) or plain cereal beverages based on barley, malt, chicory, rye, etc.

The antioxidant potential was evaluated by the 2,2-diphenyl-1-picrylhydrazyl assay (DPPH) and total polyphenol content was evaluated using the Folin-Ciocalteu reagent. The DPPH assay measures the reduction of the stable DPPH radical by monitoring the decrease in its absorption at 515 nm and the Folin-Ciocalteu method was used to estimate the total phenolic compounds [2]. The activities were expressed on a comparative mass basis per 100g of commercial sample, using trolox in the former and chlorogenic acid in the latter.

A strongest correlation was found between the Folin and the DPPH methods, suggesting the scavenging of this radical by phenolic compounds. Both methods showed higher activity in the 100% coffee samples, almost equivalent to the decaffeinated ones, highlighting that the decaffeination processes was not responsible for a reduction in the antioxidant activity evaluated by these two methods. In opposition, the antioxidant potential of beverages based on roasted cereals was reduced, except when chicory was present in the formulation. A strong positive correlation was also found between the antioxidant capacity and the amount of coffee in the blends.

Based on these preliminary results, coffee substitute beverages have a reduced antioxidant potential when compared with coffee, being therefore advisable to choose mixtures with coffee, including decaffeinated, or chicory. Other antioxidant methods are being tested in order to support these data and understand the chemical families involved.

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# The colorimetric SPADNS method is not suitable for fluoride determination in saliva

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Fluoride is an important anion present in saliva.<sup>[1,2]</sup> The ion-selective electrode (ISE) method is the most widely used for the determination of fluoride in saliva.<sup>[3]</sup> The spectrophotometric methods are still seldom used. These methods have the advantage of being easily applicable to reduced samples volumes, an important feature considering the difficulties in collecting human saliva samples. However, the colorimetric methods are susceptible to several interfering substances.<sup>[4,5]</sup> The present study aimed to evaluate the usefulness of colorimetric SPADNS for fluoride determination in saliva.

Standard solutions were prepared for ion-selective electrode (ISE) and colorimetric SPADNS methods and calibration curves were drawn. Several approaches were carried out to eliminate the presence of interfering substance in the SPADNS method, namely the turbidity: addition of acids, filtration, heating, “saliva digestion” and distillation. Wavelength scans were performed and the absorbance at 570 nm was recorded for each analyses.

In comparison to fluoride-ISE method, the colorimetric SPADNS method was much less linear and sensitive for fluoride concentrations ranging from 0.01 to 1.00 mg/l. The colorimetric method revealed inappropriate for saliva samples due to saliva intrinsic turbidity. The approaches performed to eliminate the saliva turbidity were not successful or the color complex was altered.

Our study showed that the SPADNS method wasn't accurate for fluoride determination in saliva. Also, the interferences of SPADNS method such as turbidity were not successful eliminated. The fluoride-ISE remains the most appropriate method for fluoride saliva determination.

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# The influence of craving in food choices in patients under treatment for smoking cessation

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The nicotine craving, described as a strong desire to use the substance and fear of weight gain, may trigger relapse during treatment for smoking cessation.

The objective of this work was to evaluate the influence of abstinence and craving in food choices and nutritional state (NS) of patients after one month of treatment for smoking cessation.

A longitudinal study was conducted with smokers in treatment. Questionnaires were applied to assess craving, dependence level, food intake to decrease craving and alcohol consumption. The NS was assessed by bioelectric impedance measurement (BMI), waist circumference (WC) and abdominal circumference (AC). Evaluations were performed at baseline and after one month of treatment.

Among 23 participants who started treatment, 17 completed one month follow-up, and 41.2% (n = 7) of patients were abstinent in a mean of 9 days. Drugs to aid in smoking cessation were used by 74.0%. After a month of treatment, the craving was lower among those abstinent compared to smokers. The most consumed foods to reduce the craving were fruits, coffee and candies. The candies were reported as those that generated a greater welfare. Most subjects presented overweight (mean BMI  $27.9 \pm 6.9$  kg / m<sup>2</sup>), and increased cardiovascular risk (WC:  $89.0 \pm 14.2$ ; AC:  $96.4 \pm 15.0$ ). After one month of monitoring, these parameters have not changed significantly.

Smokers in treatment for one month for smoking cessation showed a decrease in nicotine craving. Abstinence did not change the anthropometric parameters. The fruits were the most consumed foods to reduce the craving.

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# The influence of zinc stearate in the hardness of tablets

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Zinc stearate is a metallic soap primarily used in pharmaceutical formulations as a lubricant in tablets and capsules [1]. In cosmetology, zinc stearate is described also as one of the best-known dry binder used in compact powder manufacturing to makeup [2]. So the purpose of this work was to study how the zinc stearate can affect the hardness of tablets and thereby find out if it could be also used as dry binder in the production of tablets.

To conduct this study Avicel® PH 102 (95%) and povidone (5%) were granulated with water. Zinc stearate at concentrations of 1% and 5% was mixed with the granules obtained using a Turbula® shaker mixer for 5 minutes. Tablets with 500 mg of each mixture were prepared in a hydraulic press using 13 mm diameter punches (for approximately 5 s). The compaction forces used were 1 and 2 ton. The hardness of the tablets was evaluated using a hardness tester (Pharmatest® PTB 311E); 5 tablets (n=5) were tested for each compaction force. Tablets without zinc stearate were also prepared for control

To evaluate the influence of zinc stearate a comparison between the average hardness of tablets with and without zinc stearate was made. The results showed, as it was expected, that the increase of compaction force increase tablet hardness for granulates tested. It was also observed that for a compaction force of 1 ton, the zinc stearate at 1% increased the tablets hardness (66,7 N to 74,6 N) while for force of 2 ton it caused no relevant change (144,1 N to 143,7 N). For 5%, zinc stearate did not produce relevant change in the hardness of tablets, when was used a compaction force of 1 ton (66,7 N to 66,2 N), or even decrease, when force of 2 ton was used (144,1 N to 117,1 N).

Other parameters such as different times of mixture and compaction, or other compaction forces should still be evaluated, but the results obtained in this work suggest that the zinc stearate cannot be used as dry binder in tablets formulations.

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# THEMATIC SEMINARS AS METHODOLOGY FOR ADVANCED TRAINING IN NURSING: AN EXPERIENCE REPORT

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The seminar theme is a methodological approach that contributes to building and knowledge sharing in postgraduate nursing. The text that is present is a theoretical reflection on the contributions realized through thematic seminars during the learning process and the development of expertise in advanced training in science of nursing, lived during graduate school abroad. I highlight that their operationalization breaks with fragmented and compartmentalized vision of teaching, learning and the concept of student-teacher historically predominant in the formation of higher education. The curriculum is centered on hypotheses arising due to the demands of the group, a relationship of dialogue and collective integration between theory and practice, and respect the minimum theorising of the academic community. The figure of the teacher is not neglected but appears as a facilitator for initiatives from the group, keeping the co-responsibility for the contents to be saturated and internalized by all. Thematic seminars<sup>[1]</sup> as learning strategy enables a space for reflection in which ideas should germinate on the plurality of knowledge that are being sown. Therefore, I define this experience in graduate as a space where everyone could discuss and debate the issues questioning them and developing the skill of communication and freedom of opinion. For the moment graduate this strategy was a great contribution in addition to allowing for the adequacy of the study plan to the collective interests, could awaken the multiple themes of investigation still dormant. Classify seminars in advanced training in nursing as sufficient to ensure the epistemological understanding of the concepts and as a proper place for the creation of knowledge and practices of specialized intervention, given its dynamism, creativity and the emancipatory power of the student about his process learning and communing culture, planning, decision making, critical thinking and social integration, without discarding the multicultural group.

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# Thyroidal response in women with history of preeclampsia: relationship with adhesion molecules

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Preeclampsia (PRE) is a common and severe disease in pregnancy, a major cause of maternal and fetal morbidity and mortality. Thyroid disorders during pregnancy are associated with adverse health outcomes for both mother and child, including increased risk of preterm delivery, placental abruption, low birth weight and fetal death. It is well known that during pregnancy there is relationship between hypertension and thyroid dysfunction. However, the future impact on thyroid function in women with a history of preeclampsia during pregnancy is discussed. The aim of this study is to explore the relationship between thyroidal hormones (FT3–Free Triiodothyronine, FT4–Free thyroxine and TSH–Thyroid Stimulating Hormone) and adhesion molecules (ICAM-1–Intercellular Adhesion Molecule 1, VCAM-1–Vascular Cell Molecule 1) in women with history of preeclampsia.

We selected 39 women with a history of preeclampsia and also a control group of 31 normal women. In all cases, we also considered age, body mass index and the time interval after delivery as well as systolic (SBP) and diastolic blood pressure (DBP). Serum TSH, FT3 and FT4 levels were measured by autoanalyzer using the ELFA technique (Enzyme Linked Fluorescent Assay). Soluble adhesion molecules also were measured by standard commercial ELISA methods. We found no significant differences in FT3, FT4 and TSH between Normal and PRE groups; however, statistically significant correlations were found between FT4 and SBP, as well as between FT4 and VCAM-1 only for the PRE group while a significant correlation between ICAM-1 and TSH for the normal group even when adjusting for confounding factors. We also found a significant correlation between FT3 and FT4 only in the PRE group. Our findings support a change in thyroidal hormones metabolism in women with history of preeclampsia. Moreover, the positive relationship with respect to blood pressure may suggest an increased risk of thyroid dysfunction, but further studies are required.

This work was developed within the optional curricular unit “Project” of the 4<sup>th</sup> year of the Master Degree in Pharmaceutical Sciences of the Faculty of Pharmacy, University of Porto, under the responsibility of Irene Rebelo.

# Unraveling the mechanism of action of BNIPDiaminooctane on *Leishmania infantum* parasites – target identification and development of resistant strains

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Visceral leishmaniasis is a neglected and poverty-related disease that causes significant morbidity and mortality. Despite efforts developed both to find a vaccine and to combat the insect vector, chemotherapy remains the most efficient way to control leishmaniasis. Treatment options are quite limited and the development of resistance to antimonials and amphotericin B has added to the problem [1]. Bisnaphthalimidopropyl (BNIP) derivatives were recently identified as inhibitors, *in vitro* and *in vivo*, of *Leishmania infantum* parasite [2]. BNIPDiaminooctane (BNIPDaoct) was the most effective compound *in vivo* [2].

To gain some knowledge on the mechanisms of action of BNIPDaoct the objectives of this work were to identify intracellular targets of the BNIPDaoct in *L. infantum* promastigotes and to understand whether *Leishmania* parasites are able to become resistant to BNIPDaoct. In order to identify the targets of this compound, it was immobilized on epoxy-activated sepharose resin and then total *Leishmania infantum* extract was incubated. BNIPDaoct potential protein targets in *Leishmania infantum* were identified after a SDS-PAGE gel stained by silver coloration and the specific proteins bound to the compound were sent to proteomics analysis. The data obtained have identified six proteins (three of them putative) able to interact to BNIPDaoct, which are localized between the cytoplasm and the nuclei of the parasite. In parallel, a *Leishmania infantum* promastigote BNIPDaoct-resistant line was selected by stepwise drug pressure (up to 11  $\mu$ M). One resulting resistant strain was cloned and checked for stability of resistance by drug-free *in vitro* passage as promastigotes for 12 weeks. Resistance selection in promastigotes took about 20 weeks to reach the maximal 11  $\mu$ M that did not affect normal growth. Comparison of the IC<sub>50</sub> values between the parent and the selected strains revealed a 14-fold.

This study suggests six potential protein targets for BNIPDaoct on *Leishmania infantum* promastigotes and clearly describes the development of a *Leishmania infantum* BNIPDaoct-resistant strain.

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# Using yeast to search for new selective activators of protein kinase C isoforms

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The Protein Kinase C (PKC) family is an important group of serine/threonine kinases with at least ten isoforms divided into three major subfamilies: classical (cPKC- $\alpha$ , - $\beta$ I, - $\beta$ II and - $\gamma$ ), novel (nPKC- $\delta$ , - $\epsilon$ , - $\eta$  and - $\theta$ ) and atypical (aPKC- $\zeta$  and  $\lambda$ ) [1]. PKC isoforms play a critical role in the regulation of cell proliferation and death. Therefore, PKCs have emerged as striking drug targets in the treatment of several pathologies, including cancer. The complexity of the PKC family in mammalian cells, particularly the difficulty to carry out an independent analysis of individual PKC isoforms, has limited the identification of isoform-selective modulators [1]. To circumvent this limitation, the yeast-based PKC assay has been extensively used as a drug screening tool to search for selective activators and inhibitors of individual PKC isoforms [2].

In the present work, we intended to study the modulatory activity of two natural compounds (C1 and C2) and two hemisynthetic compounds (C3 and C4) from the Simões's group (iMed. UL) on individual PKC isoforms. With this goal, *Saccharomyces cerevisiae* cells individually expressing isoforms of the three PKC subfamilies were used. As previously described [2], in this assay, PKC activators induce growth inhibition in yeast expressing a PKC isoform, having no impact on the growth of control yeast (transformed with the empty vector). The results obtained showed that C1 was no effective on all the tested PKC isoforms. On the other hand, 1-10  $\mu$ M C2 and C3 induced a significant growth inhibition on yeast expressing PKC- $\alpha$ , - $\beta$ I, - $\delta$ , - $\epsilon$ , - $\eta$  or - $\zeta$  with no impact on the growth of control yeast. Finally, 10  $\mu$ M C4 significantly inhibited the growth of yeast expressing PKC- $\delta$ , having no impact on the growth of control yeast and yeast expressing PKC- $\alpha$ , - $\beta$ I, - $\epsilon$ , - $\eta$  or - $\zeta$ .

In conclusion, using the yeast-based PKC assay, compounds C2 and C3 were identified as putative non selective activators of PKC isoforms. Importantly, compound C4 was identified as a potential nPKC- $\delta$ -selective activator. Promising pharmacological applications may be therefore envisaged for compound C4 as the first non-peptide selective activator of nPKC- $\delta$ . Based on this, further studies are underway.

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## Vitamin E profile of cooked dishes for patients with Phenylketonuria

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Phenylketonuria (PKU) is a rare inherited metabolic disorder with an autosomal recessive transmission. Early diagnosed patients need a prompt dietary intervention that consists in a semi-synthetic low-phenylalanine (Phe) diet with the inclusion of free Phe aminoacid mixtures, controlled amounts of natural foods low in protein such as fruits and vegetables and also complement with low Phe dietetic products. The dietary treatment of PKU resembles a vegan-like food pattern. As a result from the dietetic restrictions, low intakes of some micronutrients have been observed in some PKU patients, namely of vitamin E [1].

Vitamin E is the common name given to a group of 8 lipophilic compounds, namely  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ - tocopherols and  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ - tocotrienols, that occur naturally in vegetable oils. Its distribution pattern is related to the botanical origin of the oil.

In this work, we studied the Vitamin E profile of 10 low protein recipes [2,3] specifically planned for PKU patients, as well as natural daily basic cooked foods. Lipid fraction of the samples was obtained by Soxhlet extraction with petroleum ether and tocopherols were analyzed by normal-phase HPLC/fluorescence [4].

Total vitamin E contents varied between 0.07 and 10.08 mg/100 g. The prevailing vitamer found in all samples was  $\alpha$ -tocopherol. The 8 vitamers were found in 3 meals cooked with vegetal margarine as a common ingredient. This suggests that the margarine used may contain palm oil in its composition.

In conclusion, cooked dishes, using good quality fats in its preparation, can be good sources of vitamin E for these patients and contribute to improve their nutritional status, concerning this particular nutrient.

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# Long-term stability of freeze-dried insulin-loaded solid lipid nanoparticles: a nanostrategy for a great solution

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Solid lipid nanoparticles (SLN) represent highly versatile protein delivery systems with numerous advantages, ranging from the superior drug stability to the easier scaling up for industrial production [1]. However, few studies have been supporting the long-term stability and shelf-life behavior of the nanoparticles formulations [2].

At the present work, a long-term study was performed to evaluate the physical and chemical stability of freeze-dried and non-freeze-dried insulin-loaded SLN during six months of storage at different conditions as recommended by *International Conference on Harmonization* guidelines of pharmaceuticals for human use. The nanoparticles were characterized by infrared spectroscopy, high performance liquid chromatography/ ultraviolet detection, dynamic light scattering, transmission and scanning electron microscopy. Furthermore, spectra similarity-based algorithms were applied to evaluate the maintenance of the insulin native-like structure over the time.

