

# BOOK OF ABSTRACTS

8<sup>TH</sup> MEETING OF YOUNG RESEARCHERS  
OF UNIVERSITY OF PORTO







**ENCONTRO INVESTIGAÇÃO JOVEM  
DA UNIVERSIDADE DO PORTO**

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# CREDITS

LIVRO DE RESUMOS IJUP'14

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UNIVERSIDADE DO PORTO

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## FOREWORD

A nova equipa reitoral da Universidade do Porto assume como compromisso envolver, cada vez com maior intensidade, os estudantes dos diferentes ciclos em atividades de investigação. Queremos promover de forma decisiva a formação de jovens abertos à descoberta científica, com capacidade para produzir conhecimento e motivados para comunicar, debater e aplicar os resultados das suas pesquisas. Por outro lado, somos sensíveis à necessidade de apoiar e valorizar os investigadores juniores da Universidade do Porto, de modo a consolidar as suas carreiras emergentes na ciência.

Com os elevados méritos que se lhe reconhecem, o IJUP – Investigação Jovem na Universidade do Porto parece-nos ser um instrumento eficaz para cumprir este nosso compromisso com a promoção da ciência entre os estudantes e com a valorização da atividade dos investigadores juniores. Isto porque o evento é, para a maioria dos participantes, uma espécie de tirocínio na apresentação pública de trabalhos de investigação, proporcionando-lhes assim uma experiência assaz enriquecedora ao nível da comunicação, divulgação e debate científico.

Os participantes no IJUP têm a oportunidade de apresentar publicamente os seus trabalhos científicos num contexto semelhante ao de um congresso internacional, o que os obriga a comunicarem em inglês e a serem claros, assertivos e concisos, mas também criativos na exposição dos resultados das suas investigações. Acresce que, no IJUP, os jovens participantes são sujeitos ao escrutínio científico quer dos seus pares, quer de professores e investigadores seniores, daqui resultando um debate interdisciplinar tendencialmente enriquecedor para os estudos apresentados.

Por tudo isto, a nova equipa reitoral está empenhada na realização do IJUP e fará os possíveis para que, ano após ano, o evento consiga reunir mais jovens investigadores e sirva de montra a investigações com crescente qualidade científica, dimensão internacional e pertinência socioeconómica. Estamos conscientes da importância do IJUP para a descoberta de novos talentos na ciência e para o ingresso na carreira científica de mais jovens qualificados, condição decisiva para a renovação geracional da investigação no nosso país. Como sabemos, o corpo de investigadores portugueses está um pouco envelhecido, o que não só levanta problemas de produtividade, como dificulta a transmissão de conhecimentos entre gerações de cientistas. Importa, pois, injetar “sangue novo” na ciência portuguesa.

Resta-me tão-só louvar a realização desta 8.ª edição do IJUP, sem esquecer que se trata de uma iniciativa criada pela anterior Vice-Reitoria para a I&D e cuja concretização se deve, em boa medida, a trabalho voluntário, realizado com escassos meios e a custos reduzidos. É justo enaltecer também as empresas e instituições que colaboram com o IJUP’15, destacando em particular o apoio do Santander Universities.

Por fim, impõe-se um agradecimento muito especial aos jovens que, certamente eivados de entusiasmo e energia, participam nesta edição do IJUP. É nosso desejo que a curiosidade científica destes jovens não esmoreça no futuro e que, com as suas descobertas, contribuam para o desenvolvimento da investigação em Portugal.

Sebastião Feyo de Azevedo

Reitor da Universidade do Porto



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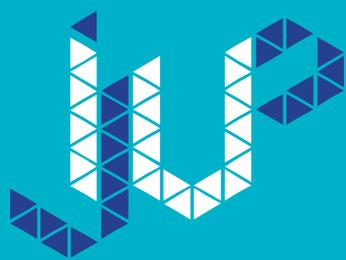
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**PARALLEL  
ORAL  
SESSIONS I**



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**A1** **ENGINEERING I**  
PARALLEL ORAL SESSIONS I

# Graphical user interface development for analysis of press bending operations

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The linear press bending of sheet metals is one of the most common industrial forming operations. This process consists of forming a blank in a  $V$  shape by using a punch to press the metal sheet into an open die. Despite a simple geometric interpretation, the control of final geometry is complex due to the interaction of different variables, either from the process or from the geometry.

The analysis of press bending operations is usually performed through analytic expressions, a fundamental one being the relation between punch penetration and sheet bending angle. An alternative to analytic equations is the use of numerical methods, e.g. finite element method, in which wider possibilities of results are available.

In this contribution an integration of analytical and numerical analysis is developed through a graphical user interface (GUI). This development opens the possibility of analyzing the press bending process by combining the efficiency of quick results from analytical expressions with the better accuracy and additional results of numerical simulation. Input variables include the material, its thickness, die opening, die radius, punch radius and the desired bending angle ( $\alpha$ ). Main results include optimized relations for tool variables, besides punch penetration, inside bending radius, forces, pressures and springback.

Additionally in this contribution some studies are included in order to compare analytic and numeric methodologies using different materials (steel and aluminium), thicknesses and different tool relations. Results are also validated by comparison with experiments.

Acknowledgements: The authors would like to acknowledge the support of FCT – Fundação para a Ciência e a Tecnologia through the project PTDC/EMS-TEC/2404/2012.

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# A Return-mapping Algorithm for an Elastic Damage Model in Concrete Materials using the Radial Point Interpolation Meshless Method

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## Abstract

An advanced discretization meshless technique, the radial point interpolation method (RPIM), is applied to analyse concrete structures using an elastic damage model. The theoretical basis of the material, model and the computational treatment are presented. The elastic model is extended with a damage formulation where both compressive and tensile damage states are based on Helmholtz free energy function connected to the damage energy release. Within the return mapping damage algorithm, the plane stress formulation is considered to obtain the required variable fields, such as the damage and the displacement fields. The enforcement of boundary conditions is guaranteed for both compressive and tensile states. This work uses the Newton-Raphson nonlinear solution algorithm to obtain the non-linear damage solution. The validation and performance of the proposed model is demonstrated by numerical relevant benchmarks available in the literature.

**Keywords.** Damage Mechanics, Return-mapping Algorithm, Concrete Materials, Meshless Method, RPIM.

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# Determination of the hardening curve of dual-phase steels using hydraulic bulge test

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In order to obtain highly reliable results from numerical simulations of sheet metal forming processes, the accurate mechanical characterization is one of the important factors.

Usually, the method used to obtain the mechanical properties is the standard uniaxial tensile test. This test provides significant properties of the materials, but the maximum value of plastic strain obtained is limited when compared to the real levels of forming operations. To overcome this problem it is necessary to extrapolate the hardening curve [1], which using the results from uniaxial tensile test will affect the reliability of the flow stress curve prediction, thus leading to inaccuracies.

In this paper it is used an approach to achieve a better extrapolation of hardening curve of sheet materials, based on uniaxial tensile test data combined with hydraulic bulge test results thus covering a larger strain range compared to commonly used tensile test.

Since the two flow curves have different strain paths, they can't be directly compared. Therefore it is necessary a transformation [2,3] of flow stress curve provided from biaxial bulge test into effective stress-strain curve and after this transformation it is possible to combine and extrapolate the data obtained from uniaxial tensile test.

Different methodologies were applied to transform the obtained flow curve from biaxial bulge test into hardening curve expressed in terms of effective stress-strain. The combination of the tensile test data and results from the bulge test proved to be an approach with excellent results in the prediction of material behaviour for higher plastic strains.

Aknowledgements: The authors would like to acknowledge the support of FCT – Fundação para a Ciência e a Tecnologia through the project PTDC/EMS-TEC/2404/2012.

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# Determination of biaxial flow curve for sheet metal materials using hydraulic bulge test

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The hydraulic bulge test has been considered an efficient method to investigate the flow curve under biaxial state of stress. This state of stress allows higher maximum plastic strain when compared with the uniaxial tensile test [1]. Bulge test, when properly instrumented, provides relevant data for important parameters used by most current constitutive equations [2]. This paper presents a comparative study between different numerical methodologies to determine fundamental bulge variables such as radius of curvature and thickness reduction at the pole as a function of the pole height [3-5]. Additionally, a different methodology is suggested which represents not only an increase of accuracy on the relationship between thickness reduction and pole height but also allows to obtain biaxial flow stress curve until the breakage point of the test. In order to compare and validate these different methodologies, experimental tests were performed using the aluminum alloys AA5754-T4 and AA6061-T6 as well as the steel alloys DP590 and DQ.

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# ASA (Aerial Supervisor for Android): a Situation Awareness Tool for Drone Pilots

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Unmanned Aerial Vehicles (or drones) are undeniably having their Tech Boom as newer, better vehicles are released every week. Costs for these vehicles have been taken down mostly because needed components are now being mass-produced and it is possible to print some of them at home [1]. This has led to the emergence of large groups of DIY (Do It Yourself) drone enthusiasts [2].

At Porto University, our lab has been developing and testing different drone generations mixing commercially off-the-shelf components with in-house electronics and autonomy software. During UAV operations, typically three user roles are needed: an operator (that designs single-vehicle plans and monitors their execution from a laptop-based console), a supervisor (that ensures viability and safety of the operations) and a pilot (that validates vehicle health, and does manual control during takeoff and landing).

Considering the stressful environment that is operating these vehicles and the short reaction time needed to avoid collisions or other dangerous situations, the objective for ASA is to improve the situational awareness of UAVs pilots by providing real-time access to UAV information during all phases of the operation.

We considered two modes of operation that may change several times during a single flight: **Manual** and **Auto** Mode.

- In **Manual**, the pilot fully controls the UAV and must be aware of its Speed and Altitude while not deviating his eyes from the drone. For this mode we use vibration feedback to ensure the pilot that there is a communication link with the vehicle (vibration stops if the link is gone) and we use Audio feedback to provide periodic information of the vehicle's altitude and speed.
- In **Auto** mode, the pilot only needs to monitor the status of the UAV while it is executing its autonomous plan. In this mode, we use visual elements to show the status of the global mission and information on the main vehicle. A map shows the vehicles' locations and their orientation, the plans being executed and an altitude bar with measured and desired altitudes. In this mode the pilot can also access a video feed coming from a camera mounted on the UAV.

The validation and testing of this project was done iteratively between requirements analysis, development, simulation and field-testing. The Interface was continuously changed/adapted/improved by request and critic of its users (refinement of requirements). In order to provide a scientific method of measuring the impact of this application in the operations, pilots were subjected to NASA TLX [3] enquiries after both flights without and with the application. By doing this we verified that the pilot's stress levels were reduced and the awareness increased quite significantly with newer versions as initially predicted.

References:

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[2] DIY Drones website - <http://diydrones.com/>

[3] NASA's TLX test - <http://humansystems.arc.nasa.gov/groups/tlx/>



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**A2** **CHEMISTRY I**  
PARALLEL ORAL SESSIONS I

# Application of gas-diffusion microextraction for determination of aldehydes in cork based products

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Emission of volatile organic compounds (VOC), particularly aldehydes such as formaldehyde and acetaldehyde from cork and wood based products, is a well-known problem [1] that can affect the air quality of indoor environments and human health. Thermal treatments, the use of resins and other industrial processes may lead to the appearance of volatile compounds. Cork products have been vastly used as building materials due to its singular characteristics, such as thermal and sound insulation and for being a renewable and environmental friendly resource.

Gas-diffusion microextraction (GDME) was initially developed for the extraction of volatile compounds from liquid samples, particularly beverages [2]. However, more recently, was successfully applied to solid samples [3]. The GDME device consists in a small dimension Teflon module, with a microporous hydrophobic membrane at its bottom that allows the diffusion of volatile compounds from the sample to an acceptor solution. The acceptor solution is, in many cases, the derivatization reagent, which helps improve the extraction efficiency and allows the detection of the analyte by high performance liquid chromatography with UV detection.

In this work, the main objective was to determine the amount of aldehydes, such as formaldehyde, in cork based agglomerates using the GDME methodology. Two different derivatization reagents were used as acceptor solution in this study: 2,4-Dinitrophenylhydrazine, which reacts with both aldehydes and ketones, and the acetylacetone reagent.

Using the GDME approach we were able to identify several aldehydes. The results showed significant differences between the samples studied and a possible correlation between the results and cork based products industrial manufacturing could be made. Similar formaldehyde concentrations were obtained using the two different derivatization methods and levels lower than 9.0 mg/kg were found in the analysed products.

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## **Influence of production system and plant age in olive oil lipophilic antioxidants**

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Vitamin E and carotenoids, namely  $\beta$ -carotene and lutein, belong to the group of lipophilic antioxidants present in olive oil. These compounds are nutritionally important and contribute to the oxidative stability of olive oils. Therefore, it is important to determine the factors that might influence the content of these antioxidants in extra virgin olive oils.

In the present study, the effect of olive trees age and type of production system (organic/conventional) on the content of these lipophilic compounds was evaluated. For this purpose, extra virgin olive oils from centenarian olive trees (n = 27), conventional (n = 22), and organic production (n = 21) were analyzed, all from the 2013/2014 crop season. Vitamin E was quantified by normal phase chromatography and fluorescence detection while  $\beta$ -carotene and lutein were evaluated by reverse phase chromatography and diode-array detection.

Olive oil from centenarian olive trees had higher amounts of total vitamin E (323 mg/kg) than conventional (262 mg/kg) and organic production (287 mg/kg). In opposition, carotenoids were present in higher concentration in the group from conventional production system, with 6.7 mg/kg vs. 5.2 mg/kg for lutein, and 4.4 mg/kg vs 3.6 mg /kg of  $\beta$ -carotene, for conventional and centenarian, respectively. The organic samples had 6.4 mg/kg of lutein and 3.6 mg/kg of  $\beta$ -carotene, being lower than the conventional group but higher than the centenarian one.

The highest concentration of vitamin E found in olive oils from centenarian olive trees may be related to the trees age, naturally producing higher amounts of secondary metabolites to counterpart the biological stress induced by senescence. However, it does not seem to influence the content in carotenoids similarly. Also, the highest amount of vitamin E in olive oil from biological production may be related with the production system, where soil fertility is ensured by natural processes without chemical fertilization. To validate these differences, it would be interesting to analyze olive oil from the same cultivar, under the same edaphoclimatic conditions, and through different years.

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# New analytical methodology based on QuEChERS followed by GC-MS to determine siloxanes in personal care products

**D. Capela, L. Santos and V. Homem**

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Siloxanes are organic compounds used in a wide variety of personal care products, such as shampoos, hair conditioners, lotions, etc. Chemically, they consist of a structural unit of alternating Si-O bond linked into a cyclic or linear way, with organic side chains [1]. They are usually incorporated in toiletries as antifoaming and conditioning agents, film formers and emollients. These compounds have sparked a growing interest in the scientific community since some laboratory studies in animals have shown that siloxanes may have toxic effects [1]. In fact, these compounds have already been detected in biological matrices such as human blood and breast milk [2]. Due to their ubiquitous occurrence, bioaccumulation and toxic potential, siloxanes are compounds of concern for human health, mainly through dermal exposure by toiletries application. Although several studies have been conducted to determine siloxanes in environmental and human compartments, few have focused on their determination in personal care products. Therefore, the aim of this study was to develop and validate a methodology based on quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction followed by GC-MS for the analysis of 8 siloxanes in different toiletries.

The selected methodology uses hexane as extraction solvent and two QuEChERS, one containing MgSO<sub>4</sub> and NaCH<sub>3</sub>COO to promote phase separation and other containing a mixture of MgSO<sub>4</sub>, PSA bonded silica and C18 to remove undesired components. Chromatographic analyses were performed by a Varian Ion Trap GC-MS system.

Personal care products were divided into different categories according to their overall composition (moisturizers, hair care products, body wash, shaving products, dentifrices, deodorants, perfumes and toilet soaps). Low detection limits, high average recoveries (>80%) and precision (RSD <15%) were determined. In general, higher levels of siloxanes were detected in shampoos and hair conditioners. Cyclic siloxanes were detected more frequently in the analyzed products.

This work was funded by FEDER funds through the Operational Programme for Competitiveness Factors – COMPETE, ON.2 - O Novo Norte - North Portugal Regional Operational Programme and National Funds through FCT - Foundation for Science and Technology under the projects: PEst-C/EQB/UI0511, NORTE-07-0124-FEDER-000025 - RL2\_ Environment & Health. Vera Homem would also like to thank FCT for the post-doctoral grant SFRH/BPD/76974/2011 co-funded by QREN-POPH.

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# Screen-printed electrodes for the detection of pharmaceutical compounds

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Screen-printed electrodes (SPEs) have gone through significant improvements over the past few decades. Intensive research activity has been focused on the development of miniaturized electrochemical sensors intended for extracellular fluid measurement, clinical diagnosis and micro total analysis [1]. In this context, the use of the screen-printing technology in the serial production of SPEs for the electrochemical detection of a wide range of substances is currently undergoing widespread growth [2]. Screen-printing techniques offer high-volume production of inexpensive, highly reproducible and reliable sensors, providing precise control over the SPEs dimensions, excellent uniformity, high reproducibility and the potential for mass production. Different substrates can be employed in the production of SPEs, resulting in highly versatile devices and the extensive range of potential modifications opens numerous fields of applications [3]. Particularly, the use of these electrodes on the analytical detection of pharmaceutical compounds in various samples can provide important advantages, such as no extensive sample processing, low detection limits, simplicity, low cost, portability and potential for miniaturization [1]. The development of electrochemical sensors based on SPEs for pharmaceutical analysis has received massive consideration since they enable the rapid screening of the pharmaceutical compounds in complex matrixes, such as biological fluids and pharmaceutical formulations, requiring small volumes of samples and no pre-treatment steps.

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# Development and optimization of printed electrochemical biosensors for microbial testing

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A biosensor is a chemical sensing device incorporating a biological recognition element (eg. an enzyme or an antibody), coupled to a transducer, which is able to detect quantitatively a specific biochemical analyte [1, 2]. Electrochemical sensors are one the most common classes of biosensors [3].

Nowadays there are several techniques for detection of microorganisms being divided in two groups, conventional methods and rapid microbiological methods [4]. In this context, the main aim of this work is the development of low cost printed electrochemical biosensors for the detection of small amounts of microorganisms, quickly and efficiently. For this study, the baker's yeast, *Saccharomyces cerevisiae*, was selected as a microorganism to be detected. This yeast has a wide industrial application, especially in baking, fermentation of alcoholic beverages and production of ethanol for biofuels.

Electrochemical sensors with three electrodes were fabricated by screen printing. After, the working electrode was electrically and electrochemically characterized, to evaluate its electrochemical behavior and to determine its sheet resistance. Additionally, the thickness and roughness of the electrode were also evaluated, by surface profilometry.

In a second phase, working electrodes were modified with cobalt phthalocyanine (CoPC), an electron mediator to allow electrochemical detection of H<sub>2</sub>O<sub>2</sub>. Currently, a further step of the electrode modification, the addition of the enzyme alcohol oxidase, is being studied. This enzyme catalyzes the reaction of primary alcohols with oxygen, being H<sub>2</sub>O<sub>2</sub> one of the final products. In future, the biosensors to detect yeast will be tested, by detecting the ethanol produced by yeast in the presence of glucose [5].

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## Biosorption of fluoroquinolones by activated sludge and aerobic granules sludge

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Antibiotic residues have been detected in various environmental matrices, such as surface water and even drinking water. Although present at low levels ( $\mu\text{g/L}$ ,  $\text{ng/L}$ ), many antibiotics are bioaccumulative, pseudo-persistent and can promote resistance/alterations in bacterial populations [1]. Recent studies on antibiotics removal by activated sludge (AS) and aerobic granules (AGS) show biosorption as the dominant process, determining the fate of these micropollutants [2-3].

In this work the biosorption of three widely used fluoroquinolones (FQ) - ofloxacin (OFL), norfloxacin (NOR) and ciprofloxacin (CPF) - to AS and AGS was evaluated. A High Performance Liquid Chromatography with Fluorescence Detection (HPLC-FD) method was validated and used to follow the biosorption of the target FQ.

Data obtained in this study contribute to a better comprehension of FQ biosorption behavior in AS and AGS. At pH 7 AS showed better performance to biosorb OFL, NOR and CPF than AGS. The higher biosorption capacity of AS was probably due to the negative charge on its surface, evaluated by a zeta potential of  $-25.65$  mV, at pH 7. OFL was the less biosorbed, both onto AS and AGS, because this FQ at pH 7 is mainly present in its anionic form. The equilibrium data for AS showed a better fit to the Langmuir model, while the model that presented better fit for AGS was the Freundlich model. The FQ could be desorbed from AGS at pH 3, pH 8 and pH 9 whereas at pH 4 the biosorption process was promoted.

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ENCONTRO INVESTIGAÇÃO JOVEM  
DA UNIVERSIDADE DO PORTO

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**A3** **BIOLOGICAL SCIENCES I**  
PARALLEL ORAL SESSIONS I

# A functional metagenomics approach in the identification of novel ultraviolet resistance genes

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Culture independent techniques have recently unveiled information on the resistance mechanisms of uncultured organisms, correcting our biased understanding of Earth's biodiversity [1]. Although extreme ultraviolet (UV) radiation is harmful to life, causing direct and indirect damage to cells, microorganisms have been found thriving under UV-light [2-3]. This has triggered the curiosity of scientists on how life has evolved to adapt and resist to such conditions and whether life can exist beyond Earth [4-5]. Metagenomic libraries of Ojo Seco (OS), Diamante (D) and Mallorca (PMB) were constructed (Table 1). The libraries were functionally screened for resistance to UV-B and to UV-C allowing the identification of novel genes involved in UV-resistance.

Library name	No. of Recombinants	Average insert size (kb)	Library size (Mb)
OS	285.000	1,9	541,5
D	170.000	1,0	170
PMB	236.250	2.9	685

Table1. Characteristics of the constructed metagenomic libraries.

A transcription factor (pML\_56) and three hypothetical proteins (pML\_6, pML\_105 and pML\_84) were found to confer resistance to UV-light. The known gene *recA* was also represented on several resistant clones, confirming its involvement on UV-resistance, which is known to be mainly within the SOS response. To elucidate the mechanism of resistance, a 4-nitroquinoline 1-Oxide treatment of UV-resistant as well as the co-localization of the expressed protein fused with Green Fluorescent Protein (GFP) is being performed. Characterization of the genes together with protein function assignment is expected to contribute to the knowledge of molecular adaptations and metabolic pathways involved in UV-resistance mechanisms of microorganisms.

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# Characterization of *Pollicipes pollicipes* cement protein genes

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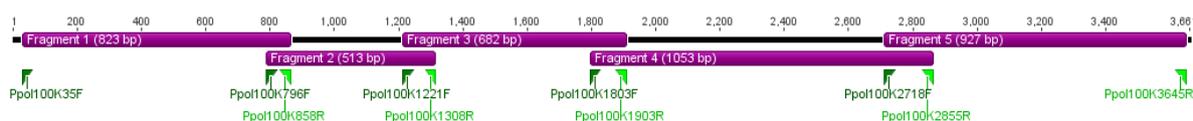
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*Pollicipes pollicipes* (Gmelin, 1789) is a goose barnacle (Crustacea: Pedunculata) abundant on exposed rocky shores [1]. They are important fouling organisms that adhere to underwater surfaces through singular cement proteins [2]. However, it is not fully understood how the mechanisms of fouling and adhesion work. Moreover, the sequence and function of most *P. pollicipes* genes remain unknown. Our aim was to characterize the cement protein genes (e.g., CP-19K, -20K, -52K and -100K) and others involved in the attachment (e. g., SIPC) of *P. pollicipes*. The long-term objective of this work is to provide the necessary data to be used on antifouling studies and induction of settlement, with possible industrial applications.

Firstly, we downloaded all cDNA sequences of target genes from *P. pollicipes* and closely related species available in public databases. The sequences were aligned in order to design primers in the conserved regions. *P. pollicipes* samples were collected in Northern Portugal and used to extract DNA and isolate RNA (for cDNA synthesis).

The target regions were amplified by PCR and the complete CP-19K, -52K and -100K cDNA sequences of *P. pollicipes* were sequenced. The cDNA sequences had some differences relative to available *P. pollicipes* EST's. Most notably, we detected several erroneous insertions and deletions in available EST's that were not compatible with the translation of a functional protein. We have also detected four heterozygotic positions across the CP-100K sequence, one of them resulting in an amino acid change. The amplification of CP-19K and of the 5' CP-100K target region (fragment 1 in Fig. 1) was not achieved in genomic DNA, which may indicate the presence of an intron.



**Figure 1** – Schematic representation of CP-100K cDNA. The primers are represented by green arrows and the purple bars correspond to the overlapping regions amplified by PCR.

These results provide useful data to understand the evolution of cement protein genes, as the polymorphic positions identified may be useful for phylogenetic and population studies. Furthermore, it will allow the design of gene expression studies at different *P. pollicipes* developmental stages and tissues, in which it will also be possible to test different compounds with potential to induce/inhibit larval settlement.

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# Conjugative Transfer of AmpC beta-lactamase gene in *Enterobacteriaceae*

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Plasmidic AmpC-producing *Enterobacteriaceae* clinical isolates have been increasing around the world. AmpC type  $\beta$ -lactamases (Ambler's molecular class C and group 1 of the Bush-Jacoby-Medeiros functional classification) are a diverse group of enzymes of broad substrate specificity that can degrade penicillins and most cephalosporins. Several genes are involved in the production of these enzymes, namely LAT, CMY, MOX, FOX, ACC, DHA, that may be located on plasmids<sup>[1,2]</sup>.

The aim of this study was the standardization of AmpC gene transfer technique in *Enterobacteriaceae* producing plasmidic AmpC enzymes, by conjugation.

We analysed pre-selected AmpC producing *Enterobacteriaceae* clinical isolates, according to the hospital information. Antimicrobial susceptibility tests were performed by disk diffusion methods according to the CLSI guidelines in order to confirm AmpC resistance phenotype. Confirmation was done by PCR amplification using primers for different relevant AmpC types.

The evaluation of the transfer ability of AmpC encoding gene was performed by conjugation using two different methods: in liquid medium (Trypticase Soy Broth - TSB) and in solid medium (Müller Hinton Agar – MHA) in order to promote mating and transfer of gene in conjugative plasmid, obtaining transconjugants expressing AmpC beta-lactamase. *Escherichia coli* HB101 a lactose non-fermenter and streptomycin resistant laboratory strain, was used as a receptor in the conjugation experiments.

Transconjugants were obtained in both methods. AmpC gene transfer was confirmed in transconjugants by expression of AmpC resistance phenotype and PCR amplification.

The two different conjugation methods tested in our study are adequate for conjugation assay with AmpC producing *Enterobacteriaceae* isolates in order to confirm plasmidic location of AmpC gene when present in conjugative plasmids. In the future, these methods will be used in AmpC-producing *Enterobacteriaceae* isolated from Brazilian mastitic milk in order to confirm plasmidic origin of AmpC genes.