The results showed that the freeze dried SLN formulations with no cryoprotectant added were able to preserve insulin secondary structure in 6-months of shelf-life even at room conditions. These outcomes prove the drug-loaded protection and stability uniquely offered by the lipid nanoparticulate matrix. However, protein structure and, morphological and release properties of the nanoparticles varied significantly when a cryoprotectant is used, and thus cryoprotectant effect is discussed. Considering that the formulations were freeze-dried by a non-optimized process, the next step must be based in an experimental design in order to find the process parameters settings that best fit with the desired product quality attributes.

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# Analysis of omega-3 PUFA content in fish-oil supplements with cardiovascular benefits

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The consumption of fish and fish-based products has increased in recent years due to the wide range of health benefits related to the high content of polyunsaturated fatty acids (PUFA) of this kind of food.

Fatty acids (FA) are the main constituents of lipids being some of them essential nutrients for humans, precursors of a variety of bioactive compounds and structural and functional elements in all cell membranes as well as a source of energy. Several scientific research has shown that n-3 FA present a range of biological effects, including reducing inflammatory responses, lowering triglyceride levels and platelet aggregation, all of which contribute to protect against cardiovascular diseases (CVD) [1]. The main n-3 FA involved in the prevention of CVD are eicosapentaenoic acid (EPA) and docosaexaenoic acid (DHA). Since the ability of the body to manufacture n-3 fatty acids from the precursor is limited, their intake is of vital importance [2]. These n-3 FA can be found in fatty fish such as herring, mackerel, sardine, tuna and salmon, and in fish oils. However, due to different circumstances, it is not always possible to consume adequate amounts of n-3 FA through the diet, leading to an increasing interest in n-3 FA dietary supplements [3].

This work aimed to study the FA profile of fish-oil supplements, commercially available, claiming protection against cardiovascular diseases. For that, fatty acid methyl esters (FAME) of n-3 fish-oil supplements were obtained by transmethylation using boron trifluoride and analysed by GC-FID. A particular relevance was given to EPA and DHA contents. Another point of interest of this work was the comparison between the manufacturer's claims on the supplement label and the measured contents.

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## Arsenic assessment in octopus species

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Cephalopods comprise a large variety of valuable commercial species being *Octopus* one of the most important. They are important target species in fisheries and have a high economic, cultural and social value. These species can represent a significant source of essential elements but may also promote exposure to toxic elements. Arsenic (As) is a metalloid and a non-essential element to humans and animals. Inorganic As, 0.2–11% of the total arsenic in seafood, is the most toxic form which can damage DNA and is considered carcinogenic causing lung, kidney, bladder, and skin disorders [1].

In this study, As levels were determined in the *Octopus* species available in the Portuguese market, namely, *Octopus vulgaris*, *Octopus maya* and *Eledone cirrhosa*. These species originated from the Northeast (NEA), Eastern (ECA), Western (WCA) Central and Northwest (NWA) Atlantic Ocean, as well as, the Pacific Ocean (PO) and Mediterranean Sea (MS). Samples were digested with suprapur nitric acid (65%) by microwave-assisted digestion (MARS-X, 1500 W CEM, Mathews, NC, USA). Quantification was carried out by High Resolution Continuum Source Graphite Furnace Atomic Absorption Spectrometer (Analytikjena ContrAA 700). The detected total As content was 7.26 (4.10 – 9.76 µg/g ww) in *Octopus vulgaris* from Northeast Atlantic Ocean (Fresh sample), 12.0 (6.45 – 18.4 µg/g ww) from Northwest Atlantic Ocean, 2.16 (0.39 – 5.55 µg/g ww) from Eastern Atlantic Ocean, 3.79 (2.34 – 6.31 µg/g ww) from Western Atlantic Ocean, 6.11 (0.53 – 19.8 µg/g ww) from Pacific Ocean and 4.71 (3.38 – 7.25 µg/g ww) from Mediterranean Sea; 1.54 (0.17 – 3.29 µg/g ww) and 4.58 (1.52 – 7.03 µg/g ww) in *Octopus maya* from Eastern Atlantic Ocean and Western Atlantic Ocean, respectively; 34.2 (24.8 – 45.7 µg/g ww) in *Eledone cirrhosa* from Northeast Atlantic Ocean. As a worst hypothesis scenario (considering 11% of the total As content), inorganic As levels may range from  $0.17 \pm 0.09$  to  $3.76 \pm 0.72$  µg/g ww. Some samples of *Eledone cirrhosa* (from the Northeast Atlantic Ocean) and *Octopus vulgaris* (from Pacific and Northwest Atlantic Ocean) exhibited higher levels than the guideline value adopted by the World Health Organization for inorganic As in cephalopod species, 2 µg/g ww [2].

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## Assessment of an anti-*Leishmania* compound loaded on PLGA nanoparticles - *in vitro* and *in vivo* studies

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The therapy of infectious diseases caused by protozoan parasites of the trypanosomatid family is a neglected area of research and drug development, including here leishmaniasis, an emerging disease in Portugal. Recently, the anticancer bisnaphthalimidopropyl (BNIP) polyamines compounds have attracted attention due to their potential activity against the parasite *Leishmania infantum* [1]. However these compounds are quite insoluble in aqueous solutions, making their testing and potential development into chemotherapeutics difficult. To overcome this, nanotechnology tools can be applied to provide enhanced formulations, namely nanoparticulate carriers, such as those based on poly(D,L-lactide-co-glycolic acid) (PLGA), that are able to tailor the spatial and temporal delivery of drugs [2].

In this context, the objective of this work was to develop a fast chromatographic method for the determination of bisnaphthalimidopropyldiaminooctane (BNIPDaoct) and further application in *in vitro* drug release studies and *in vivo* bio-distribution. In order to enhance determination throughput using conventional liquid chromatography equipment, a monolithic column (Chromolith RP-18e, 100 mm × 4.6 mm i.d., Merck) was applied, connected to a Jasco HPLC system coupled to a fluorimetric detector. Several parameters were studied, including the flow rate (up to 2 mL min<sup>-1</sup>) and the composition of the mobile phase. The operation conditions chosen comprised a flow rate of 1.5 mL min<sup>-1</sup> (pressure = 2.5 MPa) and a mobile phase containing 40% (v/v) acetonitrile and 60% (v/v) of aqueous buffer (acetic acid/acetate 0.10 mol L<sup>-1</sup>, pH 4.5, 0.010 mol L<sup>-1</sup> octanesulfonic acid). Calibration curves were linear up to 10 μmol L<sup>-1</sup>, with working ranges of 1 to 10 μmol L<sup>-1</sup> (10x gain) and of 2 to 100 nmol L<sup>-1</sup> (1000x gain). The LOD and LOQ were 0.4 and 1.1 nmol L<sup>-1</sup>, corresponding to 7 and 17 fg (20 μL). The developed methodology was successfully applied to monitor the *in vitro* release profile of BNIPDaoct encapsulated in polymeric biodegradable nanoparticles, formulated with PLGA and to quantify the BNIPDaoct free and encapsulated in several biological samples (spleen, liver, kidney, heart and lung) recovered from mice.

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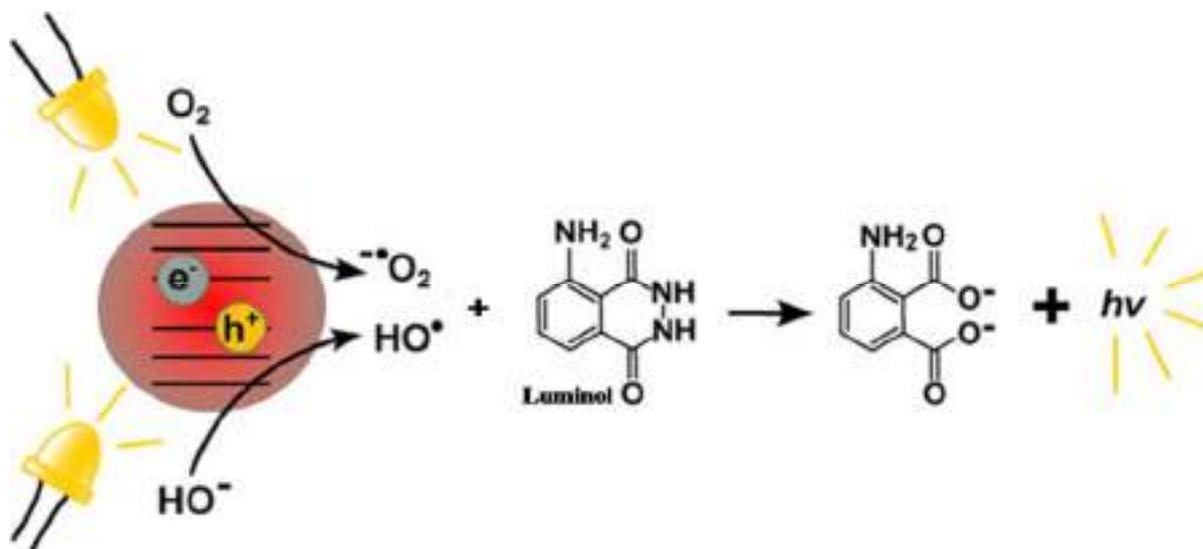
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## Assessment of capping influence on radical generation by photo-activated CdTe nanocrystals

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Quantum dots (QD) are semiconductor nanocrystals able to generate free radical species upon exposure to an electromagnetic radiation. In this work, we studied the generation of reactive oxygen species (ROS) by exposing CdTe QDs with different cappings to visible light. To do so, we used a high power visible light emitting diode (LED) as photoirradiation element, assembled in a laboratory-made photocatalytic unit [1]. The developed approach was based on the chemiluminometric monitoring of the oxidation of luminol by the produced ROS. Due to the very short lifetime of ROS and to ensure both reproducible ROS generation and time-controlled reaction implementation and development, all reactional processes were implemented inline by using an automated multipumping micro-flow system. The radical generation was studied using QD of different sizes with glutathione (GSH) and with mercaptopropionic acid (MPA) as capping agents. Three different concentrations of QDs (0.25, 0.5 and 1  $\mu\text{M}$ ) were tested in water, in the presence of NaOH and with manitol and/or tiron. The NaOH was used to assess the formation of hydroxyl ( $\text{OH}^\bullet$ ) from the oxidation of the  $\text{OH}^-$  ion. The tiron and the manitol were used as ROS scavengers to evaluate the chemoluminescence (CL) quenching, being tiron a specific superoxide ( $\text{O}_2^{\bullet-}$ ) scavenger and manitol a specific  $\text{OH}^\bullet$  scavenger. We verified increased ROS generation with greater concentrations of QDs and when QD of higher sizes were used. The studies with tiron revealed a CL quenching near 100%, indicating an extended formation of the superoxide radical. In the same study with tiron, the presence of NaOH demonstrated around a 10% smaller CL quenching, revealing the formation of small amount of the  $\text{OH}^\bullet$  radical.



## Biosensor development for the antioxidant evaluation

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To counteract the deleterious effects induced by the free radicals, the living organisms have developed complex endogenous and exogenous antioxidant systems. Endogenous antioxidant systems include enzymes, such as superoxide dismutase, catalase and glutathione-S-transferase (GST) [1,2].

GST is a powerful endogenous antioxidant enzyme that is involved in the cellular detoxification of xenobiotics substances and the endogenous products formed during the lipid peroxidation [2].

In this work it was evaluated the protective effect promoted by the GST antioxidant enzyme on the scavenger free radical activity. For that a voltammetric purine-based biosensor was designed and developed. This biosensor was based on the immobilization of the purine base (adenine or guanine) onto a carbon paste electrode (CPE).

Using the square wave voltammetry (SWV), it was, firstly, analyzed the oxidative lesions induced by xenobiotics onto the DNA bases. It was observed a decreased on the oxidative peak signal, which is indicative of damage in the DNA. Then it was studied the protective effect promoted by the enzyme. For that, the Purine-based biosensor was immersed in a solution containing xenobiotics and the GST antioxidant. In this case an increase on the electrochemical signal was observed, consequently, it was concluded that GST presents antioxidant activity by promoting DNA protection.

The developed biosensor system was used to assess the total antioxidant capacity of food and biological samples.

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# Design and synthesis of novel antioxidants based on natural scaffolds

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Reactive oxygen and nitrogen species (ROS and RNS, respectively) are important chemical modulators of both physiological and pathological cellular pathways. Though strictly regulated, ROS/RNS production can outgrow the mechanisms of antioxidant defense, causing severe and deleterious effects on vital cell structures such as the membranes, ion channels and genetic material. This redox deregulation, known as oxidative stress, is implicated in numerous human diseases such as cancer, coronary heart disease (CHD) and neurodegenerative diseases (NDs). Antioxidants are able to avoid oxidative damage caused by ROS/RNS and might be helpful in the prevention or treatment of oxidative stress-related diseases [1,2]. Hydroxycinnamic acids (HCAs) are naturally-derived antioxidants contained in fruits and vegetables and extensively present in the human diet [2,3]. Epidemiological studies show an inverse correlation between dietary HCAs intake and the occurrence and prevalence of cancer, NDs and CHD. Though exhibiting a wide range of biological properties, HCAs are extensively metabolized and rapidly excreted from the human body, which prevents them from attaining their target sites [1-3]. Hence, the development of lipophilic HCA derivatives is a rational strategy to yield membrane permeable compounds able to effectively reach their target sites within the human organism [1,3].

The goal of the present work is the synthesis of lipophilic HCA derivatives that exhibit a more favorable pharmacokinetic profile, while retaining or improving the biological properties of the natural HCA templates. The synthetic route pursued involved a two-step reaction using a benzaldehyde with the appropriate substitution as starting material. The first step was a microwave-assisted Suzuki-cross coupling reaction to introduce an aromatic ring with the selected substitution pattern. The following step was a Knoevenagel-Doebner condensation with malonic acid to yield the corresponding cinnamic acid derivatives. The compounds were isolated in moderate-to-high yields and then structurally characterized by <sup>1</sup>H and <sup>13</sup>C-NMR spectroscopy. The results obtained will be presented in this communication.

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# Design of tailored $Mn_xCo_{1-x}Fe_2O_4$ ferrite nanoparticles for magnetic hyperthermia applications

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In the last decades, there has been an increasing interest on the design and functionalization of magnetic nanoparticles (MNPs) with tailored properties due to their technological applications in a wide spectrum of research fields such as medicine and catalysis [1]. The production of MNPs with high colloidal stability, small particle size and high saturation magnetization values is of utmost importance for theranostics and imaging applications [1,2]. Pure and mixed metal nanoferrites are promising platforms for magnetic hyperthermia due to the heat power they release when subjected to an alternating magnetic field.

In this work, Mn-Co ferrite MNPs with different Mn:Co ratios ( $Mn_xCo_{1-x}Fe_2O_4$ ,  $x = 0, 0.3, 0.5, 0.7, 1$ ) were produced by a new coprecipitation method recently developed by our group [3].

The nanomaterials size and structure were studied by X-ray diffraction and Raman microspectroscopy. The colloidal stability of the MNPs in aqueous medium and their hydrodynamic size were characterized by dynamic light scattering. Furthermore, the surface charge was quantified by zeta potential. All the nanomaterials exhibited a magnetic response in the presence of an external magnetic field (Fig. 1). Finally, the effect of Mn substitution and coprecipitation base was evaluated, in order to tailor the physicochemical properties of the MNPs to maximize their heating capability.



**Fig. 1** Magnetic response of  $Mn_xCo_{1-x}Fe_2O_4$  nanomaterials.

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## Determination of phthalates in blood samples by GC-MS

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Phthalates are one of the most widely used plasticizers to improve the extensibility, elasticity and workability of polyvinyl chloride (PVC), polyvinyl acetates, etc. Considered non-toxic and harmless additives for polymers, they were used unrestrainedly all over the world for several decades [1]. However, recent studies have indicated that some phthalates and their metabolic products are reproductive and developmental toxicants in animals and suspected endocrine disruptors in humans [1] especially by mimicking estrogens [2]. The general population is widely and continuously exposed to phthalates because they are components of plastics, cosmetic products, toys, lubricants, etc.[3].