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# A new method for the detection of genetically modified plants using multiplex PCR

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Genetically modified organisms (GMOs) are those who have undergone changes in their genetic structure by means of a biotechnological process of recombination, which allows the introduction of new features in a species [1]. The most recently launched GMOs on the world market are plants that have different characteristics such as resistance to herbicides or insects. The number of cultivated GMOs has been growing in recent years despite the intense discussion about the benefits or damage that these organisms may have on humans and ecosystems [2,3]. For this reason, there is great interest in developing effective methods for the identification of GMOs in different stages of the chain of cultivation, processing and distribution [4]. This work describes the development of a rapid molecular application to detect genetically modified (GM) plants at low cost. The new multiplex PCR assay allows the detection of six transgenic sequences (P-35S, T-nos, bar, ctp2-cp4epsps, P35S-pat and FMV35S) used in most GM plant events and a chloroplast DNA (cpDNA) trnL gene as internal control. The seven target regions were designed to yield amplicons with different lengths to be discriminated by electrophoresis. Our method successfully detected the presence of transgenic elements in 19 samples of reference materials from GM plants. The seven target regions were easily discriminated both by conventional and capillary electrophoresis. The use of target regions of small length (<270 bp) allows the analysis of forensic samples with degraded DNA, as the detection of illegal GMO crops or to verify the labelling of food products.

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# Identification of transgenic fishes using multiplex PCR

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Increasing consumer demand for fish products and the resulting overfishing is leading to the saturation of the fish production through traditional aquaculture. This problem has prompted the construction of transgenic or genetically modified (GM) fishes (using “all-fish” constructs) with foreign DNA artificially inserted into their genomes, in particular the growth hormone (GH) gene used to increase growth and feed conversion efficiency. In recent years, GH-transgenic strains have been developed in a number of species, including common carp (*Cyprinus carpio*)[1], tilapia (*Oreochromis niloticus*)[2] or Atlantic salmon (*Salmo salar*)[3], among others. Biotechnology companies are getting closer to commercializing transgenic fish engineered to grow more rapidly, although strict regulatory approvals are expected, particularly in the European Union (EU) market. An important part of the EU regulatory process is the use of efficient molecular methods capable of detecting the foreign DNA used to make a GM species[4]. However, no DNA-based method for the simultaneous detection of different transgenic fishes is currently available. Here we describe the development of a molecular kit for the identification of the transgenic fishes most likely to be on the market in the near future. The method is based on multiplex polymerase chain reaction (PCR) for the detection of the transgenic elements (i.e., promoters and genes) used to produce GM-fishes. PCR primers were designed to amplify sections of the GH gene of Nile tilapia, Common carp, Grass carp (*Ctenopharyngodon idella*), Rohu (*Labeo rohita*), Rainbow trout (*Oncorhynchus mykiss*), Chinook salmon (*Oncorhynchus tshawytscha*) and Atlantic salmon, the  $\beta$ -actin promoter of Common carp, Grass carp and Mud Loach (*Misgurnus mizolepis*) and the AFP promoter of the Ocean pout (*Zoarces americanus*). All target regions were initially tested by singleplex PCR and sequenced using DNA extracted from reference samples using a conventional phenol-chloroform method. Then, a multiplex PCR was designed so that each element yields a different length (from 80 to 300 bp), allowing their identification by conventional gel electrophoresis. Overall, the method presented here allows a specific and sensitive detection of the transgenic elements used in GM-fishes and can be used efficiently in different laboratories.

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# The *N*-glycosylation Role in the Trafficking and Sorting of two Soybean Aspartic Proteinases

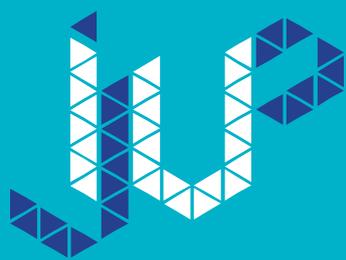
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The importance of *N*-linked glycans in aspartic proteinases has been emphasized in recent studies[1], drawing our attention to the possible influence of this post-translational modification in aspartic proteinase trafficking and sorting. Given the surprising results obtained with the extensively studied cardosin A whose Plant Specific Insert (PSI) presents the ability to direct the protein through an unconventional pathway, bypassing the Golgi apparatus[2], to reach the plant vacuole, it has been hypothesized that this ability could be influenced by the presence or absence of a glycosylation site. Soybean aspartic proteinases soyAP1 and soyAP2 [3] pose as remarkably suitable study models, as their respective PSI domains appear to possess a glycosylation pattern similar to cardosins: soyAP1-PSI possesses a putative *N*-glycosylation site in its sequence, whereas soyAP2-PSI does not. A closer study of the PSI domain independent behavior as a vacuolar sorting determinant is therefore needed. In the course of this study, molecular biology techniques were employed to obtain chimeric proteins containing each isolated soyAP-PSI, fused to a signal-peptide that directs it to the secretory pathway and also fused to a fluorescent mCherry protein to allow its detection. These constructs were transiently expressed in *Nicotiana tabacum* leaf epidermal cells in order to observe their subcellular localization using laser scanning confocal microscopy. To better understand the *N*-glycosylation role in trafficking and sorting, a glycosylation soyAP1-PSI mutant was also prepared. Pathway blockage assays were performed with the purpose of mapping the trafficking to the vacuole directed by these PSIs. Results point out that both soyAP-PSIs appear to travel through the same pathway, consisting of: Endoplasmic Reticulum – Golgi Apparatus – Prevacuolar Compartment – Vacuole. We were able to conclude that both PSI domains are capable of directing the mCherry protein to the vacuole, allowing us to classify them as valid vacuolar sorting determinants.

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**ENCONTRO INVESTIGAÇÃO JOVEM  
DA UNIVERSIDADE DO PORTO**

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**A4 BIOMEDICINE I**  
PARALLEL ORAL SESSIONS I

# A new prenylated chalcone induces mitotic arrest and cell death by mitotic catastrophe in MCF-7 breast cancer cell line

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Currently, antimicrotubule agents are amongst the most effective chemotherapeutic drugs against many cancers. Unfortunately, their use is associated with high toxicity and drug resistances, emphasizing the need for novel antimicrotubule agents to overcome or minimize these limitations.

Research at CEQUIMED-UP group aimed at discovering small molecules, with natural and synthetic origins, with potential antitumour activity has led to a synthetic prenylated chalcone, C1P, reported to have potent anti-growth activity against different tumours cell lines ( $GI \leq 10 \mu M$ ) [1].

Here, we describe the cell based assays [2] used to provide insight upon the mechanism of anti-growth action of C1P chalcone, on MCF-7 breast cancer cell line. It was verified that this compound induces the collapse of the mitotic spindle, arresting cells in mitosis, with the accumulation of the checkpoints proteins Mad2 and BubR1, in response to spindle assembly checkpoint activation. Furthermore, live cell imaging revealed that C1P treatment sustained prolonged mitotic arrest, followed by massive mitotic catastrophe. Like so, we present C1P as new prenylated chalcone with a promising potential for antitumour activity.

This work was funded by FCT under the project CEQUIMED-PEst-OE/SAU/UI4040/2014; by CESPU under the Projects 02-GCQF-CICS- 2011N and CheckTax-CESPU-2014; and partially by the European Regional Development Fund (ERDF) through the COMPETE Programme and FCT, under the project “PEst-C/MAR/LA0015/2013”. P. Silva is a PhD fellowship holder from FCT (SFRH/BD/90744/2012).

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# Discovery of a tryptophanol-derived oxazolopiperidone lactam OXAZ-1 with promising antitumor activity via dual inhibition of the p53-MDM2/MDMX interaction

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The p53 tumor suppressor protein is a major transcription factor activated in response to cellular stresses leading to cycle arrest, senescence, and apoptosis. Inactivation of the p53 tumor suppressor protein by interaction with murine double minute (MDM)2 and MDMX proteins is a common event in human cancers bearing wild-type p53. The simultaneous inhibition of the p53 interaction with MDM2 and MDMX represents an attractive therapeutic strategy for a full p53 reactivation in cancer [1]. Here, we report the identification of a dual inhibitor of the p53 interaction with MDMs, the (*S*)-tryptophanol derivative OXAZ-1, from the screening of a small library of enantiopure tryptophanol-derived oxazolopiperidone lactams (from Santos's group), using a yeast-based assay [2]. OXAZ-1 induced a p53-dependent growth inhibitory effect, involving p53 stabilization, up-regulation of p53 transcription targets, and PARP cleavage, in colon adenocarcinoma HCT116 cells with wt p53 (HCT116 p53<sup>+/+</sup>), but not in its p53-null isogenic derivative (HCT116 p53<sup>-/-</sup>). A similar antitumor activity was observed in MDMX-overexpressing breast adenocarcinoma MCF-7 tumor cells, resistant to MDM2-only inhibitors. In HCT116 p53<sup>-/-</sup> cells, OXAZ-1 potently triggered a mitochondria-mediated apoptotic pathway, involving reactive oxygen species generation, mitochondrial membrane potential dissipation, cytochrome *c* release, and Bax translocation to mitochondria. The disruption of the p53 interaction with MDMs by OXAZ-1 was further confirmed by co-immunoprecipitation, in HCT116 p53<sup>+/+</sup> cells. OXAZ-1 also exhibited a p53-dependent synergistic effect when combined with conventional chemotherapeutics, improving their antitumor outcome. In conclusion, this work opens the way for the development of a new class of dual p53-MDMs interaction inhibitors with promising anticancer applications.

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## Vaccination with GAPDH confers protection of adult mice against the disease caused by Group B Streptococcus

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Group B Streptococcus (GBS) is a commensal organism in adults but can turn into a life-threatening pathogen in newborn and in elderly or immuno-compromised adults. The only treatment currently available is based on the administration of antibiotics, which lead to the increase of antibiotic-resistant strains<sup>[1]</sup>.

Our group developed a vaccine based on the GBS's virulence factor, glyceraldehyde-3-phosphate-dehydrogenase (GAPDH). We showed that maternal vaccination with the recombinant protein (rGAPDH) is very effective in preventing the GBS-induced neonatal diseases in a mice model<sup>[2]</sup>. Moreover, the preclinical tests showed that this vaccine is safe and stable (Alves et al., unpublished). The aim of the present study was to assess the efficacy of rGAPDH vaccination against the disease induced by GBS in adults, using an adult model of GBS infection. In order to do that, we used two GBS strains, NEM2526 and NEM2433, belonging to serotypes Ia and V, respectively. These serotypes are responsible for up to 50% of all GBS infections in non-pregnant adults<sup>[1]</sup>. For the protection studies, BALB/c mice were actively or passively immunized with rGAPDH and infected with a sub-lethal dose of GBS NEM2526 or NEM2433. At different times post-infection, the animals were euthanized, several organs were collected to evaluate the colonization and the levels of several cytokines were quantified in the serum. The obtained results showed that both active and passive immunization confer protection of adult mice against GBS infections caused by the two tested serotypes. Indeed, at 18h post-infection, the levels of colonization in brain, heart and blood were significantly lower in the groups of immunized mice than in the groups of control mice. Associated with protection, we observed an increased production of pro-inflammatory cytokines (mainly IL-6), early post-infection.

In conclusion, this study showed that the GAPDH vaccine is effective against the disease induced by GBS in adult mice, as was observed in neonates. Therefore, we could predict a potential application of GAPDH vaccine as a global human vaccine to prevent GBS induced diseases.

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# Application of box-behnken design to optimize methotrexate loaded nanostructured lipid nanoparticles

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The main goal of this study was to optimize and assess the potential of lipid based nanoparticles (NLC) as capable of carrying drugs such methotrexate (MTX) [1-2]. Preliminary screening drug/lipid solubility study, allowed us to select Witepsol<sup>®</sup> E85 and Miglyol<sup>®</sup> 812 as the solid lipid and liquid lipid for the NLC loaded with MTX. Then, a 3-level, 3-factor Box-Behnken design was developed in order to study the influence of three independent variables: amount of liquid lipid ( $X_1$ ), surfactant amount ( $X_2$ ) and amount of drug ( $X_3$ ) on the dependent variables as mean particle size, polydispersity index (PDI) and entrapment efficiency (EE) values [3]. Optimized NLC loaded with MTX were produced with 45 mg of liquid lipid, 47 mg of surfactant polyvinyl alcohol (PVA) and 18 mg of drug and evaluate their physical-chemical properties such as morphology, size, zeta potential, EE, storage stability, *in vitro* drug release and cytotoxicity were investigated. NLCs loaded with MTX exhibited spherical shape with 252 nm, PDI around  $0.06 \pm 0.02$ , zeta potential of -14 mV and an EE of 87%. *In vitro* release studies revealed a fast initial release followed by a prolonged release of MTX from the NLC up to 24 h so in physiological and inflammatory environments that allow us to simulate some skin conditions. No toxicity was observed in fibroblasts up to 48 h. Thus, the optimized MTX-loaded NLC have the potential to be exploited as delivery system such topical, oral or parenteral delivery.

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# Using multifunctional Solid Lipid Nanoparticles to diagnose and treat Rheumatoid Arthritis

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Rheumatoid Arthritis (RA) is the most common autoimmune disease related to the joints and one of the most severe and finding effective and long lasting therapies that specifically target RA is a challenging task. This work proposed an innovative approach for RA therapy, taking advantage of the new emerging field of nanomedicine. This study aimed to develop a targeted theranostic system for intravenous administration, using Solid Lipid Nanoparticles (SLN) to function as a drug delivery system. The SLNs were co-encapsulated with methotrexate (MTX) and superparamagnetic iron oxide nanoparticles (SPIONs), to be used as therapeutic and imaging agents, respectively. The SLNs were also surface-functionalized with an anti-CD64 antibody that specifically targets macrophages in RA.

A total of eight different cetyl palmitate and stearic acid SLN formulations were produced using in an organic solvent-free emulsification-sonication method that combined high shear homogenization and ultra-sonication in order to compare the influence of each component present (MTX, SPIONs and anti-CD64) on NP characteristics. Particle size was assessed by dynamic light scattering and analyzed by transmission electron microscopy and surface charge (zeta potential) was measured by phase analysis light scattering. All the formulations presented sizes below 250 nm and zeta values lower than -16 mV, indicating suitable characteristics as nanosystems for intravenous administration. The encapsulation of MTX and SPIONs didn't significantly influence the NP properties, whereas the antibody conjugation caused an increased in zeta potential as expected. The stability of these formulations was also proven up to one month for the non-conjugated formulations. TEM photographs indicated that the SPIONs were encapsulated inside the SLN matrix. Also, it was possible to observe small deformity and aggregation of NPs, while formulations without SPIONs presented a spherical shape with little aggregation. FT-IR was used to confirm the presence of MTX in the SLNs as well as the successful conjugation of the antibody to the SLN. MTX association efficiency was determined by UV/Vis spectrophotometry, rendering values higher than 98% for all formulations.

In vitro studies were performed with THP-1 cells and enabled to assess the cytotoxicity of the developed formulations. MTT and LDH assays demonstrated that the formulations were biocompatible and presented low cytotoxicity a concentrations lower than 500 µg/mL, but there were no significant changes when comparing the different formulations at the same concentrations unexpectedly.

This study could provide an effective and viable approach for future theranostic strategies. It was proven that the proposed NP were not cytotoxic, that both a therapeutic and imaging agent could be co-encapsulated and the SLN functionalized for a potential future application such as anti-body specific targeting. The proposed formulations are, therefore, promising candidates for future theranostic applications.

# Hypoxia induces bladder cancer cell invasion by antagonizing the extension of protein O-glycosylation

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Invasive bladder tumours express the cell-surface Sialyl-Tn (STn) antigen, which stems from a premature stop in protein O-glycosylation. The STn antigen favours invasion, immune escape, and possibly chemotherapy resistance, making it an attractive biomarker for target therapeutics. However, the events leading to the deregulation of protein glycosylation are mostly unknown. Since hypoxia is a salient feature of advanced stage tumours, we search into how it influences bladder cancer cells glycophenotype, with emphasis on STn expression. Three bladder cancer cell lines with distinct genetic and molecular backgrounds (T24, 5637 and HT1376) were submitted to hypoxia. To disclose HIF-1 $\alpha$ -mediated events, experiments were also conducted in the presence of Deferoxamine Mesilate (Dfx). In both conditions all cell lines overexpressed HIF-1 $\alpha$  and its transcriptionally-regulated protein CA-IX. This was accompanied by increased lactate biosynthesis, denoting a shift towards anaerobic metabolism. Hypoxia was also responsible by the activation of mesenchymal/stem cell programs, giving rise to more aggressive phenotypes. Concomitantly, all cell lines overexpressed the STn antigen, in an HIF-1 $\alpha$ -dependent manner. Surprisingly, STn elevation associated with the downregulation of glycosyltransferases participating in downstream O-glycan elongation rather than the overexpression of its main biosynthetic enzyme ST6GalNAC.I. These effects were reversed by reoxygenation, demonstrating that oxygen levels act as an on-off switch for O-glycan extension. The cells behaved similarly when grown in Dfx, suggesting that HIF-1 $\alpha$  plays a key role in O-glycosylation modulation. Moreover, hypoxia enhanced cell invasion, which was inhibited by exposure to anti-STn monoclonal antibody TKH2. Associations between HIF-1 $\alpha$  and STn overexpressions and muscle invasion were further validated in tumour samples.

In conclusion, STn overexpression may, in part, result from a HIF-1 $\alpha$ -mediated cell survival strategy to adapt to the hypoxic challenge by enabling invasion. Moreover, the STn antigen offers potential to address tumour hypoxic areas that harbour more aggressive and highly resistant cancer cells and to control disease dissemination

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## Dissecting the role of Hes5 in cardiogenesis

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The adult mammalian heart has a very low regenerative potential and an active effort is being made to find better and more efficient strategies for cardiac therapies. The heart is the first organ to be formed during embryonic development and results from a complex crosstalk between various signaling pathways in a temporal and context-dependent manner. Understanding the complex signaling environment underlying cardiac development is important, especially considering that some of these pathways are activated in stress conditions (reviewed in [1]). Differentiating mouse embryonic stem cells (mESC) closely follow *in vitro* the kinetics occurring in the embryo development, thus constituting an unique platform for mechanistic studies on lineage determination and differentiation. The Notch signaling pathway has been implicated in determination of a cardiac fate from mesodermal progenitors [2]. Using a Notch1-intracellular domain (NICD1)- inducible ES cell line we have identified a novel function for the Notch effector Hes5 during the onset of cardiogenesis in gain and loss-of-function studies. In the present work the role of Hes5 in cardiac specification during mESC differentiation has been further explored in a close-to-physiological system in which Hes5 expression has not been manipulated. The dynamics and role of Hes5 in cardiac commitment will be discussed.

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ENCONTRO INVESTIGAÇÃO JOVEM  
DA UNIVERSIDADE DO PORTO

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**A5** **ECONOMICS & MANAGEMENT**  
PARALLEL ORAL SESSIONS I

# **The Determinants of Participation in R&D Subsidy Programmes: Evidence from Firms and S&T Organisations in Portugal**

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While public intervention in R&D is generally supported by economic theory and R&D subsidies are being increasingly implemented in most OECD countries, empirical evidence on their effectiveness has failed to produce conclusive results so far. A central question in the related literature is the allocation process of public funds since the participation in a R&D programme is not random and there is a potential selection bias when assessing the effects of public subsidies.

In this research, we aim to analyse the participation of firms and S&T organisations in R&D subsidy programmes by identifying the determinants of obtaining a subsidy. Using data from two R&D funding instruments in Portugal, we provide evidence for the case of an innovation follower country, contributing to fill a gap in the related literature. In order to do this, we present a characterisation of the project applications made by firms or S&T organisations to the subsidy programmes, and we estimate a binary discrete model in order to investigate the main determinants of project approval.

Our results suggest that subsidies awarded to firms are mainly aimed at large R&D projects by manufacturing firms, in both high technology sectors and in low-technology sectors that are important in the national and regional economic context. On the other hand, subsidies awarded to S&T organisations show a higher degree of selectivity, targeting mainly universities and premium public research centres, as well as privileging R&D projects in the exact sciences and engineering domains and cooperation projects. Finally, we have also found that the subsidy allocation process in Portugal presents some distinctive features compared to most countries mentioned in the related literature, which are in line with the Portuguese level of technological development and its current status of a follower country

# Analysis of perception of the quality differentiation of chicken eggs for choice modelling

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Product differentiation is a growing movement. Consumers seek products closer to their ideal preferences, restricted by their income and the availability of products on the market. Producers also have incentives to differentiate their products, once they can increase their market power to reach specific groups of demand, obtaining the so-called "premium price". This study aims to assess the egg industries' attempts to offer a product with differential characteristics, while consumers perceive and respond to alternative options.

Consumers were interviewed following the Choice Experiments Method, making a series of hypothetical choices based on the presentation of different possible combinations of features of eggs, including their prices. The modeling was made through a discrete conditional logit choice model. The results show that the characteristic potentially influences the demand is the use of antibiotics in breeding hens, linked to human health. The hens creation mode was also a factor, which indicates the value assigned to animal welfare. The heterogeneous color of the eggs, rather than indicating a higher quality related to the rustic production, generated a negative perception, probably linked to the lack of standardization of the production process. The addition of omega3 yielded no noticeable changes in demand.

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# The perception of transparency in building trust in the relationship brand / consumer

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In an era where customers have access to more information than ever, brands that are transparent can gain true and much desired consumer confidence. Today's customers are more informed, have more choices, more opportunities and can communicate their opinions with their online community with ease. For this reason, the culture of opacity of the twentieth century is not useful and it can be a disadvantage in the relation with consumers.

This study aims to measure the perception of transparency to build trust in the relationship brand/consumer. Shall assist professionals and marketing researchers interested in the brand communication strategy based on transparency and goal in building trust. To identify and define the concept of transparency, this study intends to contribute to the field of brand management. Although there are several studies about trust the focus was always on their benefits (ex.: brand loyalty) and not to what leads, what builds trust for consumers. The aim of the research is to study the perception of transparency to build trust in the relationship brands/consumers. Thus, this study will discuss/answer the following questions: 1) does the perception of transparency in building trust is different between users and non-users?; 2) does transparency influence positively or negatively building trust? To answer these questions, a model was built based on a previous study (Hustvedt et Kang, 2013) [1] that depicts the relationship among transparency, trust, attitude, word-of-mouth intention, and purchase intention. There were selected three apparel worldwide known brands for this study. Each has a set of questions that is being pretested with FEP students in brand management discipline of marketing master. After validation and purification an online survey will be applied among brand users and/or non-brand users and the data will be analyzed using the structural equation modeling method.

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# How can trust in advertising communication influences the creation of customer-based brand equity? The case of NOS

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The impact of advertising on consumer's trust sometimes manifests itself in unexpected ways. In the current context, where advertising budgets are gradually scarce, it becomes increasingly important to understand if the trust in advertising communication has impact on brand equity. Thus, the aim of this study is to identify the components of trust-ads that influence brand equity. While the Brand Equity associated with tangible goods has received a great deal of attention in the literature, a basic understanding of the nature of Brand Equity for services has yet to emerge. However, elements of a brand's equity positively influence consumer's perceptions and subsequent brand buying behaviors [2].

Generally, researchers suggests that advertising communication is successful in building consumer-based brand equity, having a sustaining and accumulative effect on this asset [1]. Furthermore, the issue of trust in advertising has been recognized as an important element, due the fact that the company attributed to advertising communication the role of informing consumers about the products, services and ideas in order to guide the moment of purchase decisions [3]. This results in an increased need to assign significance to consumer confidence in advertising communication, since there are few empirical results which prove that this is the key to build a strong brand equity. Because of this, the present investigation is based on the work of Buil *et al.* [2] and Soh [3], and proposes to measure the impact of trust-ads on brand equity from a consumer perspective of telecommunication's services company NOS. Additionally, bearing in mind studies that prove the existence of differences in the cognitive processes in the consumer's behavior, according to gender, its impact in the dimensions of Brand Equity will also be studied. In order to fulfill the goals, a quantitative study has been conducted. The information is obtained through a survey undertaken directly to the consumers in six stores NOS.

Data analysis will be initiated with a descriptive analysis of the different variables. After a Factor Analysis, by the method of Principal Component Analysis, we will be able to confirm all the seven components of trust in advertising originally envisaged and the three dimensions of brand equity. After that, a Simple Linear Regression Analysis could demonstrate a relationship between the independent variable Brand Equity, and the dependent variable Trust in Advertising. Finally, for the analysis of gender, we will apply the Tests of the Equality Averages.

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# **The Anatomy of Business Failure**

## **A Qualitative Account of its Implications for Future Business Success**

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The aim of the present study is to contribute to the empirical literature on the consequences of Business Failure (BF), resorting to qualitative methods in order to better understand the aftermath of BF.

BF is a constant in today's business world, being considered an essential and significant part of new business ventures [1]. Although there is extant literature on the topic of the costs bared by the entrepreneurs, it is undeniable that BF is essentially a learning process [2]. Although there is considerable debate regarding the narrative of the creation and performance of entrepreneurial efforts, it should be noted that failure has received much less attention [3].

Qualitative research is key to understanding the “how” of the phenomenon, especially when trying to understand the development of the individual within his/her context [4]. Thus, personal accounts and narratives are essential to understand the process, although it has only recently been applied to this field [3]. Specifically, we employ the Interpretative Phenomenological Approach [5], using a set of six selected case studies of entrepreneurs from several countries (three from the north of Europe and three from the south).

The collected evidence showed that previous failure impacted individuals strongly. Such an impact appears to be shaped by the individual's experience and age, and their perception of blame for the failure. However, for these particular individuals, it does not seem to be affected by the size of the project or the amount of financial loss. An array of moderator costs was identified, ranging from antecedents to institutions that were present in the individual's lives.

The outcomes are directly relatable to the failed experience by the individual. It was also found that the failure had a significant effect on the individual's career path.

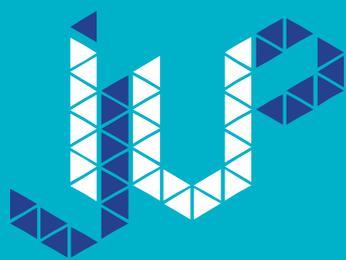
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PARALLEL  
ORAL  
SESSIONS II



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# A1 ENGINEERING II

## PARALLEL ORAL SESSIONS II

# Biological tissue recognition throughout medical images and consequent numerical mesh development and analysis

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Medical imaging is based on multiple non-invasive techniques that are used in order to create images of the interior of the human body and by which, with their analysis, is possible to perform an objective documentation for various clinical purposes. Currently these techniques, such Computer Axial Tomography (CT scan) and Magnetic Resonance Imaging (MRI), have been used increasingly, already becoming an essential tool in medical practice to diagnose lesions in human biological tissues. However, beyond these benefits and the doctor experience and qualification in making a diagnosis, there is a real need to detail the study of biological structures. For instance, in bone structures case, there is a need to further study the insertion of implants conditions or even the behavioral limitations (e.g. risk of fracture). The application of biomechanical studies for biological structures analysis arises as one of the most concrete theory to conduct a more detailed study. This project appears from the need to obtain a better geometry and shape of the structures by creating 3D models based on medical images. The creation of these models allowed the clinical, through a computational tool, to carry out numerical simulations to predict the structures behavior by improving the provision of health care.