The aim of this study was to develop a method to evaluate the presence of phthalates in human blood samples. The analyzed compounds included: dibutyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP). Chromatographic analyses were carried out in a Thermo GC ULTRA Gas Chromatograph Mass Spectrometer equipped with a ZB-XLB (30m x 0.25mm ID, 0.25µm film thickness, Phenomenex). High-purity helium (99.9999%) at a constant flow rate of 1.0 mL/min was used as the carrier gas. For injection an AI/AS 3000 auto injector was used. Injections (1 µL) were made in the split/splitless mode. Samples were analyzed using the following oven temperature program: initial temperature 90 °C (held for 1 min), increased by 20C/min to 250 °C (held for 9 min). Positive fragment ions (m/z—ions mass/charge ratio) were analyzed over 50–550 m/z mass range in SCAN mode and in selected-ion monitoring (SIM) mode. Selected ions used for quantification were 149, 150 and 223 for DBP and 149, 167, 279 for DEHP. Linear calibration curves were obtained for the two compounds. Concentrations ranging from 100 to 900 µg/L were studied.

After validation, the methodology will be applied to human samples for phthalates determination.

Acknowledgements:

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# Development of a biosensor for the assessment of kojic acid in cosmetics

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Kojic acid, 5-hydroxy-2-(hydroxymethyl)-4-pyrone is usually used in cosmetic industry due to its anti-radical and depigmenting properties. Kojic acid is a reversible and competitive inhibitor of the tyrosinase, a polyphenol oxidase which is a key enzyme on melanin biosynthesis [1]. The maximum level of kojic acid concentrations in formulations for topic application is 2%. Above 5% it can cause adverse reactions like dermatitis, erythema, and hyperpigmentation. So, analytical methods that provides high efficiency and low cost are an interesting research area.

The use of biosensors for routine analytical tasks has attracted considerable interest because these devices allow performing high selective chemical monitoring at reduced costs. With their use the amount of reagents used is very low, they have reversible response, enable portability and are capable of providing real-time response. This work aims at the development of an optical biosensor based in fluorimetric transduction to accomplish the determination of kojic acid in cosmetics resorting to the enzyme tyrosinase immobilized in a polysiloxane polymer matrix as sensing membrane. Further immobilization of the complex tris (bathophenanthroline disulfonate)-Ruthenium (II), very fluorescent when in the absence of oxygen, enables the coupling of a simple transduction mechanism. To evaluate the best conditions for the sensor implementation, several studies were performed either considering the reaction in traditional batch conditions with the enzyme and transducer in solution, when the biomolecule is chemically immobilized at the surface of controlled-pore glass beads and physical entrapment on a polysiloxane matrix obtained by the sol-gel method [2]. In the sensor, fluorimetric signal is ensured by additional immobilization.

In all cases sigmoidal shaped response curves were obtained, stressing the high sensitivity of the transducer. The signaling process was then optimized considering the main substrate, temperature, enzyme activity and amount of transducer. Under optimized conditions it was possible to attain a linear response to kojic acid in the range of 1,05  $\mu\text{mol/mL}$  up to 4,22  $\mu\text{mol/mL}$  (0,015% a 0,06% $\text{m/m}$ ), for a response time of 10 min.

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# Development of a screening test for environmental monitoring of UV filters

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The UV filters are chemical substances used to protect skin from ultraviolet rays and they can be classified in inorganic or organic filters according to the chemical structure. Currently, the organic UV filters are widely used in cosmetic products, as well as in the industrial sector [1]. Thus, the growing concern about the deleterious effects upon organic UV filters exposure, their eventual accumulation and toxicological effects require screening methods for assessing their presence in the environment [2].

For their purpose, the primary interest of this study concerns the development of a solid state screening test for rapid environmental monitoring of UV compounds in coastal bathing waters. Accordingly, commercially available solid phase extraction (SPE) disks composed by 90% of C18 sorbent and 10% polytetrafluoroethylene by weight were impregnated with a fluorescent probe X<sub>1</sub> and applied as a selective fluorimetric sensor for UV filters. The screening assay was based on the fluorescence decrease that takes place in the presence of UV filters, caused by radiation absorption from UV filters retained on the Empore membrane. Hence, preliminary experiments were directed towards evaluation of the absorption spectra of selected UV filters (benzophone-3, BZ-3; 2-ethylhexyl-4-dimethylaminobenzoate, OD-PABA; and 2-ethyl-hexyl-4-trimethoxycinnamate, EHMC), and their interaction with X<sub>1</sub> probe in liquid phase (methanol), which provided a concentration dependent fluorescence decrease. Using BZ-3 as model compound, C-18 disks (diameter of 13 mm) impregnated with X<sub>1</sub> probe (116 ng) were percolated by 10 mL of seawater surrogates containing between 14-450 µg/L of UV filter. Fluorescence inhibition was observed from 56.2 µg/L (562 ng of BZ-3) when disks were exposed to UV light (254 nm and 365 nm). Current work is focused on quantification through image analysis.

## Acknowledgements:

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# Electrochemical behaviour of ferrocene in ionic liquid [emin]BF<sub>4</sub>

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Room Temperature Ionic Liquids (RTILs) have very interesting physical properties, such as low volatility, high thermal stability, high polarity, viscosity and intrinsic conductivity, also having a wide electrochemical window [1]. They are excellent solvents for many organic reactions. However, the study of reaction mechanisms and inorganic species in these liquids is still unexploited.

The present study aims to the electrochemical system of Ferrocene (Fe(C<sub>5</sub>H<sub>5</sub>)<sub>2</sub>), an organometallic compound which is characterized by having two cyclopentadienyl rings attached to opposite sides of a central atom of iron (II). The study was conducted on a platinum working electrode, at room temperature and using the ionic liquid 1-Ethyl-3-methylimidazolium tetrafluoroborate ([emin]BF<sub>4</sub>), as solvent.

The electrochemical techniques cyclic voltammetry, square wave voltammetry, chronoamperometry, and chronopotentiometry were used to determine the following parameters: diffusion coefficient, the diffusion layer thickness, number of electrons interchanged and also studies of reversibility of the system, including some kinetic parameters such as  $\alpha$  (charge transfer coefficient) and  $k^0$  (charge transfer rate constant).

The electrochemical cell used, from Metrohm, presents holes at the top that allow the introduction of the three electrodes in different positions and at the desired height, and the entry of the inert gas (Nitrogen N-50, Air Liquide). We used two platinum electrodes as working and auxiliary electrodes and a silver wire immersed directly in the solution as a pseudo-reference electrode. Both the preparation of the solutions and the electrochemical study were carried out inside of a glove box, homemade, under inert nitrogen atmosphere into and out of the cell.

## Acknowledgements

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# Energetic study of 10-methylphenothiazine

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Polycyclic compounds (homo and heterocyclic) present relevant applications in different fields, from the modern electronic to the pharmaceutical industries. We are involved on the study of two main classes of compounds: polycyclic aromatic hydrocarbons (PAHs) and nitrogen, sulfur or oxygen heteropolycyclic derivatives, in order to evaluate the influence of these heteroatoms on the energetic of those structures.

In this context, we have been developing the thermochemical characterization of some phenothiazine derivatives which exhibit important photochemical and clinical properties, being used widely in pharmaceutical industry (antihistamines and antipsychotic drugs) and in the construction of photoelectrochemical and biochemical sensors. These studies constitute a contribution for the understanding of the reactivity of such species.

The present work reports thermal properties of 10-methylphenothiazine (Figure 1), used to derive the corresponding standard ( $p^{\circ}=0,1$  MPa) molar enthalpy of formation, in gas-phase, at  $T=298,15$ K.

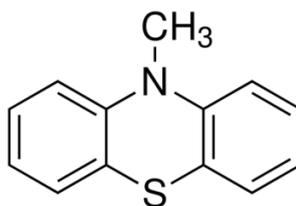


Figure 1: Molecular structure of 10-methylphenothiazine.

This study involved the use of the following experimental calorimetric techniques:

- rotating bomb calorimetry to measure the energy of combustion, and subsequent calculation of the standard enthalpy of formation in the crystalline state,  $\Delta_f H_m^{\circ}(cr)$ .
- Calvet microcalorimetry for the determination of the enthalpy of sublimation,  $\Delta_{cr}^{\circ} H_m^{\circ}$ .

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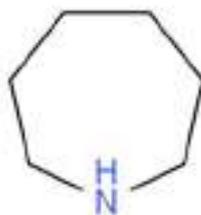
# Energetics of azepine derivatives: study of homopiperidine

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Azepine derivatives play an important role as intermediate compounds in organic syntheses of pharmaceuticals, zeolites, dyes, rubbers and textile chemicals processes, among others [1-3]. Homopiperidine (Hexahydro-1*H*-azepine, Fig. 1), also known as Azepan, is a key structure on that class of compounds and, despite the importance of the knowledge of its energetic behavior, in literature there is no report of reliable data for their thermal properties.

In order to improve the understanding of the relationship between the energetical and the structural properties on this kind of molecules, we are involved in the development of studies for their thermodynamic characterization. We have started the study of homopiperidine and the present work reports its standard ( $p^{\circ} = 0.1$  MPa) molar enthalpy of vaporization, at  $T = 298.15$  K, measured using a high temperature Calvet microcalorimeter, and the standard molar energy of combustion, in oxygen, determined using a static bomb calorimeter, at the same temperature. The combination of this two parameters for homopiperidine allowed the calculation of the corresponding standard molar enthalpy of formation, in gaseous phase, at  $T = 298.15$  K.



**Figure 1.** Molecular structure of homopiperidine.

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# Evaluation of antioxidant total capacity through photoactivation of nanoparticles “quantum dots” in an automatic system

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Quantum dots (QD) are defined as being monodisperse nanoparticles with a nucleus made of a semi-conductor material and capped with an organic layer. Their usual size range between 1-10 nm [1]. When made with a proper nucleus, QD have the ability to emit fluorescence, making them a viable option for chemiluminescence assays, playing the role of oxidative particles in aqueous solutions [2]. This can be accomplished by focus a light beam into QD in order to obtain free radicals which will then react with an antioxidant, allowing the quantification of the last one. This methodology was automated through implementation of the reaction in a multipumping flow system (MPFS) [1], allowing the automatic control of the insertion, commutation and transport of multiple reagents through time-based computer software routines.

In the present work aqueous CdTe-QD nanoparticles were synthesized in the laboratory using glutathione as capping material and NaH<sub>2</sub>Te as tellurium precursor. Reactive oxygen species (ROS) were generated upon photoactivation of CdTe-QD with visible light which are capable of oxidizing luminol, used as a chemiluminescent reagent, in an alkaline medium. The oxidation of luminol by ROS produces a strong chemiluminescent signal which is quenched in the presence of trolox used as antioxidant substance.

The automation of this reaction was accomplished through the developing of a flow analysis system involving four solenoid micro-pumps, each one responsible for handling a different reagent solution, coupled to a laboratory-made visible light source based on LEDs and a chemiluminescent detector.

The capacity to generate free radical species by QD was studied through the evaluation of the concentration of QD with different sizes (3,38 nm; 2,70 nm and 1,31 nm). Also, the concentration of all reagents was optimized. Besides this, it was also necessary to establish an optimum value for sample volume, reagents volume and flow rate (during irradiation and detection). The methodology was optimized considering the inhibition (percentage) of analytical signal based on the quenching effect of trolox on the oxidation of luminol by the ROS species generated by the QD nanoparticles irradiation.

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# Evaluation of different chelating agents on the performance of antioxidant capacity assays based on the reduction of Cu(II)

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Spectrophotometric assays, based on electron transfer mechanisms that measure the capacity of an antioxidant to reduce a chromogenic oxidant, are frequently applied for assessment of total antioxidant capacity. The degree of colour change (change of absorbance at a given wavelength) is proportional to the concentration of antioxidants in the sample [1]. There are several alternatives for total antioxidant capacity (TAC) assessment based on Cu(II) reduction as these methods usually differ in the chelating agent used: bathocuproine, bathocuproinedisulfonic acid (BCS), neocuproine (NC) [2], and bicinchoninic acid (BCA). Their complexes with monovalent copper ion have typical maximum absorption between 450 and 560 nm. In these TAC assays, the colour change is detected when, in a solution containing Cu(II) and one of these chelators, the antioxidants present in a sample reduce Cu(II) to Cu(I).

The aim of this work is to evaluate the influence of different chelating agents (BCS, NC and BCA) in the antioxidant capacity values determined by cupric ion reducing assay. Assays were adapted to a high-throughput scheme using a microplate reader and the method response towards several model antioxidant compounds like ascorbic acid, Trolox (soluble analogue of vitamin E), uric acid and glutathione were studied. The different reagents were compared in terms of sensitivity, stability, maximum absorption wavelength and reaction kinetics.

BCS was selected as the most suitable chelating agent for further studies since it provided a linear dynamic range up to 100  $\mu\text{M}$  of Trolox, and a limit of detection of 2  $\mu\text{M}$ , with within day repeatability better than 1% ( $n = 4$ ) and a between day repeatability of 3% ( $n = 5$ ). This choice was based on the highest sensitivity attained and the additive response found in the presence of several antioxidants simultaneously. Considering the concentration levels attained, this spectrophotometric method can be of great value in the assessment of antioxidant capacity at physiological concentrations.

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# Immobilization of CdTe nanocrystals capped with aminocompounds onto aminated glass

C. Frigerio, M.M. Carvalho, J.L.M. Santos, J.A.C. Barbosa, L.M.F.S. Saraiva, D.S.M. Ribeiro, J.A.V. Prior, M.L.C. Passos

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Immobilization of nanoparticles on different substrates can be achieved by using different strategies. The developed approaches were mainly fostered by the prospective exploitation of the unique physical and chemical properties of nanomaterials that made them promising tools for applications in optical, electronic, chemical and biochemical devices. Likewise others NP, QDs were immobilized onto a great variety of supporting materials by using different approaches, such as layer-by-layer (LbL) assembly, direct incorporation and covalent attachment to chemically modified surfaces. Chemically modified materials, especially silanized surfaces, have been extensively used for the immobilization of enzymes. Due to their chemical nature enzymes demand extremely soft reaction conditions in order to retain a high catalytic activity. One of the most simple and efficient immobilization processes relies on covalent linkage upon reaction between the enzyme and solid supports previously activated with glutaraldehyde. This compound can react with amine, thiol, phenol, or imidazole functional groups in proteins because the most reactive amino acid side-chains are nucleophiles. These nucleophiles can attack the carbonyl group of glutaraldehyde forming an imine. In this work we have developed a glutaraldehyde-based methodology for the preparation of CdTe quantum dots functionalized substrates. Two nucleophilic additions are used to link one of the carbonyl groups of glutaraldehyde to the amino modified glass while the second carbonyl group binds the  $\alpha$ -amine of the CdTe capping agent.

In this work we have developed a glutaraldehyde-based methodology for the preparation of CdTe quantum dots-functionalized glasses. The successful immobilization is proved by fluorescence microscopy and by FTIR. The method assures noteworthy advantages regarding similar approaches, namely in terms of simplicity of implementation and the utilization of more favorable reaction conditions that do not compromise QDs optical properties. Moreover, it can provide a straightforward process for covalent immobilization of amino-capped QDs (without carboxylic groups) that could not be immobilized by using carbodiimide chemistry or even for immobilization of these QDs onto other substrates exhibiting the same amino groups as the functionalized glass. The obtained results also showed that the establishment of a covalent linkage guaranteed improved stability under aqueous conditions when compared to alternative methods based on electrostatic interactions.

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# Immobilization of CdTe quantum dots in biosilica nanostructures

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The interest in the biomimetic synthesis of silica-based materials is increasing, being biosilicification, in the last few years an inspiration for the development of novel fabrication procedures in nanobiotechnology [1]. Diatoms and sponges are able to take up silicon from the environment in soluble form as silicic acid, store it in the cell and catalyze its polymerization into silica with precise structure architecture down to nanoscale [2]. The mimetization of these natural phenomena, avoid the use of harsh and environmentally unfriendly conditions such as toxic and expensive organic solvents, high temperature and pressure and high or low pH. Moreover, it minimizes the long reaction times and the multiple steps of complex protocols [3]. This biomimetic synthesis is then characterized by the processing of nanostructure particles in water under mild conditions, by using either synthetic or biologically-derived amine-containing macromolecules as additives, mediators or templates. The ability to manipulate all these conditions enables the controlling of the morphology of silica materials with a high level of precision and provides a powerful paradigm for the construction of biological nanostructures, involving other nanoparticles, such as quantum dots, with wide potential applications. Quantum dots (QDs) or colloidal semiconductor nanocrystals are monodisperse crystalline clusters of atoms with size normally comprised between 1 and 10 nm that due to their low dimension acquire peculiar characteristics [4]. In this work, CdTe quantum dots were synthesized, by wet chemistry using mercaptopropionic acid (MPA) as stabilizing agent by Zou's method [5] with some modifications. Thus, in this work, QDs were immobilized during the biosilica synthetic process and different parameters that could influence the biosilica particle synthesis and the QD immobilization were evaluated. Was observed that the encapsulation efficiency, the size and the shape of biosilica nanoparticles varies with experimental conditions as silica precursors, catalysts, pH and anions concentrations. Moreover, it was also tested the stability and fluorescent character of quantum dots encapsulated in silica matrix.

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# Improvements on the synthesis of catechol-based rosamine fluorescent chemosensor for metal ion sensing applications

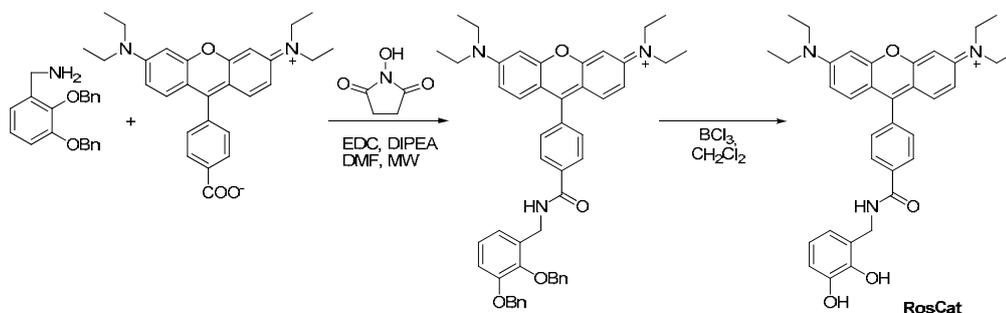
**Maria G. M. Couto,<sup>1</sup> Solenne Paiva,<sup>1</sup> Carla Queirós,<sup>1</sup> Andreia Leite,<sup>1</sup> Ana M. G. Silva,<sup>1</sup> Paula Gameiro<sup>1</sup> and Maria Rangel<sup>2</sup>**

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Fluorescent chemosensors have been developed to be a useful analytical tool to sense environmental,[1] medical and biochemical important metal ions,[2] because of their high sensitivity, quick response, simplicity of measurement and quantification of the analyte. A typical fluorescent chemosensor contains a receptor (chelating unit) linked to a fluorophore (fluorescent molecule) which translates the recognition event into the fluorescence signal. The fluorescence quantum yield, chemosensor chelating capacity and sensibility are crucial parameters to be considered in order to reach promising candidates for chemical sensing purpose.

Here we are presenting the synthesis, characterization, UV-Vis and fluorescence spectra of a novel catechol-based rosamine fluorescent chemosensor - **RosCat** (Scheme 1). Improvements on the synthesis of this compound were achieved when the amide coupling reaction of the catechol derivative with the rosamine scaffold was performed using the coupling reagent pairs *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC)/*N*-hydroxysuccinimide in the presence of *N,N*-diisopropyl-ethylamine (DIPEA), under controlled microwave heating. Debencylation was successfully performed with BCl<sub>3</sub> in dichloromethane, under argon atmosphere, affording the expected **RosCat** in good yield. Photophysical characteristics of **RosCat** such as UV-Vis absorption and emission fluorescence intensity and its affinity for metal ions will be presented and discussed.



**Scheme 1.** Synthetic approach for the preparation of **RosCat**.

Financial support from FCT through project PEst-C/EQB/LA0006/2011 is gratefully acknowledged. We also thank Dr. Andrea Carneiro from CeNTI, V.N. Famalicão, for making available the CEM Discover microwave reactor.

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# ***In vitro* evaluation of salicylate ionic liquids toxicity through the inhibition of human carboxylesterase and *Vibrio fischeri***

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The emergence of ILs with biological activity has revolutionized the scientific focus of these compounds and opened interesting perspectives regarding their pharmaceutical application [1]. The development of ILs that can be used as active pharmaceutical ingredients seems to overcome some of the drawbacks associated with solid forms (traditionally adopted by the pharmaceutical industry) and has already resulted on compounds with interesting pharmacological activity and modulated chemical properties [1, 2]. The research on this field has been focused mainly on the synthesis of novel IL-APIs by association of active ions (anion and cation) and on the evaluation of the expected pharmacological activity. As far as we know, there are no consistent studies on the toxicity of these compounds and thus, there are no evidences that can guarantee their safe utilization *in vivo*!!

In this work we studied the toxicity of salicylate ILs [2] through the inhibition of human carboxylesterase (CE), widely accepted as toxicity biomarker [3] and *Vibrio fischeri* [4], a marine bacterium used as standard ecotoxicological bioassay. Both assays were implemented in sequential injection analysis (SIA) systems. CE inhibition was evaluated through the hydrolysis of 4-methylumbelliferyl acetate, to produce the fluorescent compound 4-methylumbelliferone ( $\lambda_{exc}=365$  nm;  $\lambda_{em}=460$ nm). The *Vibrio fischeri* assay comprised a simple protocol that guaranteed adequate aspiration and handling of the solutions as well as the precise implementation of a computer controlled stop period. Automation assured the precise control of the contact time between *Vibrio fischeri* and test compound.

We expect that the obtained results can provide important information about the safety of the studied salicylate ILs and thus contribute to their future utilization in the pharmaceutical field.

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# Oxidation of acridine with hydrogen peroxide catalyzed by a Mn(III) porphyrin

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The oxygenation mechanism performed by the prosthetic group of cytochrome P450 has inspired the use of metalloporphyrins as catalysts for oxidation reactions. Based on the fact that this mono-oxygenase mediates oxidations even of inert and apolar xenobiotic substrates, the metalloporphyrins have been used as biomimetic catalysts for the oxidation of polycyclic aromatic hydrocarbons, terpenes and alkylbenzenes. [1,2] In this work, acridine, a polycyclic aromatic hydrocarbon with three fused rings and a nitrogen heteroatom, was efficiently oxidized in mild conditions using  $H_2O_2$  as a green oxidant, in the presence of the (*meso*-tetrakis(2,6-dichlorophenyl)porphyrin)manganese(III) chloride [Mn(TDCPP)Cl] (Figure 1a) and ammonium acetate as the co-catalyst. [3] The reaction (Figure 1b) proceeded at room temperature leading to various oxidized compounds, such as diepoxydes and tetraepoxydes, that may have important properties for future biological and photochemical applications.

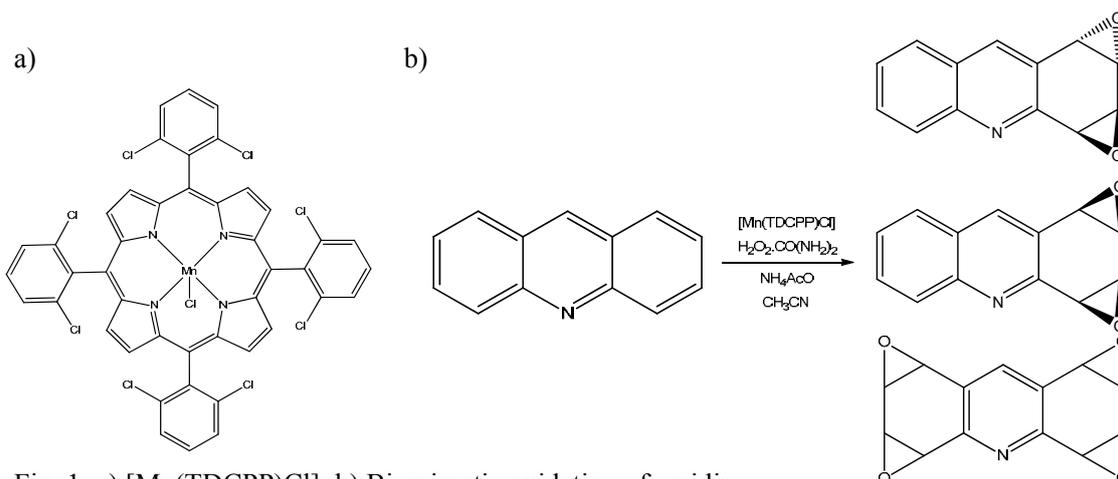


Fig. 1: a) [Mn(TDCPP)Cl]; b) Biomimetic oxidation of acridine.

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# Oxidative desulfurization of model fuel oil using environmental friendly conditions

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The deep removal of sulfur-containing compounds from fuel oils is an important part of the petroleum refining industry. According to the legislation of many countries, the sulfur content in diesel and gasoline could not exceed the value of 10 ppm. [1] The actual process to remove the organosulfur compounds from fuels is the hydrodesulfurization (HDS). However this process is poorly efficient to remove benzothiophene (BT), dibenzothiophene (DBT), 4,6-dimethyldibenzothiophene (4,6-DMBT) and others derivatives, requiring more energy which causes more costs. So new methods, such as oxidative desulfurization (ODS) have been investigated as an alternative to HDS. The ODS technology have several advantages such as very mild reaction conditions as low temperature (50 °C) and atmospheric pressure and no requiring expensive hydrogen as HDS process. The ODS is a two-stage process, an initial oxidation of sulfur compounds, followed by their liquid-liquid extraction. In the first step, the sulfur-containing compounds are oxidized to their corresponding sulfoxides and/or sulfones which can be more easily extracted. The oxidant most successfully used in ODS process is the H<sub>2</sub>O<sub>2</sub> because this is environmental friendly, water is the only sub-product, it is cheap and commercially available. [2]

The polyoxometalates (POMs) are metal oxide anionic clusters with unique properties that make them good catalysts to be used in green oxidative systems. [2,3]

Ionic liquids (ILs) have showed to be efficient solvents for ODS process. These solvents are designated as “green solvents” by their unique physical-chemistry properties.

In the present work, a Keggin-type POM (TBA<sub>5</sub>[PW<sub>11</sub>Zn(H<sub>2</sub>O)O<sub>39</sub>], PW<sub>11</sub>Zn) will be present as efficient catalyst for the ODS process. The synthesis and characterization of PW<sub>11</sub>Zn will be present. This compound showed to be a selective and efficient catalyst for the ODS process using a model fuel oil containing DBT, 1-BT and 4,6-DMBT, H<sub>2</sub>O<sub>2</sub> as green oxidant and a IL as solvent (1-Butyl-3-methylimidazolium hexafluorophosphate, BMIPF<sub>6</sub>). The studied system showed to be recyclable and approximately total sulfur removal was achieved after 2h for three consecutive cycles.

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# Phase solubility studies of the inclusion complexes of terbutylazine with cyclodextrins: an HPLC approach

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Human population is constantly exposed to numerous chemical species present in the environment. Pesticides present the only group of chemicals that are purposely applied to the environment with aim to suppress plant and animal pests and to protect agricultural and industrial products. However, the majority of pesticides is not specifically targeting the pest only and during their application they also affect non-target plants and animals. Many pesticides are not easily degradable, they persist in soil, leach to groundwater and surface water and contaminate wide environment. Therefore, it is crucial to reduce the environmental and economic costs of pesticides.

One approach to solve this problem is to design controlled-release formulations, based on cyclodextrin complexes, which could decrease the dose and rate of release of the active ingredient. Cyclodextrins (CD) are cyclic oligosaccharides containing containing 6 ( $\alpha$ -CD), 7 ( $\beta$ -CD) or 8 ( $\gamma$ -CD)  $\alpha$ -(1,4)-linked glucose units and can be represented as a truncated cone structure with a hydrophobic cavity. The ability of the cyclodextrins to form inclusion complexes with a variety of organic compounds is based on their ability to provide a hydrophobic cavity in aqueous solution for a hydrophobic guest molecule or hydrophobic moieties in the guest molecule. The inclusion complexes formed usually shows improved physicochemical properties like water solubility, solution stability and bioavailability [1]. The phase-solubility technique permits the evaluation of the affinity between cyclodextrins and guests molecules in water. Phase solubility studies are usually performed according to the method reported by Higuchi and Connors based on UV-Vis spectrophotometry [2].

The aim of this study was to investigate the formation of inclusion complexes of herbicide terbutylazine with different cyclodextrins and the development of an HPLC methodology for the determination of the association constants. The results obtained were compared with Higuchi and Connors' UV-Vis spectrophotometric method. The results obtained will be presented and discussed in this communication.

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# Polyoxometalates catalysts for oxidative desulfurization processes

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The combustion of the transportation fuels remains the major source of sulfur oxide (SO<sub>x</sub>), which contributes to refinery catalyst fouling, corrosion, air pollution, and acid rain. This problem, has placed pressure not only on refiners but also on governments and legislatures. Therefore the limit value for sulfur in gasoline and diesel fuel has been set globally, in order to achieve smaller values and in the next few years is aimed the sulfur-free fuels. The catalytic oxidative desulfurization (ODS) is regarded as the most promising process to meet the future environmental regulation for low sulfur diesel, as there are several advantages in its use such as mild reaction conditions, high selectivity and no use of expensive hydrogen. The ODS process combines the oxidative desulfurization and the extractive desulfurization. The first step consists in the oxidation of organic sulfur compounds (such as dibenzothiophene, DBT) into its corresponding sulfoxides and sulfones that are then removed by an extractive process [1-3].

In this work, the Keggin-type polyoxometalates TBA<sub>3</sub>[PW<sub>12</sub>O<sub>40</sub>] (TBAPW<sub>12</sub>) and TBA<sub>4</sub>[PW<sub>11</sub>Fe(H<sub>2</sub>O)O<sub>39</sub>] (TBAPW<sub>11</sub>Fe) were tested as catalysts for the oxidation of DBT and H<sub>2</sub>O<sub>2</sub> was used as oxidant. Two different biphasic catalytic systems were investigated using two different systems: n-octane/acetonitrile and n-octane/ionic liquid (1-butyl-3-methylimidazolium hexafluorophosphate, BMIPF<sub>6</sub>). The tested Keggin-type catalysts revealed to be efficient. The BMIPF<sub>6</sub> used as solvent enabled the achievement of better results (the conversion of DBT in the corresponding sulfone was almost complete after 30 minutes of reaction using both catalysts) than acetonitrile and also allows the recyclability of the system. An efficient ODS catalytic process working under eco-sustainable experimental conditions is here proposed, based on a recyclable green solvent (BMIPF<sub>6</sub>) and green oxidant (H<sub>2</sub>O<sub>2</sub>), using two robust the Keggin-type catalysts.

## Acknowledgments

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# Post-synthetic modification of MIL-101(Cr) with monovacant polyoxometalates: novel heterogeneous catalysts for oxidation

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Metal-organic frameworks (MOFs) are materials formed by metal centers interconnected by organic molecules, leading to infinite hybrid networks that can be one-, two- or three dimensional (1D, 2D or 3D, respectively). In addition to their notable structural characteristics, the properties give them high potential for industrial and technological applications, such as heterogeneous catalysis, gas storage and separation, and others.[1] Polyoxometalates (POMs) are metal-oxygen clusters that have been extensively investigated as homogeneous catalysts for a large variety of liquid-phase oxidations.[2] Their catalytic behaviour can be fine-tuned by varying metal elements in their structure without loss of oxidative stability. As consequence of their versatility and accessibility, POMs can be used in a wide range of organic reactions, such as epoxidation, sulfoxidation and others.

In the present work, a porous 3D MOF material, chromium(III) terephthalate, known as MIL-101(Cr), was explored as solid-support for the preparation of heterogeneous catalysts. MIL-101(Cr) was isolated by hydrothermal synthesis and two monovacant polyoxometalates  $K_7[PW_{11}O_{39}] \cdot n(H_2O)$  ( $PW_{11}$ ),  $K_8[SiW_{11}O_{39}] \cdot m(H_2O)$  ( $SiW_{11}$ ) were immobilized in their porous network leading to the formation of novel composite materials,  $PW_{11}@MIL-101$  and  $SiW_{11}@MIL-101$ , respectively.[3] All materials were characterized by vibrational (FT-IR and FT-Raman) and  $^{31}P$  solid-state NMR spectroscopies, powder X-ray diffraction (PXRD) and scanning electron microscopy (SEM) with energy-dispersive X-ray spectroscopy (EDX). Afterwards, it was studied the catalytic performance of these composite materials for the oxidation of cis-cyclooctene, geraniol and R-(+)-limonene using the  $H_2O_2$  as oxidant. Recyclability tests were also performed and the stability of the composites after several catalytic cycles was confirmed. Remarkably, the MOF framework was found to play an important role in the stability of the  $PW_{11}$  in the presence of  $H_2O_2$ .  $PW_{11}@MIL-101$  and  $SiW_{11}@MIL-101$  revealed to be active, selective and recyclable heterogeneous catalysts.[3]

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## Preliminary study of biosensor optimization for the detection of polycyclic aromatic hydrocarbons

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Polycyclic aromatic hydrocarbons (PAHs) are environmental contaminants which originate primarily from the incomplete combustion of wood or petroleum products, with the majority coming from anthropogenic activities. The health concerns of PAHs have been traditionally focused on their potential cytotoxicity, mutagenicity and carcinogenicity in humans. PAHs are genotoxic compounds and their carcinogenicity is probably mediated by their ability to damage the DNA [1].