The main objective of this project is to create a software capable of recognizing distinct biological tissues, such as bone tissue, through the analysis of medical images. Simultaneously, the software must have the ability to create a nodal mesh with the geometry and shape of the biological structure in study, which can be used for analysis of biomechanical problems by using numerical methods, such as finite element method (FEM) and meshless method. For this project was used MATLAB software, where a set of images in DICOM format was processed. The set of images is grouped into two sets according to the acquisition plan: sagittal and axial. The differentiation in these two subsets will allow the validation of the reconstruction method, knowing that the model will be created with just one of the subsets.

The first step is based on histogram analysis, through which is achieved the threshold value for each binary image ( $bw = [0, 1]$ ). In the initial image is also created an array with density values for each pixel. Then, the binary image is compared with the matrix containing the density values in order to save only the bone values ( $bw = 1$ ). The second stage is based on achieving the mesh nodes based on the calculation of distance between pixels (in x and y coordinates) and the distance between slices (z coordinate).

The representing structure was the bone structure and the result was a nodal mesh, where each node contains its characteristic bone density (cortical or trabecular bone). Also, a graphic interface was developed in order to ensure greater interoperability. As future results, it is expected the application of the model with a numerical method.

To conclude, in the future, this software will allow the inclusion of biomechanical studies in hospitals (more specifically in Orthopedics) to allow a better clinical decision.

# Recognition of Cancer using a Bag-of-Words Random Forest for Gastroenterology

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In gastroenterology, Computer Aided Diagnosis techniques have allowed physicians to analyze endoscopic images as a first or second opinion, or even as an educational program [4]. Cancer recognition in the gastroenterology track is such a difficult problem that only trained physicians can easily detect. Some pattern recognition solutions have already been published in the past [1-4]. However, these solutions have to be invariant to acquisition constraints (rotation, scale and luminance), goals which are not always achieved.

We extract features from pre-processed endoscopic images, extracted pixel intensities of some regions, and then use Random Forests to create a Bag of Feature Words (BoW). The BoW is built by counting how many times each feature appears on each image, thus giving its information content, and used to build a classification model using Support Vector Machines.

Our dataset 176 chromoendoscopy images of the oesophagus (30 for training and the remainder for testing), exhibiting three different conditions (normal and the pathological tissues with dysplasia and metaplasia). After 50 runs, we achieve  $22.03 \pm 3.09\%$  of average error. This results are promissory, especially when compared to the usage of SIFT descriptor and k-means ( $32.20 \pm 3.58\%$ ) to generate the BoW for classification.

Our methodology seems to deal well with few data train samples and it is statistically different and better then the results of the existent methodologies.

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# Using Speech-to-Text and Parsing to Extract BI-RADS descriptors from Mammography Reports

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Mammoclass (<http://cracs.fc.up.pt/mammoclass/>) is a web tool that allows users to enter a small set of variables that describe a finding in a mammography, and produces a probability of the patient having breast cancer. The classifier [1] was trained using data collected from the School of Medicine and Public Health of the University of Wisconsin, Madison, WI, USA, which consisted of a set of 348 patients whose BI-RADS<sup>®</sup> (Breast Imaging and Reporting Data System) descriptors [2] were annotated by a radiologist specialist in mammography. Very often, the BI-RADS descriptors are available in the text-free medical reports, and are not in a structured format. Moreover, some descriptors that can be relevant for posterior analysis are not annotated.

In this work, we use a Speech-to-text [3] tool to facilitate physicians to enter information about the findings seen in a mammography. After studying several tools, we decided for Google's Web Speech API (Application Programming Interface) (<https://dvcs.w3.org/hg/speech-api/raw-file/tip/speechapi.html>), which is easy to use and, most importantly, supports the Portuguese language. This API takes audio content and transcribes it into written words. Our integrated system takes the user's input voice, parses its contents, extracts BI-RADS descriptors [4], and automatically fills up a form. This form can be checked and corrected by the user. We also performed feature selection on a larger set of mammograms than the one used to generate a classifier to Mammoclass, in order to rank the most relevant. If the user misses some descriptor that is considered important by our feature selection, the system emits an alert, highlights the variable box in red, and suggests that the user manually chooses a value for that variable.

Ultimately, the data entered using our tool will be used to build new classifiers that are more suitable to model the Portuguese population.

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## Particulate matter in rural and urban nursery schools in Northern Portugal

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Studies have been showing strong associations between exposures to indoor particulate matter (PM) and health effects on children [1]. Children spend more time in schools (or nursery schools) than in any other indoor microenvironment besides home [2, 3]. Urban and rural nursery schools have different known environmental and social differences which make relevant their study [2, 4]. Thus, this study aimed to: i) evaluate indoor concentrations of particulate matter (PM<sub>1</sub>, PM<sub>2.5</sub>, PM<sub>10</sub> and TSP) on different indoor microenvironments (ME) (class and lunch rooms) of rural nursery schools and in an urban nursery; ii) compare PM concentrations between rural and urban nursery schools; and iii) analyse those concentrations according to Portuguese legislation and WHO guidelines for indoor air quality (IAQ) and children's health. The measurements were performed in several classrooms and lunch rooms in four nursery schools (three rural and one urban) on weekdays and weekends. PM concentrations were continuously measured (at least 24h for each ME) with a TSI DustTrak particle monitor using light-scattering laser method. Hourly averages were calculated from a set of 4 measurements per hour (each 15 minutes). Outdoor PM<sub>2.5</sub> and PM<sub>10</sub> were also obtained and indoor/outdoor (I/O) ratios have been determined. Indoor PM mean concentrations for all fractions were higher in urban nursery schools (PM<sub>1</sub> – 26.78 µg m<sup>-3</sup>; PM<sub>2.5</sub> – 27.14 µg m<sup>-3</sup>; PM<sub>10</sub> – 39.02 µg m<sup>-3</sup>; TSP – 65.15 µg m<sup>-3</sup>) than rural ones (PM<sub>1</sub> – 17.38 µg m<sup>-3</sup>; PM<sub>2.5</sub> – 17.57 µg m<sup>-3</sup>; PM<sub>10</sub> – 24.28 µg m<sup>-3</sup>; TSP – 37.87 µg m<sup>-3</sup>), which might have been related to outdoor traffic emissions. Despite this, results from I/O ratios allowed to conclude that the recorded concentrations depend more significantly on indoor sources than outdoor ones. WHO guidelines and Portuguese legislation exceedances were observed for PM<sub>2.5</sub> and PM<sub>10</sub>, mainly in the urban nursery school (100% in some cases). So, to mitigate these situations it was recommended to improve the air exchange ratios (higher and more efficient ventilation habits) and to change cleaning activities schedule for after the occupation period to promote children's and childcare workers overall life quality.

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# Independent Component Analysis (ICA) performance to bathymetric estimation through water column correction using high resolution satellite data in estuarine environment

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With the current techniques available it is possible use satellite remote sensing data as an alternative to the classical survey bathymetric methods. To produce bathymetric maps using multispectral satellite data, it is possible consider the pixel reflectance as a depth indicator. Teodoro et al., (2010) [1] already proposes a model for the estimation of depth based on Principal Component Analysis (PCA) of an IKONOS-2 image for the Douro River estuary (Porto, Portugal). Teodoro et al., (2014) [2] also considers the use of Independent Component Analysis (ICA), and the Fast ICA algorithm used in Matlab. The results were compared with the bathymetric estimation through PCA. The models used with ICA showed better results.

Teodoro et al.,(2010) [1] and Teodoro et al.,(2014) [2] already used PCA and ICA but they computing directly through the digital values. The main purpose of this work is to investigate if it is possible to obtain better results using the deep-water reflectance as depth indicator and consider the water column correction.

Audrey et al., (2007) [3] measures the water attenuation by estimating the deep-water reflectance and the water depth with the assumption that the bottom reflectance attenuation is only due to water column and therefore to the depth. The water attenuation model is given by (1):

$$\rho_s(\lambda) = [\rho_b(\lambda) - \rho_w(\lambda)]e^{-2k(\lambda)z} + \rho_w(\lambda) \quad (1)$$

The surface reflectance ( $\rho_s$ ) and depth ( $z$ ) are already known and the seabed reflectance ( $\rho_b$ ) does not be known, therefore, and because the main purpose of this work is the estimation of deep-water reflectance our model is given by (2):

$$\rho_w(\lambda) = \frac{\rho_s(\lambda)}{e^{-2k(\lambda)z} - 1} \quad (2)$$

The diffuse attenuation applied was estimated considering the work developed by Raymond et.,al (1981) [4].

Since is a work still in progress the finals results are not presented in this abstract.

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# A2 CHEMISTRY II

## PARALLEL ORAL SESSIONS II

## **Chemical analysis of legal high products containing Kratom (*Mitragyna speciosa*) sold in smartshops or in the Internet**

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The traditional market of recreational drugs has been suffering drastic changes in the last few years in consequence of the emergence of numerous new psychoactive substances (NPS), also entitled “legal highs”. In recent years, the legal high most popular worldwide among young people, is the Kratom - a product extracted from the *Mitragyna speciosa* plant, mainly constituted by alkaloids, the most important being mitragynine (MG). The use of Kratom, among other NPS, has alerted the authorities for consumer safety due to absence of information on the real composition and their potential health risks.

The present study aimed to determine and compare the real composition of 13 commercial samples of Kratom acquired in smartshops and in the Internet (before the approval of Decree 54/2013) by gas chromatography mass spectrometry (GC-MS).

A mixture of chloroform: methanol (1:2) with ultrasonication and overnight incubation of the samples proved to be the most complete extraction procedure, allowing the identification and quantification of their alkaloid content: MG and its analogs 7-hydroxymitragynine (7-OHMG), paynantheine (PAY), speciociliatine (SPC), speciogynine (SPG) and corynoxine A/B (CORY A/B). Statistically significant differences in the MG concentration (0.640 – 187 mg/g) were found among the different purchased Kratom samples, although there is no information about the amount of MG and other alkaloids on the products packaging. Besides, these obtained concentrations do not match with the “power” and “dose” described on the label. The concentrations of other alkaloids proved to be significantly lower than those of MG: 0.157-19.3 mg/g for 7-OHMG, 0.112-34.7 mg/g for PAY, 0.090-28.8 mg/g for SPC, 0.169-64.0 mg/g for SPG and 0.077-136 mg/g for CORY A/B.

Our results demonstrated that package information of Kratom products does not reflect its true composition with consequent for risk consumers.

# Forensic Electrochemistry: Study of Piperazine-based Psychoactive Drugs

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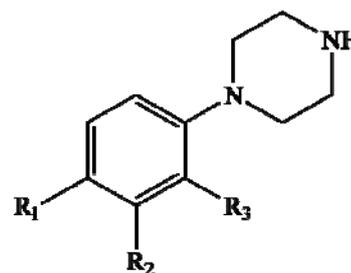
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Phenylpiperazine derivatives have been known as structural backbones for pharmacologically active compounds since 1960's. A number of these derivatives were investigated, and in some cases patented, as appetite suppressants, antidepressants, physical and neuroenhancers [1]. In the last decade phenylpiperazine derivatives have been reportedly used as recreational drugs due their amphetamine-like CNS stimulant properties and were also detected as adulterants of well known psychoactive drugs (eg. MDMA). The search by underground drug entrepreneurs for new psychoactive substances (NPS) with ability to circumvent drug legislations brought a substantial number of 'failed pharmaceuticals' and experimental substances from pharmaceutical research to the illegal or non-regulated market, such as *m*-chlorophenylpiperazine (mCPP), its isomers and analogs [2] (Figure 1).

Progresses in the area of analytical chemistry are aimed at bringing data close to the 'field' (eg. parties where the drugs are used, emergency rooms of hospitals, etc.) to overcome several limitations, as the lag time of identification, difficulties in drug sample collection or transport due to legislative restrictions. A real time *in situ* detection and identification system is highly desirable for the fast and efficient monitoring of NPS. Electrochemical methods have been shown to be advantageous techniques to study psychoactive substances [3,4]. Besides the importance of redox data for areas such forensic chemistry, helping in the development of new and faster forensic assays, is also noteworthy its interest in toxicologic studies, helping to reveal metabolic pathways [4].

This project consisted on the synthesis of phenylpiperazine derivatives and the study of their electrochemical profile by voltammetric techniques. The results gathered allowed to build a preliminary model for the oxidation mechanism of these piperazine-based psychoactive drugs that in the near future could be used in the development of new electrochemical sensors. The results obtained will be presented in this communication.



**Figure 1.** Backbone of phenylpiperazine derivatives.

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# Strategies for Improved Ligand/Decoy Discrimination in Virtual Screening of Large Databases for Drug Discovery

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Computational methods occupy a prominent place in current Drug Discovery efforts. In particular, Protein-ligand Docking can be used in Virtual Screening (VS) to evaluate databases with millions of compounds to identify molecules that could bind to a particular protein or receptor of pharmacological interest [1,2]. This strategy is often used to limit the amount of molecules that has to be tested experimentally and to reduce the cost in the identification of new lead molecules for drug development.