This work aims to develop an electrochemical purine-based biosensor for detection of PAHs in real samples.

The development of this biosensor consists of three steps:

(i) Guanine or adenine (DNA purine bases) electro-immobilization on the carbon paste electrode (CPE).

This immobilization was performed by the application of an adsorptive accumulation step. For that, the activated CPE was immersed in phosphate buffer solution (PBS) pH 4.8 containing adenine or guanine and it was applied a positive potential of +0.4 V for 180 s, after this the electrode was washed with water.

(ii) Damage of the purine base by the immersion of purine-based CPE on the PAH solution.

Oxidative damage of the purine base was carried out by immersing the biosensor in a freshly prepared PAH solution in PBS pH 7.4 [2]. After a fixed period of reaction time, the purine-based biosensor was rinsed with water.

(iii) Detection and measurement of the electrochemical peak current of adenine or guanine in a phosphate buffer solution (PBS) at pH 7.4.

The electrochemical signal was obtained by using square wave voltammetry (SWV) technique. SWVs were carried out between +0.2 V and 1.4V (frequency 50 Hz, step potential 4.12 mV and amplitude 0.09 V).

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# Production of zero valent iron nanoparticles through the valorization of by-products of the food industry

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The interest in zero-valent iron nanoparticles (nZVI) has been increasing exponentially since the first developments on green production methods in which extracts from some natural products. However, this field of research is yet poorly studied and lacks knowledge to allow a full understanding of the production methods and the forthcoming application processes. These methods are based on the production of aqueous extracts of natural products that, because of its high antioxidant capacity, are capable to react with an iron (II) solution and form the nZVI [1].

The aim of this work was to evaluate the viability of the utilization of some by-products of the food industry (lemon processing wastes, namely leaves) to produce extracts that are capable to form zero valent iron at a nanometer scale and to optimize the production methodology, namely the type of solvent, extraction temperature, contact time and  $\text{mass}_{\text{product}}:\text{volume}_{\text{solvent}}$  ratio. The obtained results allow concluding that the proposed method was capable to produce nZVI with ranges within 20 and 80 nm, as presented in Figure 1, representing an excellent and promising alternative to the traditional methods that are more expensive and uses toxic reagents (sodium borohydride).

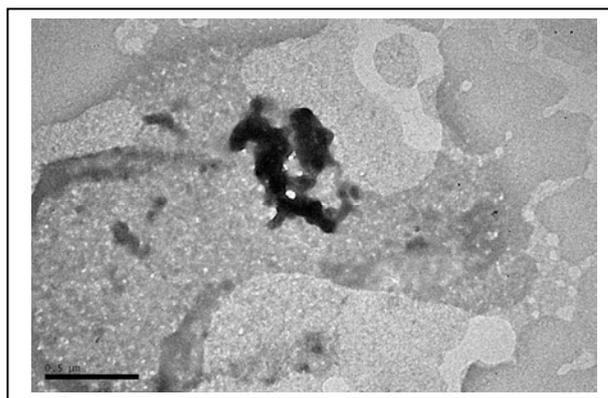


Figure 1 – nZVI produce with lemon leaves extract.

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# Studies on the Catalytic Mechanism of Human Heparan Sulfate-3-O-Sulfotransferase - A Computational Approach

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Heparan Sulfates (HS) are highly sulfated polysaccharides, which consist of repeating 1-4 linked sulfated glucuronic/iduronic acid and sulfated glucosamine units. HS are involved in several biological interactions, such as assisting viral infection, suppressing tumor growth or regulating blood coagulation. They are a component of proteoglycan present in large quantities on the surface of mammalian cells and in the extracellular matrix. HS are initially synthesized as polymers of a disaccharide and later they undergo several modification processes, which result in a variety of disaccharide isoforms. These different isoforms are specific for individual ligand proteins and the different biological functions of HS seem to be dictated by the sulfated saccharide sequences [1].

HS D-glucosaminyl-3-O-Sulfotransferase (3-OST) is one of the enzymes involved in the HS biosynthesis. It is responsible for the generation of 3-O-sulfated glucosamine linked to different uronic acid residues, which is the last step in the biosynthesis of HS. The different 3-OST isoforms have different substrate specificity, which results in diverse biological functions of the HS modified by 3-OST. HS produced by 3-OST-1 and 3-OST-3 enzymes regulate the blood coagulation cascade and serve as an entry receptor for the Herpes Simplex Virus type 1 (HSV-1), respectively. HS modified by 3-OST-5 have both anticoagulant activity and serve as receptors for HSV-1 [2].

The final goal of the present work is to establish the catalytic mechanism [3] of Human Heparan Sulfate 3-O-Sulfotransferase with atomic detail, using computational methods. The model system includes a simplified reaction centre, a small substrate molecule and the cofactor, 3'-phosphoadenosine-5'-phosphosulfate (PAPS). Using semi-empirical calculations we were able to confirm and provide molecular-level detail to the mechanism proposed in the literature. On the other hand, in order to allow for detailed and accurate studies on the enzyme mechanism, while taking into account the influence of the protein as a whole, we used ONIOM (B3LYP:AMBER), which is an integrated Quantum Mechanics and Molecular Mechanics two-layered method, capable of geometry optimizations. We are presently performing calculations on a large enzymatic model, which allows for the inclusion of environmental effects. This work will enable further studies on the inhibition of this enzyme, which is a useful target for drug design.

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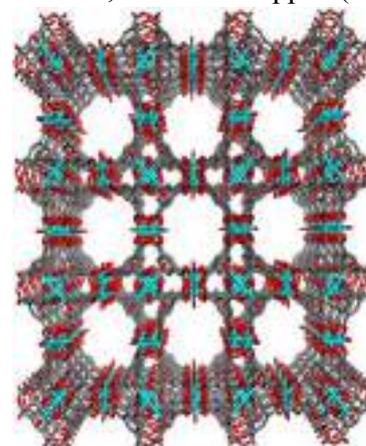
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# Synthesis and characterization of metal-organic frameworks: Cu-btc and Cu-bdc

**Joana Barbosa<sup>1</sup>, André Barbosa<sup>1</sup>,  
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Metal-organic frameworks (MOFs) are a relatively recent group of porous materials composed of metal or metallic clusters centers that are linked by multidentate organic molecules (ligands). These compounds are also known as coordination polymers or coordination networks and reveal extended metal-ligands network structures that can be one-, two- or three dimensional (1D, 2D or 3D). MOFs have unique properties like pore sizes and shapes making them a new high performance adsorbents, ideal for gas storage and heterogeneous, among other applications.[1,2] In this investigation, two compounds were synthesized and characterized, based in copper (II) and benzene-polycarboxylic acids, Cu-btc (Figure 1) and Cu-bdc. The two MOF were obtained by hydrothermal and microwave assisted synthesis. While the Cu-btc was prepared with trimesic acid and copper (II) nitrate trihydrate, in the Cu-bdc was used terephthalic acid and copper (II) nitrate trihydrate.[2,3] These materials were characterized by infrared spectroscopy (IR), powder X-ray diffraction (PXRD) and scanning electron microscopy (SEM) with energy dispersive spectroscopy (EDS). Attempts to incorporate polyoxometalates in the porous network of these materials are currently carried out in order to obtain novel composite materials to be evaluated as heterogeneous catalysts in oxidation reactions, using sustainable oxidants, hydrogen peroxide and oxygen.



**Figure 1.** Representation of the crystal structure of Cu-btc.[2]

## Acknowledgments:

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## Synthesis by MAOS of some chalcone derivatives

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Chalcones are naturally occurring flavonoids composed by two aromatic rings connected by a three-carbon chain containing an  $\alpha,\beta$ -unsaturated carbonyl group. They are pharmacologically relevant because of their ability to exert a wide range of biological activities, namely as antitumor, antioxidant, anti-inflammatory and antimicrobial [1,2]. Having such a variety of biological activities, these natural compounds have attracted the attention of medicinal chemists and therefore several classic and non-classic methodologies, have been developed for their syntheses. One of these methodologies is microwave-assisted organic synthesis (MAOS), which offers considerable advantages concerning substantial enhancements of rate of heating and of the reactions, compared with conventional heating. On this basis, we used MAOS to synthesize several chalcone derivatives by a base-catalyzed aldol reaction of appropriated substituted acetophenones with benzaldehydes. Structures were established by IR, <sup>1</sup>H and <sup>13</sup>C NMR techniques.

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[1] Go, M. L., Wu, X. and Liu, X. L. (2005), *Chalcones: An Update on Cytotoxic and Chemoprotective Properties*, *Curr. Med. Chem.*, 12, 483-499.

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# Synthesis of 2'-hydroxy-3,4,4',5,6'-pentamethoxy-3'-propylchalcone analogues with potential antitumor activity

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During our research work on the search for potential antitumor compounds, several chalcones were synthesized and evaluated for their inhibitory activity against the *in vitro* growth of several human tumor cell lines [1,2]. Particularly, a chalcone derivative, 2'-hydroxy-3,4,4',5,6'-pentamethoxy-3'-propylchalcone, revealed to have a potent growth inhibitory effect [1]. Additionally this chalcone interfered with the cell cycle distribution of MCF-7 cell line [1]. Taking this into account, several analogues of this chalcone were synthesized by a base-catalyzed aldol reaction of appropriated substituted acetophenones with benzaldehydes under microwave irradiation. The synthesized compounds were characterized by spectroscopic techniques (<sup>1</sup>H NMR, <sup>13</sup>C NMR, HSQC, HMBC). In the future all synthesized compounds will be evaluated for their ability to inhibit the *in vitro* growth of human tumor cell lines. For the most promising compounds further studies will also be carried out in order to clarify if the growth inhibitory effect is associated with cell cycle arrest and/or induction of apoptosis.

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[1] Neves, M. P., Cravo, S., Lima, R. T., Vasconcelos, M. H., Nascimento, M. S. J., Silva, A. M. S., Pinto, M., Cidade, H., Corrêa, A. G. (2012), *Solid-phase synthesis of 2'-hydroxychalcones. Effects on cell growth inhibition, cell cycle and apoptosis of human tumor cell lines*, *Bioorganic Med. Chem.*, 20, 25-33.

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## Synthesis of 3,4-oxygenated xanthone derivatives

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Xanthenes are a class of natural products which have been gathering much attention due to their interesting biological activities. They are capable of interacting with several different receptors being an interesting starting point for the discovery of new potential drug candidates [1]. The synthesis of functionalized xanthone derivatives allows the access to structures acting as building blocks, that otherwise could not be reached in order to get appropriated molecular modifications [2]. Oxygenated xanthenes showed to be potential candidates in a study involving the investigation of the inhibitory effect of natural and synthetic xanthenes on several human tumor cell lines [3] and interesting models for the development of new antitumor xanthone derivatives [4].

The aim of the present work was to scale-up the synthesis of an oxygenated xanthone, 3,4-dihydroxyxanthone, and to pursue the development of a library of analogues with diverse substituents patterns, for structure-activity relationships studies.

The synthetic strategies involved the condensation of an appropriated phenol with a substituted benzoyl chloride followed by cyclization of a benzophenone. Several derivatives with a 3,4-oxygenated pattern were obtained, namely, prenylated, methoxylated, hydroxylated, and brominated xanthenes. The structure elucidation of the xanthenes was achieved based on spectroscopic methods (IR and <sup>1</sup>H NMR).

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## Synthesis of alkylated derivatives of a bioactive natural flavone

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One of the main interests of CEQUIME-UP is the search of new pharmacologically active compounds from natural and synthetic origin, focusing on potential antitumor compounds. In this respect, several small-molecules have been evaluated for their activity as inhibitors of the growth of human tumor cell lines. From these studies several hit compounds have been emerged, particularly those with flavone scaffold [1]. Inspired by the potential of these flavones as antitumor agents, we have synthesized several alkylated analogues using a natural flavone as the starting material. The synthetic approach was based on the reaction with alkyl bromides in alkaline medium under microwave irradiation. The structure elucidation of synthesized compounds was established on the basis of NMR techniques (<sup>1</sup>H NMR, <sup>13</sup>C NMR, HSQC and HMBC).

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# Synthesis of New Proline-mimetics of PLG with Potencial Neuroprotective Activity

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Peptides biological importance is linked to its broad range of functions in living organisms acting as hormones, neurotransmitters or neuromodulators, with straight intervention on respiratory, reproductive and immunological systems.<sup>[1]</sup>

In recent decades, investigators deal with synthesizing structurally modified peptides in order to improve the stability and biological activity of these compounds to obtain new potential pharmaceuticals with improved pharmacokinetics and dynamics scores.<sup>[2]</sup>

This project aims the synthesis of new class of mimetic compounds for neuropeptide PLG (*L*-prolyl-*L*-leucyl-glycinamide) which perform important roles in central nervous system. Therefore, it is proposed the synthesis of new class of tripeptides structure- related to PLG containing proline-mimetics derivatives. This work might make an enormous contribution to the discovery of new potential therapeutic agents for the treatment of neurodegenerative diseases such as Alzheimer, Parkinson or Huntington.

Summary:

- Type I and II adducts synthesis, **P\*** (Fig. 1): The methodology in synthesizing this type of bicyclic, proline-mimetic precursors, is well known in our research group.<sup>[3,4]</sup>
- Synthesis of dipeptide III, *L*-valyl-*L*-alanine methyl ester, **VA** (Fig. 1) through coupling reaction with TBTU between Boc-*L*-valine and *L*-alanine methyl ester.
- P\*VA** tripeptides synthesis by coupling the dipeptide **VA** in b) with the corresponding proline-mimetics derivatives by the same methodology of condensation. Biological evaluation of the tripeptides obtained will be held at Faculty of Pharmacy, University of Santiago de Compostela, Spain.

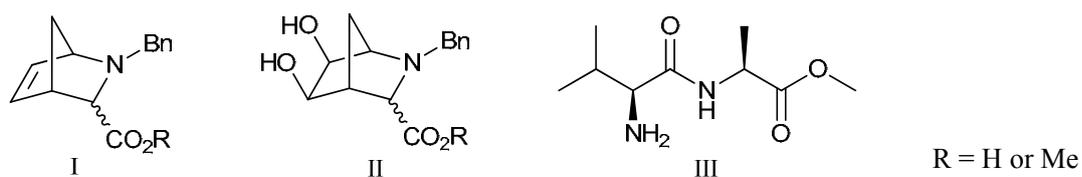


Figure 1 – Proline-mimetic precursors proposed (I and II) and the dipeptide **VA** (shown in III).

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- [1] Fletcher, M.D., Campbell, M.M. *Chem. Rev.*, **1998**, *98*, 763-795.
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# Synthesis of novel derivatives embedded with privileged benzopyran scaffold as potential MAO-B inhibitors

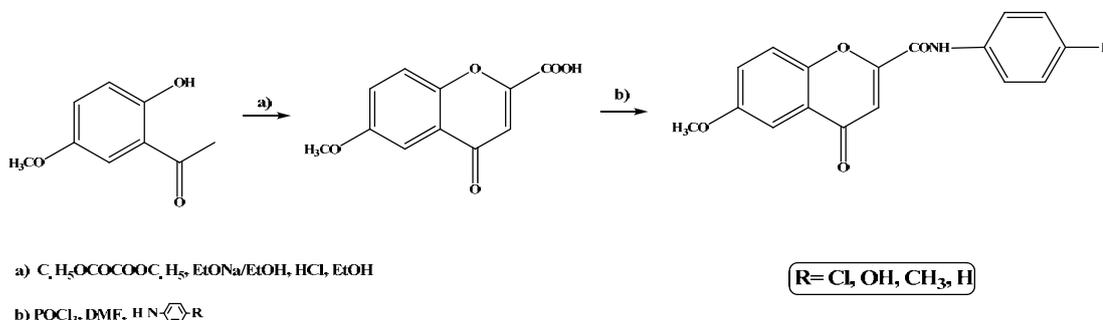
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Parkinson's disease (PD) is a progressive neurodegenerative disorder. Although some drugs are available to control the symptoms and/or slow down the progress of the disease, it remains incurable with no effective treatment. The main symptoms are tremors, bradykinesia, hypokinesia, postural instability and mobility problems. An evidence of PD is the decrease of the activity of dopamine, a neurotransmitter with a stimulating role in the Central Nervous System (CNS). The most common treatment is the use of levodopa (*L-Dopa*) even though with prolonged use complications [1].