The challenge in VS is discriminating real ligands from similar molecules that do not bind (decoys). Currently, one of the big problems in VS arises from the large number of false positives, i.e. molecules that are erroneously suggested, from docking, to bind strongly to the target. An even worse problem in VS comes from the large number of false negatives, i.e. molecules that docking fails to identify as strong ligands, despite their high affinity. Both problems result from the imperfections in the currently available scoring functions.

In this study we tested the performance of several scoring functions available in popularly used docking programs such as Autodock, Vina and GOLD and evaluate their ability in discriminating between real ligands and structurally similar decoy molecules, for different types of targets and ligands. Based on these results we highlight the strengths and weaknesses of different scoring functions and provide a set of guidelines on the limits of validity of currently available scoring functions with the type of target and ligand.

In addition, we present a strategy to obtain improved ligand/decoy discrimination in VS runs for particular types of targets and ligands, combining different scoring functions based on the idea of consensus scoring

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## Xanthone as a promising scaffold to develop new potential inhibitors of p53:MDM2 interaction

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The growth inhibitory activity of tumor suppressor p53 is tightly controlled by interaction with two negative regulatory proteins, MDM2 and MDMX, which are overexpressed in about half of all human tumors. The knowledge of crystallographic structures of MDM2/X complexes with p53 has led to the identification of several classes of inhibitors of p53:MDM2/X interaction [1]. Xanthone derivatives have been reported as potential anticancer agents with the ability to target p53:MDM2 interaction. In fact, the natural xanthenes,  $\alpha$ -mangostin and gambogic acid, and the synthetic derivative pyranoxanthone 1 (LEM1) exhibited potent cytotoxic activity against several human tumor cell lines accompanied by an inhibitory effect on p53:MDM2 interaction [2,3]. Overall, the xanthone scaffold may represent an important basis for the development of new disruptors of p53:MDM2 interaction.

In this work, the synthesis and structural elucidation of a library of new aminated xanthone derivatives will be presented. Based on the xanthone scaffold and on known inhibitors of p53:MDM2 interaction, a hybridization strategy was followed to obtain potential inhibitors of p53:MDM2 interaction with antitumor activity. The use of different xanthone building blocks and different reaction conditions allowed us to develop new aminated xanthenes. An *in silico* docking study was performed to rank these derivatives according to their binding affinity towards MDM2. Future work will consist in the investigation of their potential to disrupt p53:MDM2 interaction using yeast-based assays. These studies will contribute to the knowledge of structural requirements for the construction of novel potent and selective p53 activators with antitumor activity.

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# Understanding diclofenac's interaction with membranes: *in vitro* study

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Nonsteroidal anti-inflammatory drugs (NSAID) are one of the most widely consumed pharmaceuticals worldwide due to their anti-inflammatory, analgesic and antipyretic properties. However, their chronic consumption is associated with a wide spectrum of unwanted effects, from gastrointestinal to cardiovascular and renal adverse effects. [1] The NSAID mechanism of action is the inhibition of the prostaglandins biosynthesis through the cyclooxygenase pathway; yet, it does not fully explain the drug's toxic and therapeutic effects. Hence, other mechanisms including the drug's action at the membrane level have been suggested. [2]

In this sense, this work intends to unravel diclofenac's interactions with biological membranes, using liposomes made of DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine) as membrane models. The preparation of large unilamellar vesicles of DMPC was accomplished through lipid film hydration, followed by extrusion. The drug's partition coefficient was assessed by derivative spectrophotometry and its effect on the thermal behaviour of DMPC was evaluated by fluorescence anisotropy. To study the drug's location within the liposome, fluorescence quenching assays were executed. All experiments were performed under 3 pH conditions (7.4, 5.0 and 3.0) to mimic different microenvironments found *in vivo*.

In general, diclofenac interacts with phosphatidylcholine bilayers and the drug's effect is highly dependent on its ionization state. As so, the medium pH is a key factor to understand diclofenac's behaviour when associated with the membrane. At lower pH, when the drug is in its protonated form, the affinity to the bilayer is greater and consequently there are more pronounced alterations on the lipid phase transition parameters as well as a higher ability to quench fluorescent probes. In sum, this work corroborates the importance of studying the interaction of NSAID with membranes and suggests that diclofenac action at the membrane level is more relevant in more acidic environments, such as the gastrointestinal tract and inflamed cells.

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# Resveratrol gastric ulcerogenic properties: are lipid membranes the answer?

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Resveratrol is a natural polyphenol found in grape skin and seeds. This phytochemical has a great therapeutic potential because of its anticarcinogenic and antioxidant properties. Also, it is an excellent cardiovascular and neuronal protector [1]. Though, resveratrol has also undesirable effects such as ulcerogenic properties [2]. In fact, it was demonstrated that resveratrol delayed the healing of gastric ulcers possibly by the inhibition of cyclooxygenase (COX), which leads to the decrease of prostaglandin (PG) E2 synthesis being this PG responsible for the protection of the gastric mucosal barrier [3]. The modulation of COX by resveratrol may be related to the inhibition of protein kinase C (PKC) signal transduction pathway at multiple levels [3]. Although the biophysical mechanisms underlying these effects are far from being fully understood, they seem to be closely connected with the interaction of this compound with lipid membranes.

In this context, the current work aims to study the interaction between resveratrol and biomembranes to elucidate the effects of this compound in gastric mucosal barrier. The membrane model systems used in this study were large unilamellar vesicles (LUVs) made of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), which represented a typical membrane cell at physiological pH of 7.4 and 37°C and a protective membrane lining of the gastric mucosa at acidic pH of 5.0 and 37°C.

The studies performed included the determination of partition coefficients of resveratrol in the membrane model systems using UV–vis derivative spectrophotometry, the membrane location of resveratrol by fluorescence quenching of probes by steady-state and time-dependent measurements and fluorescence anisotropy which provided insights into the fluidity of the membrane and lipid order.

The results obtained suggest that resveratrol compromises the integrity of the gastric mucosa by chemical interaction with the phospholipids. In fact, resveratrol showed a strong interaction with lipid membranes which resulted in a change of membrane fluidity, which, thereby, may condition the function of many transmembrane proteins such as PKC and, consequently, COX activity.

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# Development of new peptide-drug conjugates for cancer therapy

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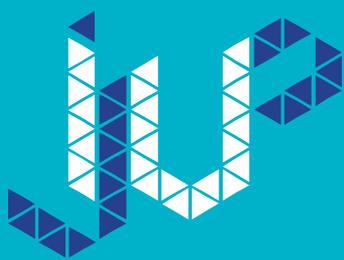
In developed countries, cancer is one of the major causes of death. Gemcitabine (2',2'-difluorodeoxycytidine), commercially available as Gemzar® by Eli Lilly and Company, is a nucleoside analogue which has been proven efficient against a wide range of solid tumors [1]. The use of gemcitabine hydrochloride was approved by the FDA in 1996 as first-line treatment for patients with locally advanced (non-resectable Stage II or Stage III) or metastatic (Stage IV) pancreatic adenocarcinoma or for patients previously treated with fluorouracil (5-FU). Gemcitabine is activated *in vivo* via phosphorylation of its 5'-monophosphate by deoxycytidine kinase, and is subsequently phosphorylated by intracellular kinases to the triphosphate form [2]. However, gemcitabine may be deaminated to its inactive uridine metabolite, 2',2'-difluorodeoxyuridine, by cytidine deaminase, which is present at high levels in both human plasma and liver [3].

This project aims at the chemical modification of gemcitabine and subsequent conjugation to Cell Penetrating Peptides (CPP), in an effort to facilitate delivery of that drug into cancer cells, taking advantage of the fact that all CPP are able to efficiently pass through cell membranes while being non-cytotoxic and carrying a wide variety of cargos inside cells [4].

Two different CPP were synthesized by Solid Phase Peptide Synthesis (SPPS), purified and characterized chromatographically. Gemcitabine was successfully modified and conjugated to both CPP. These new conjugates were purified and characterized by HPLC and LC-MS. The stability of the hydrolysable bonds was studied in PBS buffer at physiological pH and temperature and the results show a different time-dependent kinetics of gemcitabine release.

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ENCONTRO INVESTIGAÇÃO JOVEM  
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**A3** **BIOLOGICAL SCIENCES II**  
PARALLEL ORAL SESSIONS II

# Antiquorum sensing and biofilm inhibitory activities of citronellol and carveol

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Over the last century, excessive and indiscriminate use of antibiotics has led to the emergence of multidrug-resistant bacteria. This is considered a serious public health problem that is worsened when microorganisms form biofilms [1]. Therefore, there is an urgent need for the development of therapies that can provide sustainable and long-term effectiveness against pathogenic bacteria [2]. Quorum sensing (QS) is an important mechanism that regulates bacterial behavior within biofilms. In addition to its role in biofilms, QS regulates the expression of genes involved in processes related to survival, virulence, and pathogenicity. So, the disruption of QS signaling pathways can affect biofilm development and make bacteria more susceptible to antimicrobials [1]. One key advantage proposed in targeting QS is based on the fact that QS inhibitors do not suppress the growth of cells and hence will not exert selective pressure [3]. Some natural compounds have demonstrated distinctive properties that could help to overcome the resistance problem and make them perfect candidates for these much needed therapeutics. In this sense, with this study it was intended to evaluate the QS inhibition (QSI) and antibiofilm potential of two selected essential oils (EOs) components, carveol and citronellol, to prevent the onset and severity of bacterial infections. The QSI activity of both EOs was evaluated using the bacterial model *Chromobacterium violaceum* CV12472 in a disc diffusion assay based on the violet pigment inhibition (QS-dependent phenomenon). Additionally, the ability of selected EOs to interfere with the QS response of opportunistic pathogen *Pseudomonas aeruginosa* was also evaluated. This was achieved by a high-throughput QSI screening system based on the co-cultivation of *P. aeruginosa* PA14 wild-type strain with the biosensor *P. aeruginosa* PA14-R3. Biofilm inhibition studies were carried out with PA14 wild-type strain using a microtiter plate assay. Citronellol (800 and 1000 µg/ml) and carveol (400, 800 and 1000 µg/ml) inhibited violacein production in *C. violaceum* with QSI halos ranging from 3.3 to 3.8 mm and 2.5 to 7.5 mm, respectively. A dose-dependent anti-QS activity was also obtained with carveol against *P. aeruginosa*, without significantly affecting the bacterial growth. Additionally, inhibition of biofilm formation was observed with this compound. The overall results demonstrated the ability of carveol to interfere with QS systems of both *C. violaceum* and *P. aeruginosa*. Hence, this compound has potential to be developed as a new QS inhibitor to treat emergent infections, including those biofilm related.

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## **Cannabinoids impact in placental cytotrophoblasts: changes in the oxidative state of BeWo cells**

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Cannabinoids (CBs) are the active compounds of *Cannabis sativa*. The most prevalent psychoactive substance is  $\Delta^9$ -tetrahydrocannabinol (THC), whose effects are achieved through the activation of specific cannabinoid receptors – CB1 and CB2. Due to its medicinal properties, some cannabinoids started to be synthesized artificially. On the other hand, there are endogenous lipid compounds, called endocannabinoids (eCBs), that are capable of binding and activate the same cannabinoid receptors. Until now, the most bioactive eCBs are anandamide (AEA) and 2-arachidonoylglycerol (2-AG). The eCBs, their receptors, the proteins that transport, synthesize and degrade them, represent the endocannabinoid system (ECS). Placenta development requires a tight regulated proliferation, differentiation and apoptosis of cytotrophoblasts, the specialized placental epithelial cells. Studies in the last few years have shown that ECS is implicated in pregnancy events such as implantation and decidualization. However its function during placentation requires further investigation [1].

In this work, we studied the effects of different cannabinoids in BeWo cells, a choriocarcinoma cell line and a well-accepted cytotrophoblast cell model. Cells were treated for 24 h with different concentrations of 2-AG, THC and WIN (a synthetic cannabinoid). We performed MTT assay to access cell viability and measured LDH release as a biomarker for cytolysis; ROS/RNS species generation and mitochondrial membrane potential ( $\Delta\psi_m$ ) were evaluated by fluorimetry assays; glutathione levels were measured by spectrophotometry; morphological changes were analyzed by H $\ddot{o}$ chst and Giemsa staining. All cannabinoids induced a decrease in the mitochondrial membrane potential, but only 2-AG led to an increase of ROS/RNS intracellular levels. The eCBs also induced changes in glutathione levels. Treatment with low concentrations of WIN (5 $\mu$ M) resulted in a great loss of cell viability, while for 2-AG this effect was only observed above 20 $\mu$ M. THC did not affect cell viability.

Here we show that cannabinoids affect placental cytotrophoblasts. The endocannabinoid 2-AG is able to induce changes in the oxidative state and, since placenta develops in hypoxia, these may contribute to the pathophysiological mechanisms of some pregnancy complications. Thus, the study of the effects of cannabinoids on maternal-fetal interface may be relevant to understand the impact of exo- and endogenous cannabinoids in pregnancy.

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# Exploring Cinnamic Acid Scaffold: Development of Promising Neuroprotective Lipophilic Antioxidants

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Polyphenols represent a structurally heterogeneous group of compounds with a large spectrum of biological activities [1]. Their antioxidant activity, either by direct radical scavenging activity and/or indirect mechanisms (*e.g.* metal chelation) may counterbalance the disproportioned production of reactive species in oxidative stress-related diseases [2]. In particular, oxidative stress has been implicated in the pathogenesis of neurodegenerative disorders [3]. Since polyphenols' bioavailability is limited by their physicochemical properties, structural refinement is a valid approach to improve their efficacy and potency [2].

Here, we describe the design and development of new hydroxycinnamic acid (HCA) based antioxidants with neuroprotective activity. To achieve the goal the *in vitro* antioxidant, cytotoxicity and neuroprotection properties of the synthesized compounds were evaluated.

HCA based derivatives were obtained in a three step sequence comprising phenol protection, chain elongation and phenol deprotection. The antioxidant activity of synthesized compounds was evaluated by DPPH<sup>•</sup>, ABTS<sup>•+</sup> and GO<sup>•</sup> methods. Ferrozine assay was used in the evaluation of iron (II) chelating properties. Cell-based assays were performed in SH-SY5H neuroblastoma cell lines in order to evaluate the cytotoxicity and neuroprotective properties via MTT endpoint.

HCA derivatives were successfully synthesized. The chemical modification introduced in cinnamic acids amplified their lipophilicity and in general increased antioxidant activity when compared to natural models. HCA derivatives did not display cytotoxic activity and a neuroprotective effect in the cellular model of Parkinson's diseases was recorded.

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## Production and characterization of curcuminoid-loaded lipid-based nanoparticles

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Curcuminoids (CUR) are highly pleiotropic molecules. They are involved in hippocampal neurogenesis by inhibition of pro-inflammatory responses in the brain, reduction of  $\alpha$ -amyloid production and promotion of neurotrophic factor release. However, they possess low aqueous solubility, photoinstability, rapid hydrolysis and high in vivo enzymatic degradation [1,2]. To circumvent these limitations, lipid-based nanoparticles, i.e. solid lipid nanoparticles – SLN and nanostructured lipid carriers – NLC, were developed. SLN possess a lipid matrix which is solid at room and body temperature, while NLC also contain a liquid lipid component.

CUR-loaded and unloaded nanoparticle formulations were produced by high-shear homogenization followed by ultrasonication. These formulations were completely characterized to evaluate the quality of the developed curcuminoid-loaded nanoparticles for brain-targeted drug delivery. Dynamic light scattering measurements demonstrated a Z-average around 200 nm, with a polydispersity index below 0.2 and a reasonable negative zeta potential of around  $-19$  mV. The encapsulation efficiency studies, for which an HPLC protocol was developed and validated, showed around 90% and 81% of curcuminoid encapsulation in NLC and SLN respectively. Over the course of the study (10 months) the encapsulation efficiency for the NLC formulations suffered a slight decrease of around 5%, whereas the encapsulation efficiency for the SLN formulations had a superior decrease of around 11%. Differential Scanning Calorimetry thermograms of lyophilized samples demonstrated that the curcuminoids were molecularly dispersed in the lipid matrix, since for both CUR-formulation samples there was only one phase transition at the lipid mixture's approximate melting point.

The results suggest that the inclusion of CUR in these lipid-based nanoparticles not only increases their solubility but also their stability through time when compared to the free drug. We can also conclude that NLC nanoparticles provide a more suitable matrix for CUR inclusion since they maintained superior encapsulation efficiencies over time. With these results we expect to develop a formulation with a superior brain bioavailability for the treatment of cerebral degeneration.

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# Solid lipid nanoparticles and nanostructured lipid carriers as a vehicle for brain-targeted delivery of curcumin

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Alzheimer's Disease (AD) is the most frequent neurodegenerative disorder. At the moment there are no drugs available that can prevent or cure this disease. For this reason there is a lot of research going on to find a good treatment for AD [1]. Curcumin is derived from the dietary spice turmeric and has antioxidant, analgesic and anti-inflammatory properties. It is one of the compounds that can be promising as a potential agent for both prevention and treatment of AD. Due to its poor water solubility, low bioavailability, rapid elimination and the challenges of crossing and transposing the blood-brain barrier, a nanotechnology based drug delivery system can be a tool for an effective brain delivery of curcumin.

Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) loaded with curcumin were successfully produced by a modified hot homogenization technique [2]. Thereafter, the nanoparticles (NPs) were characterized using dynamic light scattering, transmission electron microscopy (TEM) and spectrophotometric analysis to evaluate the entrapment efficiency and stability of the developed formulations. At the same time, curcumin-loaded nanoparticles were functionalized with transferrin, in order to mediate the transport of these particles through the BBB endothelium to the brain. The same parameters as described above were determined for these functionalized NPs.

TEM images revealed spherical and uniform shape with smooth surfaces. In both cases (SLNs and NLCs) dynamic light scattering measurements gave a Z-average under 200nm, with a polydispersity index below 0.2. The zeta potential was around -30mV. The functionalization of the NPs with transferrin didn't change the mean diameter, but lead to a slight reduction in the zeta potential of the NPs (-25mV). The entrapment efficiency studies showed an average curcumin entrapment efficiency of about 49% for SLNs and 58% for NLCs. A stability study revealed these characteristics remained unchanged for at least 1 month. Bradford assays confirmed the presence of transferrin in the formulations.

These results suggest that the SLNs and NLCs both can be an effective nanodelivery system for curcumin. These systems are capable of being used as controlled-release models for brain delivery of curcumin, increasing its bioavailability, protect the incorporated curcumin and targeting to the brain by the addition of transferrin to the NPs. This creates opportunities to continue research and further improve the possibilities of the use of curcumin as potential therapeutic agent for AD.

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## Seeking marine bacteria as potential bioactive resources

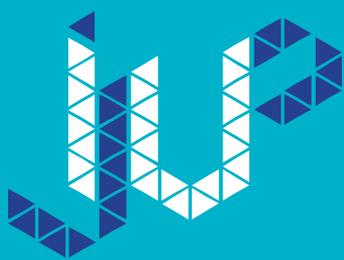
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The resistance by microorganisms to currently used antibiotics has led to the urgent need for new drugs [1]. Marine natural bioactive compounds can be isolated from a wide range of commonly found organisms in the Portuguese coast, like sponges and macroalgae, and their symbiotic microbial community [2,3]. *Planctomycetes* (38), *Alphaproteobacteria* (30) and *Actinobacteria* (41) isolated from these associations were selected for bioactivity screening studies. *Planctomycetes* are still an unexplored phylum of bacteria and due to their unique characteristics are a group with high potential for production of bioactive compounds [4]. *Actinobacteria* are, on the contrary, groups well studied regarding bioactivity and, consequently, their genomes [5]. The potential biological activity was assayed through the analysis of genes related to secondary metabolites production, the nonribosomal peptides synthetases (NRPS) and polyketide synthases (PKS-I) genes and against a panel of human pathogens and environmentally relevant microorganisms for the planctomycetes. Approximately 50% of the 107 bacterial strains amplified PK-I and/or NRPS genes, indicating potential bioactivity in different genera and bacterial groups. Regarding the planctomycetes bioactivity assays performed in strains which previously demonstrated presence of PKS-I genes, *Candida albicans* was the target microorganism more significantly inhibited by almost all of the strain extracts followed by *Bacillus subtilis*. Lower levels of inhibition were obtained for the other Gram negative bacterium assayed, *B. cereus* and two strains of *E. coli* and *P. putida* (all Gram positive). These results not only demonstrated the antimicrobial potential of the screened bacteria but also highlighted the correlation between the presence of these specific genes and bioactivity.

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**A4** **BIOMEDICINE II**  
PARALLEL ORAL SESSIONS II

# Adenosine Equilibrative Transporters: Distribution and quantification profile in mesenteric vessels

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Adenosine is an ubiquitous molecule that participates in several physiological and pathophysiological states. Once produced, adenosine exerts its biological activity by interacting with four adenosine receptor subtypes: A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> e A<sub>3</sub>. Adenosine availability is dependent of ATP metabolism, inside and outside cells, but also on the ability of transporters, mostly Equilibrative Transporters (ENT), to mediate adenosine transport across cell membranes. Until now, only four subtypes were identified: ENT-1, ENT-2, ENT-3 and ENT-4. The levels of adenosine seem to be increased in hypertension. The present study intends to quantify and determine the ENT-1, ENT-2, ENT-3 and ENT-4 distribution profile in vascular territories of normotensive rats (Wistar Kyoto rats; WKY) and spontaneously hypertensive rats (SHR). Consequently, will also be assessed the existence of adenosine dynamic alterations in hypertension.

Experimentally, after intracardiac perfusion, mesenteric vessels (artery and vein) of SHR and WKY rats were fixed in Bouin and embedded in paraffin. Sections immunolabelled with a primary antibody directed against individual adenosine ENTs were detected with a biotinylated secondary antibody, the ABC complex, and using 3,3'-diaminobenzidine as chromogen. In control sections primary antibody was omitted. Digital images of tissue samples were acquired using the same microscope/CDD camera operating conditions. Quantitative analysis and processing of the images were carried out using PAQI software (CEMUP.UP). Intensity level of the control images was determined and used for threshold segmentation of the objects of interest (regions of intensity <150). Results are expressed as mean±s.e.mean; P<0.05 was taken to reflect statistically significant differences (ANOVA followed by Holm-Sidak t test).

In SHR arteries, a lower immunoreactivity for all ENTs were detected: ENT-1( 0.67±0.26, n=6); ENT-2 (8.60±2.72, n=9); ENT-3 (3.75±1.41, n=10); ENT-4 ( 0.28±0.09, n=3) comparatively with the results observed in WKY arteries (ENT-1( 19.03± 3.30, n=8); ENT-2 (26.86±2.96, n=13); ENT-3 (14.32±2.23, n=13); ENT-4( 16.79 ±4.81, n=4); p<0.001). In veins, by contrast, adenosine ENTs immunoreactivity was unaltered except for adenosine ENT-2 expression which was lower in SHR (7.87±3.99, n=6) than in WKY (21.81±4.90, n=10); p<0.05.

Hypertensive vascular tissues exhibited a different profile for ENTs expressions both in arteries and veins supporting the occurrence of changes in adenosinergic system dynamics in hypertension.

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## Evaluation of the antimitotic activity of a new prenylated chalcone in NCI-H460 non-small cell lung cancer cells

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Cancer is one of the leading world health problems, with high overall mortality and morbidity, its incidence increasing yearly. Consequently, there is need for development of new cancer therapies, along with improvement of the current therapies, such as chemotherapy, in order to minimize side effects and resistance acquired in the course of patient treatment. CEQUIMED-UP has been involved in the search of small molecules from natural and synthetic origin with potential antitumor. In this context, C1P, a prenylated chalcone with potent antiproliferative activity against human cancer cell lines ( $GI_{50} < 10 \mu\text{M}$ ) was previously reported [1]. The present work describes the characterization of the mechanism of antiproliferative action of C1P on NCI-H460 non-small lung cancer cell line. Phase contrast microscopy and quantification of NCI-H460 cells treated with C1P showed an increase of mitotic-arrested cells after 24h of incubation. Immunostaining these C1P treated cells showed accumulation of the proteins BubR1 and Mad2, indicating spindle assembly checkpoint activation of these mitotic arrested cells. Immunostaining with anti-tubulin antibodies also demonstrated a predominance of monopolar/collapsed mitotic spindles. Live cell imaging revealed that C1P-mediated prolonged mitotic arrest lead to mitotic catastrophe and cell death mainly by apoptosis. As such, these results suggest the compound employs its antiproliferative activity by interfering with microtubules, causing prolonged mitotic arrest and mitotic catastrophe, revealing its potential as anticancer agent.

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# Assessment of the Effect of Daunorubicin on the Biophysical Properties of Model Membranes

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Drug screening involves an assortment of steps. Drug design is followed by in vitro studies, usually in cells. However, cells are time consuming, expensive to maintain and include a variety of confounding factors, so the use of model membranes such as liposomes as a first front for drug screening could be immensely beneficial.

That being said, the aim of our study was to assess the effects of daunorubicin and on the lipid membranes of four LUV formulation models, two of them constituted by DMPC with and without cholesterol at pH 7.4, mimicking the normal cell membrane, and the other two simulating the tumoral cell membrane, constituted by a mixture of DMPC:DOPC:DPPS (3:1:1) also with and without cholesterol at pH 6.3.

Size, zeta potential, membrane location and fluidity were assessed for the four formulations of liposomes mentioned before. Membrane location and anisotropy techniques were also performed on tumoral cells, the line MDA-MB-231, to assess the validity of the designed models of mimicking the actual biomembranes.

Size and zeta potential results confirmed that the models were prepared as intended.

The drug partitions very well into all models except normal with cholesterol. While in this case cholesterol seems to impair partitioning, the opposite occurs in the tumoral models. Daunorubicin appears to localize between the acyl chains of phospholipids in the membrane but still interacting through electrostatic interactions with the polar heads, so it appears to locate at an intermediate region.

In terms of fluidity, the normal model with cholesterol appears to be the most rigid of all and remains unchanged by the drugs tested, while the normal model is highly fluid. Contrarily to what was expected, the tumoral model with cholesterol becomes less fluid with the presence of drug, which does not happen in the tumoral model without cholesterol. Similar results were found for tumoral cells. S

Summarily, it could also be observed that the designed model membranes, although simple, replicated biomembranes quite well. This study and follow-up work can be a big step towards the validation of liposomes as models for cell membranes, and in the future allow the facilitation of drug-interaction studies.