Monoamine oxidase (MAO) is an enzyme present in mammals in two isoforms, MAO-A and MAO-B. The MAO-B isoform has a crucial role in neurotransmitters metabolism, representing an attractive drug target in the therapy of neurodegenerative diseases like PD. [2]

In this project new derivatives based on the chromone scaffold have been synthesized using an acetophenone derivative as starting material. The carboxylic acid of the chromone was synthesized through a Mannich reaction with a subsequent chromone amidation involving the carboxylic acid and an aniline derivative (Scheme1). The method involves the previous activation of the carboxylic acid through the coupling agent POCl<sub>3</sub> (phosphoryl trichloride). The activity of the potential MAO-B inhibitors will be evaluated for all the synthesized chromones.



Scheme 2. Synthetic strategy used to obtain the chromone derivatives

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# Synthesis of prenylchalcones with potential antitumor activity

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Chalcones represent an outstanding class of naturally occurring compounds with interesting biological activities, being the antitumor effect one of the most reported in the literature [1,2]. Among chalcones, prenylated derivatives have been attracting the attention of the scientific community because of their myriad biological activities [3,4]. In fact, it has been demonstrated that isoprenylation of chalcones significantly increased their growth inhibitory effect on human tumor cell lines [4]. Considering this, we decided to synthesize several prenylated chalcones. The synthetic approach for prenylation was based on the reaction of the chalcone building block with prenyl bromide in presence of tetrabutylammonium hydroxide at room temperature. The structure elucidation of synthesized compounds was established on the basis of NMR techniques. In the future all synthesized compounds will be evaluated for their ability to inhibit the *in vitro* growth of human tumor cell lines. For the most promising compounds further studies will also be carried out in order to clarify some of the molecular and cellular mechanisms involved in the growth inhibitory effect.

## References:

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# The effect of carbohydrates on the interaction between salivary proteins and condensed tannins

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Polyphenols (also known as polyphenolic compounds) are an important group of secondary metabolites widespread in plants with significant biological and sensory properties, contributing directly to color and flavor. Tannins, a complex group of polyphenols, are the primary source of astringency in foods and beverages reported to be astringent. Astringency is believed to be due to the interaction between tannins and salivary proteins, resulting in the formation of protein-tannin insoluble aggregates that precipitate in the mouth [1,2]. Several studies demonstrated the ability of some neutral and anionic carbohydrates to inhibit the binding of tannins to proteins. So, in this study, it was investigated the effect of different carbohydrates commonly used in food industry (pectin, arabic gum (AG) and polygalacturonic acid (PGA)) on the interaction between human salivary proteins and condensed tannins (grape seed fraction, GSF).

Human saliva was collected from volunteers, treated with 10% trifluoroacetic acid (TFA) and centrifuged (10500 rpm, 5 min). The supernatant (acidic saliva, AS) was analyzed by high-performance liquid chromatography (HPLC) before and after interaction with GSF. Stock solutions of each carbohydrate were prepared in water: pectin (2.5; 5.0; 7.5; 10.0 g.L<sup>-1</sup>), AG (5.0; 10.0; 20.0; 25.0 g.L<sup>-1</sup>) and PGA (10.0; 20.0; 30.0 g.L<sup>-1</sup>). The same volume of these stock solutions was added to GSF fraction to obtain a final volume of 50 µL. The mixture was shaken and kept at room temperature for 30 min. After this time, 150 µL of AS was added to this mixture (final volume, 200 µL) which was shaken and kept for 5 min at room temperature. Then, the mixture was centrifuged (10500 rpm, 5min) and the supernatant was analyzed by HPLC [3].

The results show that salivary proteins were affected by tannins in absence and presence of the different carbohydrates. It was also observed that all carbohydrates reduce the precipitation of salivary proteins induced by tannins, in especially for aPRPs and statherin proteins. This effect increases along with carbohydrate concentrations. Pectin was the most efficient carbohydrate in inhibiting salivary proteins precipitation while PGA demonstrated the lowest effect.

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## The Location of 2,4,6-Trichloroanisole in Cork Stoppers

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Cork is a natural, renewable and sustainable raw material. Cork has proved to be the most effective closure for wine, protecting wine quality and allowing wine to develop and improve over time.

One of the most important problems in wine world is cork taint. 2,4,6-trichloroanisole (TCA) (Figure 1) is often identified as a compound responsible for that taint, having a perception limit, in alcoholic solution, very low (1,5 - 3 ng/L). TCA can be originated from 2,4,6-trichlorophenol (TCP) produced from phenol and chlorine, but there are other causes that explain the presence of TCA in cork, namely the use of fungicides, biocides, herbicides and wood preservatives [1, 2].

To reduce TCA and compounds related in cork stoppers, the cork industry has adopted some measures: a) prevent contamination of cork by chlorinated compounds b) prevent microbial growth on the cork, and c) introduce corrective practices to avoid TCA contamination.

In present study, cork samples (cork stoppers normal, downgraded and cut) are submitted to a period of maceration in an alcoholic solution (12%), for 24h. The TCA released from cork stoppers to the alcoholic solution is then determined by solid phase micro-extraction - gas chromatography with mass detection (SPME-GC/MS). The TCA concentrations found on surface of corks stoppers normal and downgraded showed significant differences.

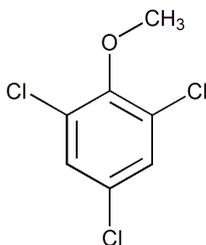


Figure 1. 2,4,6-Trichloroanisole (TCA)

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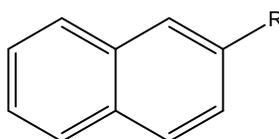
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# Thermodynamic study of three 2-halogenated naphthalenes

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Persistent Organic Pollutants (POPs) are organic compounds which are resistant to environmental degradation through chemical, biological or even photolytic processes.<sup>1</sup> Halogenated naphthalenes are a very important group of POPs, mainly due to their high toxicity as well as their significant impact on human health and the surrounding environment. In order to evaluate the behavior of these environmental contaminants, the knowledge of thermodynamic properties of these compounds is essential. A static apparatus based on an MKS capacitance diaphragm manometer<sup>2</sup> was used to measure the vapor pressures of the condensed phases of 2-chloronaphthalene, 2-bromonaphthalene and 2-iodonaphthalene, in the ranges (280.4 to 384.4) K, (291.3 to 380.5) K and (302.3 to 363.7) K, respectively. The results obtained for each compound, enabled the determination of the standard molar enthalpies, entropies and Gibbs energies of sublimation and of vaporization, at  $T = 298.15$  K, as well as phase diagram representations of the  $(p,T)$  experimental data. The gas phase standard entropies of the compounds were calculated using computational methods, enabling the determination of the standard entropy of their condensed phases at 298.15 K.



R - Cl, Br, I

**Fig. 1.** Chemical structure of 2-halogenated naphthalenes.

Gaseous phase heat capacity of all derivatives were estimated by statistical thermodynamics calculation based in a combinations of the experimental and quantum chemical calculations vibrations frequencies at the B3LYP/6-311++g(d,p) level of theory, using the methodology described previously in the literature.<sup>3</sup>

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# Utilization of CdTe-MPA quantum dots as fluorescence sensors for the determination of iron in biodiesel

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The determination of trace elements in biodiesel is essential to assess the oil qualities, identify some adulteration and prevent undesirable effects in environment and in humans. However, due to its low concentration in samples, sensitive instrumental techniques are required. In this work an effective and simple quantification method using water-soluble mercaptopropionic acid (MPA)-capped CdTe quantum dots (QDs) was for the first time implemented for the fluorescence quantification of iron in biodiesel synthesized from different vegetable oils and fat. The developed methodology was based on iron facility to establish surface interactions that result in quenched nanocrystals fluorescence intensity, proportional to iron concentration. Size and concentration of QDs, concentration and pH of the buffer solution showed a strong effect on the quenching efficiency influencing linear working range and sensitivity of the methodology. An ultrasonic bath was used for the extraction of iron from oil samples with a mixture of 1:1 (v/v) concentrated HCl and H<sub>2</sub>O<sub>2</sub>. The extraction efficiency was approximately 100 % after 50 min of ultrasound. For comparison, the samples were also analyzed using an High-Resolution Continuum Source Flame Atomic Absorption Spectrometry (HR-CS FAAS). Under the optimized experimental conditions, a linear working range was obtained for iron concentrations from 6 to 100  $\mu\text{g L}^{-1}$  ( $r = 0.9991$ ,  $n=6$ ). The determined detection limit (LOD) was about 1.24  $\mu\text{g L}^{-1}$ . Six biodiesel samples were analyzed by the proposed methodology, and the results revealed good agreement with those obtained through a HR-CS GFAAS comparison procedure. Relative deviations lower than 3.1% were achieved, and the application of a paired Student's t-test, confirmed the absence of any statistical difference for a confidence level of 95% ( $n = 5$ ). Recoveries varied from 90% to 105%. The slopes of the analytical curves obtained with different matrices showed that the quantification of the analytes was not influenced by matrix effects. The quenching mechanism of CdTe QDs is discussed. The accuracy, precision and operational simplicity, without additional electronic devices, demonstrated for this method can be an excellent alternative for determination of iron in biodiesel samples.

## Acknowledgments

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# Planctomycetes as a food source for *Daphnia magna*: preliminary findings

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*Daphnia magna* is widely used as a standard organism in ecotoxicology assays, as its life cycle is well known. It plays a key role in the energy transfer in freshwater food webs as a primary consumer (filter feeder), grazing on microalgae, yeast and bacteria [1]. *Daphnia* are commonly reared in the lab using microalgal cultures as food source; alternative or complementary sources of carbon are important to reduce the dependency on a single food source [2]. The potential of planctomycetes as a nutritional source is still unknown. So, in this work we intended to evaluate the potential of the planctomycete *Rhodopirellula* sp. Strain LF2 as nutritional source of *Daphnia magna*. This is a microorganism isolated from the microbial community of marine microalgae and it is easy to rear in the laboratory in large quantities [3].

Two life history assays were conducted with *Daphnia magna*, following a bifactorial design: daphniids were fed with three different concentrations (1/1000; 10/1000; 100/1000; v/v) of *Rhodopirellula* sp. Strain LF2 at two different growth phases (exponential and stationary). These treatments were compared with the standard microalgae-fed *Daphnia* (control treatment consisting of *D. magna* individuals fed with *Pseudokirchneriella subcapitata*, a green microalga). Assays were checked daily for mortality and reproduction along 21 days [1].

Preliminary results showed high mortality in the lowest concentration of *Rhodopirellula* sp. Strain LF2 in both growth phases. All concentrations tested revealed some mortality and *D. magna* was overall unable to produce offsprings when fed with *Rhodopirellula* sp. Strain LF2. Only in the highest concentration of the stationary phase *D. magna* was able to reproduce in a single reproductive event. Results also show that the somatic growth rate was reduced in all concentrations for both phases. Facing these results, we infer that *Rhodopirellula* sp. Strain LF2 is a poor nutritional source for *D. magna* as the sole source of carbon under the conditions tested; however future research will focus on its use as a complement in microalgae-reared daphniids.

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## Polymerase chain reaction as a tool for diet studies of crustaceans

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In this work we used genetic tools to study the diet of crustaceans, specifically the shore crab *Carcinus maenas* Linnaeus 1758 and the brown shrimp *Crangon crangon* Linnaeus 1758. Both crustaceans are common epibenthic species in European shallow coastal waters, including estuaries, from as far north as Norway to Morocco in the south limit. Crabs' native distribution extends further south to Mauritania; yet this species is highly invasive and is now present almost worldwide. Due to their generally high abundance, these crustaceans play a relevant role in the ecosystem functioning both as preys and predators. However, because they grind the preys into pieces, hardly any remain can be identified in the stomachs. Therefore, other indirect analysis, such as the genetic studies of stomach contents, can be a useful tool for diet investigations.

We started by doing an extensive literature review on diet studies of these species and on genetic studies of their preys, followed by field sampling to collect the biological material (*C. crangon*, *C. maenas* and their prey). Since both species are opportunistic predators, preying upon 67 different species, we decided to restrict the studies to common potential preys in Minho estuary (the common goby *Pomatoschistus microps* (Krøyer, 1838) and the ragworm *Nereis diversicolor* (O.F. Müller, 1776)), and to two commercially important species, the flounder *Platichthys flesus* (Linnaeus, 1758) and the peppery furrow shell *Scrobicularia plana* (da Costa, 1778).

The laboratory work consisted first on sample sorting, preservation in ethanol 80% and biometry. For a total of 389 shrimps and 127 crabs, the stomach was removed, classified according to its fullness (0 for empty; 1 for up to half full; and 2 for full), and its content roughly inspected and preserved in ethanol 80%. Then, the DNA extraction (PureLink™ Genomic DNA, Invitrogen) was conducted to prey samples (positive control) and to stomach content samples. It was followed by PCR amplification with specific primers for each prey (already developed), electrophoresis in agarose gel, and data treatment.

Preliminary results allowed us to confirm the most appropriate methods to detect the potential prey (positive controls), which were then applied to stomach content samples. We were able to confirm the presence of *P. microps* and of *N. diversicolor* in the stomachs of both crustaceans. *S. plana* was not present in any of the analyzed stomachs and the presence of *P. flesus* has not been tested yet. We conclude that this technique can be useful to study the crustaceans' diets as long as the potential preys are known by previous analysis of stomach contents.

## Polymorphisms that might explain the variation in *Drosophila americana* lifespan

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In recent years, genetic research has been putting a great deal of effort in the study of phenotypical effects of specific mutations as a way of identifying genes with interesting characteristics. In *Drosophila melanogaster*, genes which enhance the flies' longevity by decreasing or increasing their activities have been described. One of these genes, the *methuselah* (*mth*), is essential for normal development in *Drosophila* [1] and is related with phenotypes such as enhanced resistance to various forms of stress and increased lifespan [2]. Associations between naturally occurring *mth* nucleotide polymorphisms and lifespan differences have been shown in *D. melanogaster* [3] but it is still unclear whether this contributes to genetic variance for lifespan in natural populations, therefore contributing to lifespan evolution [4].

The *mth* gene encodes a putative G protein-coupled receptor, which plays a central role in signal transduction and is activated by a diverse array of ligands. It was only identified in insects, and in *D. melanogaster* it has paralogs called *mth*-like genes. To show whether polymorphism at *mth*-like genes account for the observed variation in lifespan differences in natural populations, we used *D. americana*, a species distantly related to *D. melanogaster*. The two species have been diverging for about 40 million years [5], and thus there is no *mth* orthologous gene in *D. americana*, but there are genes encoding proteins with typical Mth features (despite having less than 50% of homology with the *mth* gene) (unpublished results).

In this study, we identified a relationship between two *D. americana* *mth*-like genes (*GJ12490* and *GJ23561*) and longevity. We also identify amino acid differences that could explain the observed relationship.

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# Protective mechanism of xanthohumol against genotoxicity of heat generated compounds: PhIP and MeIQx in HepG2 cells

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An important class of heat generated compounds from the diet are heterocyclic aromatic amines (HAAs) classified by IARC as a dietary risk factor for human cancer [1]. Deleterious effects from HAAs can be minimized by the action of specific antioxidant compounds from diet.

Beer is rich in antioxidant compounds, including that xanthohumol (XN), a hop derived prenylflavonoid, characterized as a potential “broad-spectrum” chemo-preventive agent, very efficiently protects against genotoxicity and potential carcinogenicity of the HA 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) [1]. However, studies on other HAAs, namely, the most abundant HAAs found in the diet, the 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-3,8 dimethylimidazo[4,5-f]quinoxaline (MeIQx) are scarce [2].

In the present study we evaluated PhIP and MeIQx genotoxicity in the human hepatoma cell line, HepG2, analyzing the induction of DNA strand breaks, with the alkaline comet assay. In addition, changes in gene expression of the main enzymes involved in HAAs metabolism were determined, using quantitative real-time PCR.

In HepG2 cells XN completely prevented PhIP and MeIQx induced DNA strand breaks at nanomolar concentrations. With the QRT-PCR gene expression analysis of the main enzymes involved in the biotransformation of HAAs in HepG2 cells we found that XN up-regulates the expression of phase I (CYP1A1 and CYP1A2) and phase II (UGT1A1) enzymes. However, gene expression analysis in cells exposed to MeIQx and PhIP in combination with XN revealed that XN mediated up-regulation of UGT1A1 expression may be important mechanism of XN mediated protection against HAAs induced genotoxicity.

Results obtained confirm the evidence that XN displays strong chemopreventive effects against genotoxicity of heat generated compounds, and shed light for the first time the modulator effect of XN on the expression of genes involved in HAAs biotransformation.

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# Red Fox (*Vulpes vulpes*) in Portugal

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The Red Fox, *Vulpes vulpes*, is one of the most widely distributed Canidae (Mammalia, Carnivora). It occurs naturally over most of the northern hemisphere because they are medium-size omnivore animals, extremely adaptable, and capable to inhabit a very wide range of habitats [1, 2]. In Portugal the Red Fox is considered a common species, dispersed all over the country, but information on the species abundance is still scarce [3].

The purpose of our project is to understand the distribution and abundance of the Red Fox. Our data source is the hunter community, as in some hunting zones they are obliged to announce all fox battues for each hunting season. This fact enables us to estimate the hunting effort and gives us access to the hunting bag. Hunters are also our source of fox carcasses, allowing us to characterize the biometrics (weight, height, length of body and tail, ear and hind paw, maximum head perimeter and width, chest perimeter), to determinate reproduction parameters (sex ratio and fecundity) [2] and to estimate the age of the Portuguese population [4].

The results obtained show that more than 60 % of the Municipal Hunting Zones organize fox battues, but so far, and for a number of different reasons (bad weather, no hunters, economic crises, etc.), only 14% of the planned battues until the present date took place; in those battues hunter efficiency seems low. However the major hunting effort is still unapprised as most fox battues are planned to take place during January and February. Until now 15 carcasses were obtained but most were not still analysed; sexual dimorphism seems confirmed in spite of the scarcity of the available data.

The available results are still preliminary at the moment. The planned battues follow the same trend of previous hunting seasons. At the present we detect a small reduction of the number of battues planned but a very important decline of those that effectively took place, when comparing our data with those of the 2009/10 hunting season [3]. Data on the fox population characteristics is still insufficient for any meaningful analysis. We hope however that data accumulated till the end of the hunting season will allow a better understanding of the fox population in Portugal.

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## Screening of antimicrobial activity of bacteria isolated from the marine sponge *Erylus deficiens* collected in São Miguel, Açores

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With the emerging microbial antibiotic resistance and the need to search for new effective drugs, marine sponges became organisms of biological interest. Their associated microorganisms have a great potential for producing unique bioactive compounds, which can have medical applications. To test the bioactivity of bacterial associates of the marine sponge *Erylus deficiens*, the production of antimicrobial substances was screened and showed that 50 % of the isolates were active against at least one of the target organisms (*Bacillus subtilis*, *Escherichia coli*, *Vibrio anguillarum* and *Candida albicans*). The presence of genes that might be associated with the production of bioactive metabolites (polyketide synthase and non-ribosomal peptide synthase genes) was also detected. In both assays, *Firmicutes* were the bacteria with higher bioactivity. The results also suggest that other genes might be involved in the expression of antimicrobial activity. This study combined traditional and molecular approaches in order to characterize the microbial isolates that might be useful for finding new bioactive metabolites.

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# Screening of NRPS and PKS genes of bacteria associated with marine sponges *Erylus cf. deficiens*

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Nonribosomal peptide synthetases (NRPS) and type I polyketide synthases (PKS-I) are biosynthetic systems involved in the synthesis of secondary metabolites produced by microorganisms [1]. These metabolites are known for their wide-ranging biological activities. Aiming to obtain information on the potential production of bioactive compounds by a collection of bacteria isolated from three specimens of the marine sponge *Erylus cf. deficiens* from the Gorringe Ridge (Atlantic Ocean), a screening of NRPS and PKS genes was performed. A total of 115 bacterial strains, 19 of the sponge #66, 57 of the #91 and 39 of #118 were studied. For PKS-I PCR amplification, primers MDPQQRf and HGTGTr [2] were used and for NRPS, primers DKF and MTR [3] were used. We obtained 63.16% (12 bacteria) putative strains containing the PKS-I gene and 15.8% (3 bacteria) containing the NRPS gene in the #66 collection, confirming previous bioactive positive results in co-culture diffusion bioassays. From #91 and #118 bacterial collections a much lower presence of these genes was detected (1.75% (1 bacteria) PKS and 5.26% (3 bacteria) NRPS for #91 and 5.13% (2 bacteria) PKS e 0% NRPS for #118). The main genus that amplified for the PKS and the NRPS was the *Pseudoalteromonas* sp. belonging to the phylum *Gammaproteobacteria*. Further work on PCR optimization will allow confirmation of the results.

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## Seasonal variation in the energy content of the brown shrimp *Crangon crangon* from Minho Estuary

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The brown shrimp *Crangon crangon* (Linnaeus, 1758) is a very common epibenthic species in the European shallow coast from Norway to Morocco and in the Mediterranean and Black Seas. Due to its high abundance the species is a very important component in the ecosystem's trophic web, both as prey and predator. During its short life (1.5 to 3 years), it experiences periods of food abundance such as during warmer periods (spring and summer) when the system is more productive, as well as periods of food shortage like during winter. As a consequence of this seasonal variation in food availability shrimps might be able to store energy reserves during warm seasons which might be used later to pay for body maintenance costs during colder periods.

Although reproduction usually occurs throughout the year, two distinct peaks can be recognized, the first one in the winter and the second one in the summer. Since females carry the eggs till larvae are released which takes about 1.5 to 2 months, energy allocation in reproduction must vary between sexes. As such, energy reserves vary not only according to the season but also with sex and age. Yet, in the case of the brown shrimp life cycle, the winter reproductive season is also a period of food scarcity when reserves might be at a minimum. Therefore, it would be very interesting to understand the species' energy allocation along an annual cycle separately for males and females which invest differently in reproduction.

In the present study, the energy reserves of *C. crangon* Minho population were studied during one year. Samples were collected monthly since January till December 2012 with a beam trawl at 4 sites within Minho estuary. Besides sex determination, the biometry of the analyzed shrimps included the total length, the wet and dry weight determination. Then, for each month, pools of shrimps per sex and 5mm length classes were randomly selected and macerated for the calorimetry analysis which will take place at NIOZ, Netherlands. At the moment, samples up to November 2012 are ready for the calorimetry and hence only preliminary results on population dynamics can be presented and discussed.

# Seasonal variations in the lipids content of the brown shrimp *Crangon crangon* from Minho estuary, Portugal

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The brown shrimp, *Crangon crangon*, is a crustacean found in sandy and silty bottoms of estuaries and bays in the northeastern Atlantic and as such in the Minho estuary. This crustacean features a gray body, transparent and without frontal antennules; it reaches a maximum length of 8 cm. This species has two or more reproduction peaks, normally between February and June [1]. The brown shrimp is an opportunistic predator feeding on small animals, sediments and algae. However, during its life cycle, food availability and quality varies according with the system's productivity [2]. Therefore, the species might suffer periods of starvation like during winter when prey are scarce [3]. In these periods they have to rely on energetic reserves to pay for maintenance costs. Since lipids are the body compounds with higher energy, this project's goal is to study changes in the lipids' content of brown shrimp along an annual cycle.

Monthly biological (*C. crangon*) and abiotic (water temperature, salinity, oxygen concentration, pH and chlorophyll) samples will be obtained in four sampling stations (three replicates per location) within the Minho estuary, starting in January 2013. Those samples will be submitted to a sorting process in the laboratory. The shrimps will be measured, weighed and identified by gender. In the following step, the shrimps will be dried (48h at 60°C in an oven) and its dry weight registered. For each month a set of ten males and ten females per 5mm length class will be randomly separated for later lipids' analysis using a Spinreact<sup>®</sup> kit.

In this project it is expected that the brown shrimp lipids' content will follow the system's productivity. In general, estuarine productivity is highest in the warmer seasons, spring and summer. Therefore it is expected that fat will be accumulated and thus its percentage will also be highest in these seasons. However these are also reproductive periods and hence the energy investment in reproduction might differ between sexes.

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## Spatial distribution of free-roaming cats (*Felis silvestris catus*) in urban environment (Porto, Portugal)

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Free-roaming cats include the domestic ones (not confined to a yard or house) [1] and those who have no owners and who are accidentally/deliberately provided with food, commonly known as feral cats; these latter can be further distinguished as semiferal or strays, if socialized toward humans [2]. In an urban habitat, directly influenced by humans controlling the distribution/availability of resources, cats usually have access to clumped food resources (garbage cans/feeding sites) and typically live in groups. These groups are not casual aggregations but defined social groups that may differ in size and social structure. With this study we aim (1) to understand free-roaming cats spatial distribution and its determining factors, (2) to estimate the cat population size in an urban area as well as the number and constitution of cat feeding groups. This knowledge is fundamental for the control of feral cat groups and for the management of the public health problems associated.

A small area, 0,942 km<sup>2</sup> within Porto city limits was chosen for our study. Weekly observations during the day (morning, afternoon, night) began in October 2012. For each cat/group observed the time of the day, location, sex, state of development and their behaviour is registered; all cats are also photographed to build a cat database. Environmental data (feeding stations, garbage cans and shelter) are also registered.

Until now 53 different free-roaming cats were identified, showing a wide distribution in the study area. Our density estimate (47 cats/km<sup>2</sup>) is smaller than referred to another city zone [3], but the number of colonies found (6 colonies/km<sup>2</sup>) is similar. Both estimates are however more than the double of those possible with animal welfare NGO's data on cats for the city of Oporto. Cat colony location is intimately related to cat lovers feeding spots and, in a smaller degree, to garbage cans; cat presence tends to be near residential areas and not in nearby scrublands. Cat movements (in and out of the study area) are probably limited by major urban artificial barriers, (eg, major traffic roads and train/metro lines).

The results must be considered preliminary, but they will allow a better knowledge of the urban cat population in the city and we hope they will also allow, among other aspects, to appraise the success of an on-going neutering program that is being implemented in one of the colonies.

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# Symbiotic interaction between prokaryotes and eukaryotes

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Symbiosis is a widespread phenomenon evident within wide environments and among different lineages. Marine ecosystems are particularly prone for studies of cooperative interactions among organisms of different species. Ancient basal metazoans, sea sponges are characteristic examples of symbioses as their sessile nature prompted them to filter feed the dissolved particles in water. During long evolutionary events, sponge host procured some microbial partners for the nutritional as well as defensive benefits. In this work, we employed microbiology and molecular biology techniques to identify the heterotrophic bacterial communities in the most common intertidal marine sponge, *Hymeniacidon perlevis* sampled from the Atlantic coast of Portugal. The culture dependent techniques followed by 16S rRNA gene analysis revealed the presence of two types of sponge associated heterotrophic bacteria: *Pseudovibrio* sp. and *Shewanella* sp. These two genera have been reported before for being linked with the sponge survival capacity and for the production of molecules such as nitrogen, key production for sponge survival, or for helping in the elimination of possibly toxic molecules such as halogenated organic products. Bioinformatics tools were employed to identify the species of bacteria belonging to previous referred genera and to construct a phylogenetic tree to understand the relation between the previously reported bacteria and the ones now found. Our results show that the bacteria species found are related to the ones reported as symbionts before. The current study will be a foundation for future work with more emphasis on searching for more sponge associated bacteria and novel pharmaceutical compounds.



## Take a look at *Sphaerococcus coronopifolius* Stackhouse (Rhodophyta)

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Natural products play an important role in the discovery of bioactive molecules. Although oceans cover two-thirds of the earth surface, possessing a great variety of organisms, marine products are still underexploited. Nevertheless, in the last years it has been noticed a growing interest on marine organisms as source of compounds with pharmacological and economical potential. Many bioactive molecules have been detected in red algae (Rhodophyta), but concerning *Sphaerococcus coronopifolius* Stackhouse the knowledge is scarce. As so, in this work we used an ethanol extract prepared from this species to approach its metabolic profile and biological activity.

HPLC-DAD analysis (Fig. 1) revealed the presence of carotenoids, lutein being identified. Cholesterol and palmitic acid were characterized by GC-MS. The presence of alkaloids in this alga was not confirmed.

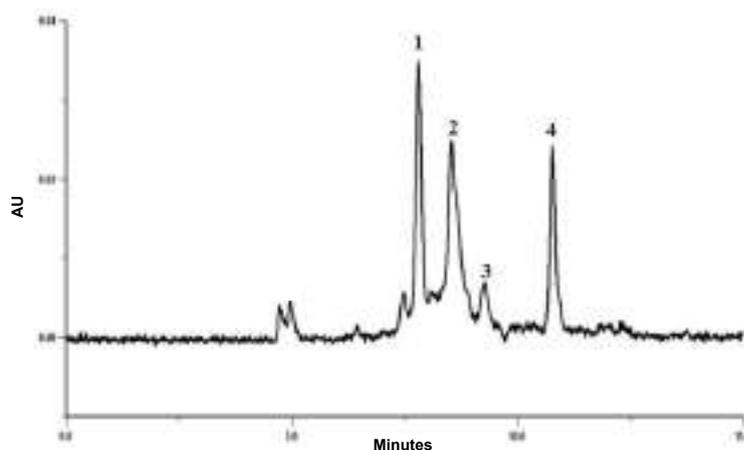


Fig. 1. HPLC-DAD profile of *S. coronopifolius* ethanolic extract. Detection at 450 nm. Compounds: 1-3, unidentified carotenoids; 4, lutein.

The antioxidant capacity was checked against 1,1-diphenyl-2-picrylhydrazyl radical (DPPH<sup>•</sup>) and nitric oxide and antibacterial activity was assessed against selected Gram positive and Gram negative species. Nevertheless, no protective effect was observed. It would be interesting to test other extracts from this alga to ascertain its biological potential and also to improve the knowledge on its carotenoids composition.

This work was developed within the optional curricular unit “**Bioactivity of Natural Matrices**” of the **5<sup>th</sup> year of the Master Degree in Pharmaceutical Sciences of the Faculty of Pharmacy**, University of Porto, under the responsibility of Paula Andrade (Head), Patrícia Valentão and Carla Sousa.

# **Tardigrades as descriptors of forest fire impact: Assessment of survival capability under experimental conditions (preliminary results)**

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Forest fires are an important destructive factor in the terrestrial ecosystems with severe consequences on biodiversity loss. Portugal, where large areas are burnt each year, is a country particularly affected by this problem. Over the years, our knowledge about the impact of forest fire has increased considerably. Actually, faunal soil succession after fire is a relatively well studied topic. However, the majority of the studies is fragmented or deals with macroscopic species which is a significant weakness for the elaboration of conservation strategies and management plans. For many other zoological groups, tardigrades included, effects of habitat loss are under evaluated. Tardigrades are a group of microscopic animals (body length about 500 µm) very common on terrestrial ecosystems, namely in the litter, mosses and lichens. They have interesting abilities to survive in extreme environmental conditions, resisting in a criptobiotic state, by instance, to high temperatures and low humidity levels. So their potential use as descriptors of forest fire impact seems to be very promising. With this final purpose, we have started a series of experiments aiming to understand the response of tardigrade communities to fire. We want to detect eventual changes on diversity, population structure and dynamics. In particular, in this work, we aim to assess the survival capabilities of a cryptogam tardigrade community submitted to a fire simulation.

To accomplish this objective, mosses and lichens were collected from 2 sampling sites in Serra do Gerês (Site 1 – 8°7'52''W, 41°47'42''N; Site 2 – 8°7'54''W, 41°47'44''N). For each site, five sets of 3 sub-samples weighting 1g each were submitted to different temperatures. One of these sets, the control, was kept at air temperature. The remaining four sets were burnt, in a muffler respectively at 150° C, 190° C, 225° C and 300° C. Then, tardigrades were extracted, counted and mounted on slides in Hoyer's medium for further identification to the species level. Measurements, given in micrometers (µm), and photomicrographs were made using a Zeiss Axioskop Phase Contrast Microscope (100x oil immersion) equipped with digital camera and using Axiovision 4.7 Imaging System Software.

The results showed that although a significant decrease in the rate of survival of tardigrades with increasing temperature ( $F=37.32$ ;  $p<0.01$ ;  $d.f.=4$ ) they were able to survive at temperatures higher than 225° C. On the other hand, small sized specimens were more resistant to higher temperatures when compared with larger specimens.

The amount of data obtained in the aim of this study is still scarce and further studies are needed. However, the preliminary results suggest that tardigrades are able to survive to forest fires and, therefore, they can be the first colonizers of burnt ecosystems.

# The Black-billed Magpie, *Pica pica*, vocal communication

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The Corvidae family (Order Passeriformes) includes a number of successful birds with a wide geographic distribution [1]. Four species are present in the northwest part of Portuguese territory (Black-billed Magpie, Eurasian Jay, Carrion Crow and Raven) [2, 3]. Being birds that live in socially complex groups, an elaborate system of vocalisation skills system was developed to allow communication. This tool (with varying expressions between individuals, populations age and sex) has a major role as bird songs and sound are fundamental breeding success, territory defense and to communicate predator detection or of food resources [4].

The present study main aim is to contribute to the catalogue of wild Black-billed Magpie vocalizations, but other Corvid as the Eurasian Jay (*Garrulus glandarius*), the Carrion Crow (*Corvus corone*) and Raven (*Corvus corax*) songs and sounds were also recorded. The Oporto City Park is our main study site, but several other locations at Pinheiros, Mazedo and Ceivães parishes (Monção municipality) were also visited. Vocalizations were registered in magnetic support with a Marantz PMD 222 cassette recorder using a Sennheiser directional microphone. Sonograms were obtained with Avisoft software.

Field trips began in October 2012 and have allowed registering several Magpies, Eurasian Jay and Carrion Crow songs and sounds. Black-billed Magpie most common/know vocalizations is the characteristic chatter-call (shrak-ak-ak-ak-ak) [1, 5]. Several other calls, alarm calls and some songs were also registered; they are being analyzed in the available software. Our recording effort is at the beginning so only preliminary results are available at the moment.

We hope however to be able to give an important contribution to Black-billed Magpie vocal catalogue and we will try to understand the context in which vocalizations are used in and if eventually we can find any inter-individual or inter-population differences in the structure of Black-billed Magpie vocalizations.

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## The invasive bivalve *Corbicula fluminea*: role and importance as a food resource for native species

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*Corbicula fluminea* (Müller, 1774) is a freshwater bivalve originated from Asia to become an invasive species of aquatic ecosystems widespread worldwide [1]. This species was introduced in Portugal in the mid-eighties and it has invaded Minho River since then. Here the species finds a suitable habitat as revealed by the high densities reported in last years [2]. Therefore *C. fluminea* Minho population seems to be settled and well adapted already for some time. Interactions with native species however might have negative impacts due to competition, but can also be profitable [1]. Despite its hard shell which avoids predation, dead *C. fluminea* can be available for predators particularly during periods of drought in warm dry summers when a large part of the population might die off like in Minho 2005 [2].

In this project, we intend to determine whether Minho native species are able to feed on this bivalve. In order to accomplish this, an experimental set up has been established. Potential consumers to use in the trials were selected due to their high abundances in Minho River: the crustaceans *Carcinus maenas* (Linnaeus, 1758), *Crangon crangon* (Linnaeus, 1758), and the teleosts *Pomatoschistus microps* (Krøyer, 1838), *Platichthys flesus* (Linnaeus, 1758), and *Anguilla anguilla* (Linnaeus, 1758). For this experiment specimens were measured and placed individually in a closed circulation aquaria system kept at controlled room temperature (mean water temperature 14°C). The animals were fed every other day with one of four prey species – *C. fluminea*, *Mytilus* sp., *P. microps* and *C. crangon*. The offering order was randomly chosen for each animal to avoid any influence from the feeding sequence. The predators' behavior towards the food piece was observed when the food was offered, 10min and 2h after.

Up to now, only two predators have been tested: *C. maenas* and *P. microps*. Preliminary results show that both species adapt well to feeding in aquaria conditions. The crustacean is very voracious and eats all the offered prey species, with no indication of any preference. Some of the gobies did not eat in one or more observations. However 98% of the crabs and 80% of the gobies ate *C. fluminea*. This suggests that the invasive species can be a food source for at least these two species. This study will be continued with observations on the other potential consumers enunciated and it is expected that data altogether will give some indications on which of these native species are able to feed on the invasive bivalve in the areas where their distribution overlaps.

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## The nutritional condition of the population of *Pomatoschistus microps* and *P. minutus* in Minho Estuary

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Two species of gobies, *Pomatoschistus microps* and *P. minutus*, live sympatrically in European shallow coastal areas and have a very important role in the balance of the estuarine system, like in other similar areas. Both are an important food source for birds and larger fish species, and are also considered great opportunistic predators. Adults feed at the surface of the sediment on amphipods, isopods, polychaetes, and chironomid larvae, while the juveniles' diet consists largely of interstitial copepods. These two aspects of gobies' biology suggest us their major role in the food chain of this environmental system.

During their short life cycle, food availability will vary according to the system's productivity. In periods of prey abundance like during warming seasons, gobies might have more than enough food and thus might store energy reserves. These can be consumed later when food is scarce like during autumn and winter. Few studies however were dedicated to the nutritional status of the gobies populations and no information exists on the fluctuations of the energy content of these two species.

In the present project, specimens of *P. microps* and *P. minutus* were collected monthly since September 2012 in 3 different locations within Minho Estuary with the aim of studying seasonal changes in their caloric content along an annual cycle. The areas chosen to sample were selected considering their characteristics; it was chosen an area with greater salt water influence (near the river mouth), other located further upstream in the river and one intermediate. These three different areas are probably also difference in prey abundance and quality due to their differential habitat conditions.

After collected, fishes are frozen for later examination. Then in the laboratory, fish were identified, measured (total length, mm), weighted (wet weight, g), eviscerated for sex determination and dried. A pool of animals of the same sex and per 5mm length class collected in the same month will be selected for later calorimetric analysis.

Presently, only part of the September 2012 sample is under analysis. The first step of the work – species identification – revealed to be requiring a detailed attention because the two species are very similar and frequently misidentified. Therefore, an exhaustive list of morphological features was made. Then it was tested which features were better to use in a routine species' identification using the sample from September. In the future we expect to obtain an evaluation of the changes in the energy content of the two species along an entire year. This might elucidate on the nutritional status of their populations and highlight periods when the animals may be under food deprivation and hence more vulnerable to environmental effects like climate change.

# Toxicological Assessment of Marine Cyanobacterial Extracts in Human Cell Lines – Proteomic and Gene Expression Approach

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Cyanobacteria are known to synthesize secondary metabolites that may have technological applications and are potential drugs to the treatment of different human diseases including cancer. [1] At the Laboratory of Ecotoxicology Genomics and Evolution – CIIMAR, Porto we are screening for biological activity in cyanobacteria isolated from the Portuguese coast, and maintained in culture. A recent result demonstrated growth inhibition of human tumor cells exposed to crude extracts in two marine cyanobacterial strains. [2] In line with this result there is a need of further characterization of the molecular events responsible for the bioactivity reported. This information will help to understand the mode of action of cyanobacterial secondary metabolites and their potential pharmacological applications.

In order to accomplish this goal research project has started to investigate the bioactivity of secondary metabolites from the cyanobacterial strains LEGE06113 and LEGE06155, in the human cell lines HepG2 (liver), T47D (breast), RKO (colon) and fibroblasts (control) using molecular biology and proteomic analysis. After culture of the human cell lines cytotoxic assays are performed with the cyanobacterial extracts. The main tools employed in the analysis are real-time PCR to analyze gene expression and also two-dimensional gel electrophoresis for proteins expression. [3]

Cyanobacterial cultures have been performed and the bioactive compounds extracted from the lyophilized biomasses. Moreover in vitro cultures of the human cell lines have been established. Conditions were also optimized for the analysis of the human cell's proteome. Patterns of proteins with isoelectric points between 4-7 and molecular masses of 20-80 kDa have been separated with high resolution in polyacrylamide gels (2DE).

With the conclusion of the toxicity assays and the proteomics and genomics analyses we expect to identify key biochemical pathways in human cells by the action of cyanobacterial extracts and insights into the molecular mechanisms leading to growth inhibition in human carcinoma cells.

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# Validation of a new method to study in situ growth rates of crustaceans

**J. Pereira<sup>1,2</sup>, P. Loureiro<sup>1,2</sup>, I. Vaz<sup>1,2</sup>, S. Mendes<sup>1,2</sup>, C. Moreira<sup>1</sup>, and J. Campos<sup>1</sup>**

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Despite the economic and ecological importance of the brown shrimp *Crangon crangon* (L.), available studies do not clarify entirely its growth and reproduction cycle. As crustaceans, shrimps grow by moulting or ecdysis, in which the animal absorbs water causing an increase on its size, and the release of the exuvia, and with it also a great percentage of the mineral compounds is discharged. The mineralization of the new exuvia starts right after the ecdysis. In this way the water percentage will increase drastically during the moulting process while the dry weight percentage will drop. A new method to study in situ growth rates of crustaceans was then based on this assumption: beyond a certain dry weight or water percentage the animal has to moult or has moulted recently [1]. This enables to assess a threshold separating moulting from intermoulting shrimps when comparing the dry weight (or water) percentage of shrimps collected in the field.

To test this method, shrimp samples were captured monthly from Minho estuary (North of Portugal) with a 1m beam trawl starting in September 2012. Shrimps were then measured, weighed (wet weight, WW) and individually placed in bottles, which were placed in a larger container with water at about 18°C. Other samples from four locations throughout the estuary were frozen and later analyzed the same way for comparison. The shrimps were observed for any moult occurred in the next few days (up to 3 days). When the moult occurred, the new exuvia's condition after moulting (soft, paper-firm, hard), and the shrimp's length and weight were recorded. After this period about 10% at least of the shrimps moulted. All individuals were then sacrificed (frozen) and equally measured and weighted (WW). Sex was determined by observation of the appendices (endopodite) of the first two pairs of pleopods. The dry weight of all shrimps (moulted and non moulted) as well as the wet and the dry weight of the exuvias were determined. Using these parameters (shrimp's and moult's dry weight, shrimp's size and the new exuvia's condition after moulting) we expect to find a relation between the size, dry weight percentage and moulting state for this particular geographical area, that can be applied to the shrimps caught and frozen to estimate the fraction of the population which was most contributing to growth..

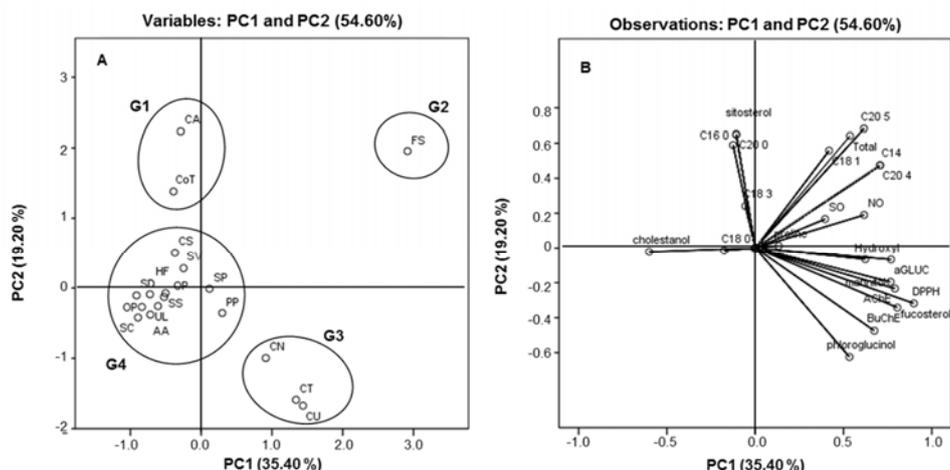
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## Valuable compounds in macroalgae extracts

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Bioactive compounds present in ethanolic extracts from 18 macroalgae of the Portuguese coast were analysed by gas chromatography–mass spectrometry (GC–MS), leading to the characterization of 14 compounds: proline, phloroglucinol, mannitol, 8 fatty acids and 3 sterols [1]. A dose-dependent response against enzymes with biological significance ( $\alpha$ -glucosidase, acetylcholinesterase and butyrylcholinesterase) and free radicals (DPPH, nitric oxide, superoxide and hydroxyl) was found, Phaeophyta being the most promising group [1]. A PCA analysis was performed and allowed the establishment of a correlation between the algae chemical composition and the biological activity (Fig. 1). *Cystoseira tamariscifolia* (Hudson) Papenfuss, *Cystoseira nodicaulis* (Withering) M. Roberts, *Cystoseira usneoides* (Linnaeus) M. Roberts and *Fucus spiralis* Linnaeus are among the most active species, which is in accordance with their higher contents in phloroglucinol, mannitol, oleic, arachidonic and eicosapentaenoic acids, and fucosterol.



**Fig. 1.** Projection of the studied macroalgae (A) and loadings (B) by chemical composition and bioactivities, into the plane composed by the principal components PC1 and PC2 containing 54.60% of the total variance.

The results point to the potential interest of the use of Phaeophyta species as food additives, due to their potent antiradical activities, and especially highlights the importance of *F. spiralis* in the food chain of Mediterranean countries. Moreover, the incorporation of the extracts of these species in food products, nutraceutical and pharmaceutical preparations for human health should also be instigated, since they can suppress hyperglycemia and inhibit cholinesterases [1].

### Acknowledgements:

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## ***Xantho incisus* Leach: crab power**

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Natural matrices are a valuable source in drug discovery, great merit being attributed to plants. Considering the diversity of species, Oceans are extremely rich, being the object of exploitation in the past few years for their chemical composition. *Xantho incisus* Leach is a crab species found in the Portuguese coast, feeding on algae. The objective of this study is to establish the relationship between the chemical composition and the biological activity of *X. incisus* using an ethanolic extract.

There were identified two carotenoids (astaxanthin and lutein) by HPLC-DAD (Fig. 1). By GC-MS were characterized three compounds: two fatty acids (oleic and palmitic acids) and one sterol (cholesterol).

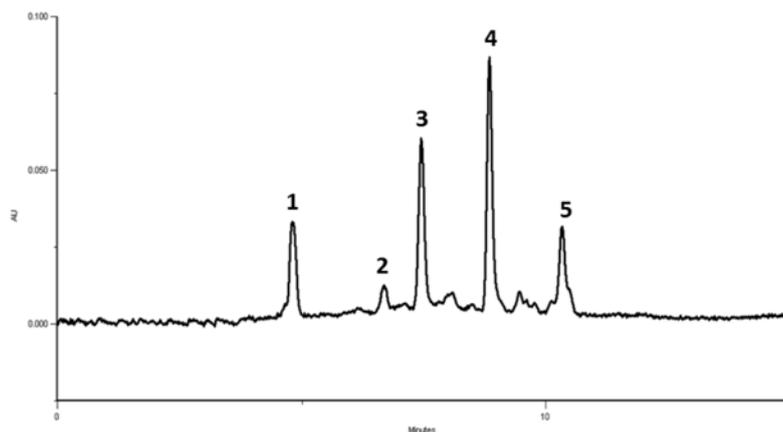


Fig. 1. HPLC-DAD chromatogram of the ethanol extract of *X. incisus* (450 nm). (1) unidentified; (2) and (3), unidentified carotenoids; (4) astaxanthin; (5) lutein.

It was also evaluated the antioxidant activity against both 1,1-diphenyl-2-picrylhydrazyl radical (DPPH<sup>•</sup>) and a reactive nitrogen species (nitric oxide) and the antimicrobial activity. In the DPPH<sup>•</sup> assay it was not observed any radical reduction. The extract revealed some activity against nitric oxide. In the tested range concentrations, it showed no antimicrobial capacity against the four bacteria species used (*Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium* and *Escherichia coli*).

This research suggests that *X. incisus* might be a source of biologically active compounds and a quite promising raw material for the pharmaceutical industry.

This work was developed within the optional curricular unit “**Bioactivity of Natural Matrices**” of the **5<sup>th</sup> year of the Master Degree in Pharmaceutical Sciences of the Faculty of Pharmacy**, University of Porto, under the responsibility of Paula Andrade (Head), Patrícia Valentão and Carla Sousa.



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