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## Expression of *Candida albicans* secreted aspartic proteases influences host kidney fungal burden and histopathology in a Treg/IL-10 dependent manner

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*Candida albicans* secreted aspartic proteases (Saps) have been considered virulence-associated factors. Here, we assessed the importance of *SAP4*, *SAP5* and *SAP6* expression, associated with *C. albicans* pathogenicity in disseminated infections, in the murine model. Organ fungal burden and the elicited immune response were evaluated in BALB/c and C57BL/6 mice infected i.p. with the wild-type (WT) *C. albicans* strain SC5314 and the SAT1-flipping mutant  $\Delta sap456$  lacking Saps 4-6. Mice infected with  $\Delta sap456$  presented lower kidney fungal burden and myeloperoxidase activity than the counterparts infected with WT strain. However, in infected C57BL/6 lymphocyte deficient (*RAG2*<sup>-/-</sup>) mice, fungal burden was lower than in WT C57BL/6 controls and no differences between mice infected with the WT and mutant strains were observed. This suggested that the influence of Saps expression on kidney fungal burden was lymphocyte-dependent. As mice of both genetic backgrounds infected with the WT *C. albicans* strain presented higher proportions of splenic Treg cells (CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup>) than  $\Delta sap456$ -infected mice, we assessed the effect of Treg depletion using PC61 mAb prior to infection. Depletion of Treg abolished the above reported kidney CFU count differences between mice infected with the WT and mutant *C. albicans*. Also, IL-10-deficient mice infected with the WT and mutant *C. albicans* strains presented no different kidney fungal burdens. Altogether, these results implicate Sap expression in the modulation of the host immune response to *C. albicans* in a Treg and IL-10-dependent manner.

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## Role of adenosine A<sub>2B</sub> receptors in right ventricular failure secondary to pulmonary arterial hypertension: friend or foe?

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Pulmonary arterial hypertension (PAH) is a severe progressive disease that overloads the heart by increasing the resistance of pulmonary circulation. PAH-induced pressure overload of the right ventricle (RV) leads to myocardial tissue remodelling and eventually RV failure. Adenosine is an anti-inflammatory agent that reacts against stressful stimuli by acting on four G protein-coupled receptors (AR; A<sub>1</sub>AR, A<sub>2A</sub>AR, A<sub>2B</sub>AR and A<sub>3</sub>AR). Interestingly, blockade of the A<sub>2B</sub>R protects lung from inflammation and fibrosis in interstitial pulmonary diseases [1], although the precise role of this adenosine receptor subtype on progression of ventricular remodelling to heart failure in PAH is still unexplored.

PAH was induced in male Wistar rats by a single subcutaneous injection of monocrotaline (60 mg/kg; MCT group); control animals received the same volume of saline (NaCl 0.9%; CTRL group). Myographic recordings were performed in right ventricle (RV) strips paced electrically at 2 Hz-frequency. Immunolocalization studies were performed by confocal microscopy.

Selective activation of the A<sub>2B</sub>AR with BAY 60-6583 (0.01–10 μM) was devoid of effect in RV inotropy in both CTRL (*n*=4) and MCT (*n*=5) rats. Pre-treatment of RV strips with the A<sub>2B</sub>AR antagonist, PSB 603 (100 nM), that displays >17000-fold selectivity over other AR (*K<sub>i</sub>* = 0.55 nM), converted the negative inotropic effect of the non-selective AR agonist, NECA (0.01–100 μM), into a mild positive inotropic action in the MCT group (*n*=5), but no effect was observed in CTRL rats (*n*=5). Interestingly, the positive inotropic effect of NECA (0.01–100 μM) in the presence of PSB 603 (100 nM) was abrogated by the simultaneous blockade of the A<sub>1</sub>AR with DPCPX (10 nM, *n*=5), suggesting that activation of both A<sub>1</sub>AR and A<sub>2B</sub>AR may cooperate to decrease RV contractile activity. The effect of PSB 603 (100 nM) was not mimicked by inhibition of adenylate cyclase, phospholipase C and protein kinase C with SQ 22356 (30 μM, *n*=3), U73122 (3 μM, *n*=2) and chelerythrine (3 μM, *n*=4), respectively; it was also not mimicked by the A<sub>2B</sub>AR inverse agonist, MRS1706 (10 nM, *K<sub>i</sub>* = 1.39 nM). That is, none of these drugs were able to modify the concentration-response curve of NECA (0.01–100 μM) in both MCT and CTRL animals. Immunolocalization studies showed that RV cardiomyocytes from CTRL and MCT rats express small amounts of A<sub>2B</sub>AR. The A<sub>2B</sub> immunoreactivity increased significantly in cells infiltrating RV interstitial spaces, which also stain positively for CD11b (macrophages) and vimentin (fibroblasts).

Data show here for the first time that A<sub>2B</sub>AR may cooperate with A<sub>1</sub>AR to reduce inotropy in overloaded RV secondary to PAH. Further studies are, however, required to investigate the functional role of the A<sub>2B</sub>AR-positive cells infiltrating the RV of MCT animals and whether they constitute a novel target for therapeutic management of PAH.

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# Distribution profile of adenosine receptors in the mesenteric vessels of spontaneously hypertensive rats

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Adenosine is an endogenous purine with an ubiquitous distribution whose action is mediated by a group of G protein-coupled receptors, the adenosine receptors. By now, four subtypes of adenosine receptors were identified: A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub>. These receptors were found in different kind of tissues and seem to have an important role in both physiological and physiopathological conditions. In vascular tissue, they seem to have an important role in vascular flow control by acting upon neuromodulation and smooth muscle contraction. In addition, previous studies reported differences in adenosine levels and its receptors signaling in hypertension. In this work, we aim to characterize the distribution profile and density of adenosine receptor subtypes (A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub>) in normotensive (WKY) and hypertensive (SHR) mesenteric artery and vein, in way to better understand the putative changes of adenosinergic system dynamic in hypertension.

In the experimental protocol used in this study, after intracardiac perfusion, mesenteric vessels (artery and vein) of spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) rats were fixed in Bouin and embedded in paraffin. Sections immunolabelled with a primary antibody directed against individual adenosine receptors, were detected with a biotinylated secondary antibody, the ABC complex, and using 3,3'-diaminobenzidine as chromogen. In control sections primary antibody was omitted. Digital images of tissue samples were acquired using the same microscope/CDD camera operating conditions. Quantitative analysis of the images was carried out using PAQI software (CEMUP). Boundaries were delineated to extract the object of interest. Lower intensity level of the control images was determined and used for threshold segmentation of the objects of interest (regions of intensity <150). Results are expressed as mean±s.e.mean; P<0.05 was taken to reflect statistically significant differences (ANOVA followed by Holm-Sidak t test).

Results indicated that in veins only adenosine A<sub>2A</sub> receptor immunoreactivity was higher in SHR (38.97±2.96, n=18) than in WKY (23.61±3.92, n=12, P<0.05). By contrast, on SHR arterial territory, adenosine A<sub>1</sub> (29.46±3.75, n=21) and A<sub>2B</sub> (20.29±4.23, n=23) receptor's immunoreactivities were reduced in comparison with data obtained in WKY mesenteric arteries (43.69±3.91, n=14, P<0.05 and 45.99±4.24, n=15, P<0.05 for adenosine A<sub>1</sub> and A<sub>2B</sub> receptor, respectively). Furthermore, during qualitative analysis we also noticed alterations in the distribution profile of A<sub>2A</sub> receptor in SHR's arteries. Qualitative analysis revealed that all the differences reported occurred in vessel's adventitial layer.

All subtypes of adenosine receptors were found in hypertensive mesenteric vascular tissues thought with a different profile of immureactivity for A<sub>1</sub>, A<sub>2A</sub> and A<sub>2B</sub> adenosine receptors. These results support the occurrence of changes in adenosinergic system dynamic in hypertension, which may have a significant role in the pathophysiology of this disease.

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ENCONTRO INVESTIGAÇÃO JOVEM  
DA UNIVERSIDADE DO PORTO

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**A5** **PSYCHOLOGY &  
EDUCATION SCIENCES I**  
PARALLEL ORAL SESSIONS II

# Exploring *praxe* among peers: Participation, voice and power issues

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*Praxe* should facilitate students' inclusion in higher education communities and transitions between cultures, valuing diversity. It's a niche economy and issues regarding power, voice, or legitimate and peripheral participation (Lave & Wenger, 1991) need further research. Based in social interactions (Vygotsky, 1934/1962), it's seen as a way to promote socialization and learning (Frias, 2003). Students' social representations of higher education institutions and themselves as learners are dynamic (Marková, 2005; Moscovici, 2000), shaping their life trajectories of participation (César, 2013). Their dialogical self assumes different I-positions (Hermans, 2001). The main goals of this work were: (1) to identify higher education students' social representations regarding *praxe*; and (2) to study their participation in it. We assumed an interpretative approach (Denzin, 2002) and developed an exploratory intrinsic case study. The participants were 841 higher education students, opinion leaders from organised groups pro and against *praxe*, journalists and decision makers. Data collecting instruments were documents, two questionnaires, interviews, and informal conversations. The results illuminate that 75% of these students participated in *praxe* and 10% did not participate at all. Friends, parents and families are those who influenced them the most. The way these students conceive power relations, legitimate or peripheral participation, and voice issues is controversial. Thus, these are expanding interactive practices needing further studies to avoid their negative effects: deaths and dropouts to avoid being humiliated.

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# Integration of New Media in educational paths: the perspective of students from the University of Porto

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As society changed over the years, technological advancements, "information and communication media, new platforms and new digital and technological devices invaded the daily lives of millions of people (...)" (Pimenta, 2012-2013:22), particularly the lives of almost all youngsters. These advancements have become indispensable in the lives of each and everyone, helping them build teaching and learning processes, by using these new technologies in their academic career.

Young as we are, we are aware that new technologies are not only part of everyday life of each youngster, but it also has an influence on it. Thus, in our research work we defined the use of the New Media in the academic career of students as the object of study, and we chose the students of the University of Porto. For equality purposes, we decided that students would be of both genders, and from the 3<sup>rd</sup> grade of the respective courses. For the semi-structured interviews to be carried out, we developed a script based on the intended criteria, and by resorting to qualitative research. After analysing the results we have achieved, we see that youngsters use the New Media not only for social networking, but also to communicate, to study and for other purposes.

Students, and society in general, are faced with an undeniable change in the ways of studying, working and relating to each other. This is a changing society, perhaps because the "technological revolution centred on information technologies, began to reshape, at an accelerated pace, the material basis of society" (Castells, 2002: 1). As such, the manner and purpose for which students use new technologies is changing with society. We thus live in transformation.

**Acknowledgements:** This study is the result of a semester long class, and all the students of the class participated.

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# Background experiences and motivations underlying volunteer experience

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Living in a time of economic and financial crisis, volunteering can prove to be one of the most significant resources of our society. And, being it also a crisis of values, becoming our society increasingly individualistic, what is it that leads some people to devote, for free, part of their time and energy to others? Research shows that altruistic motivations are on the basis of this involvement, focusing, however, most studies on specific contexts and groups of volunteers, none having been found which assesses the Portuguese volunteering phenomenon in a more transversal way. However, literature tends to present a blank as to what, in the lives of individuals, may have acted as a trigger event to the beginning of this practice. Thus, this study's main objectives are (a) to determine the motivations identified as having been decisive at the beginning of his involvement in the role of volunteer and (b) to explore life events perceived as influential in this. Secondly, we analyzed, on an exploratory basis, the relation between motivations and life events. To do so, we adopted a mixed methodology (quantitative and qualitative) having collected data from 231 volunteers (62.3% belonging to Health entities and 37.7% to Religious and Social Solidarity entities). In the assessment of the initial motivations it was used the Volunteer Motivation Inventory (VMI), Esmond and Dunlop (2004) [1]. The exploratory factor analysis applied did not validate the 10 factors of the original scale, reducing them to the following five motivational dimensions: Social Benefits, Direct Benefits for the Self, Coping Strategies, Identification with the Cause and Selflessness. The study acknowledged the Identification with the Cause as the most salient motivational dimension for the participants. Age, marital status, professional status, the Entity and the Time of volunteer work have shown to have significant effect on some of the motivational dimensions. The results related to life events led to the emergence of (positive and / or negative) "memories of childhood, adolescence and youth significant episodes" as the most influential background experiential category in the participants' decision to become volunteers.

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# Academic identity (re)construction

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This paper discusses a master's thesis entitled *Academic identity construction: trajectories, contexts and roles*, developed at the Faculty of Psychology and Educational Sciences of the University of Porto. The contemporary academic setting is characterized by continuing alterations considering that the educational system follows the social, political and economic changes of a country. Thence, it's possible to understand that exterior agencies reconfigure the concerns, structures and goals of the university [1]. These external transformations may impact the academic identity.

The research aims at comprehending the (re)construction of this academic identity regarding this scenario of continuing alterations. The theoretical framework for this study consists of the discussion about identity conceptions and about teaching in higher education. Identity is considered as a social process, in a continuing movement of (re)construction, developed on the subject-society dichotomy [2]. The research is also interested in the four areas of work of a lecture: research, teaching, knowledge exchange and academic management, considering their articulation [3].

A focus group and four biographical narratives were conducted with lectures from the Educational Sciences field in a Portuguese university, as methodological strategies. Content analysis was defined as this research method for data interpretation.

Results consider the identity construction all through lectures' life. The professional identity construction emerges, such as familiar core, initial training and professional experiences. Results show that academic identity is characterized by work intensification. Analysis highlights that lectures consider the research as priority, advantage and essence of higher education. Secondly, Teaching emerges as an committed and important area, but undermined by the lack of time, a reason why all professors demand its recognition and its articulation with research. Academic management is linked to obligatoriness and precedence. Participants made an evaluation that the effective participation in the leadership activities is narrowed since these activities are administrative and bureaucratic. Knowledge exchange represents a point of articulation between interests and goals of a university, concerning production, presentation and discussion of scientific knowledge.

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PARALLEL  
ORAL  
SESSIONS III



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**A1** **ENGINEERING III**  
PARALLEL ORAL SESSIONS III

# Rheological behaviour of Olive Oil: Temperature vs Viscosity

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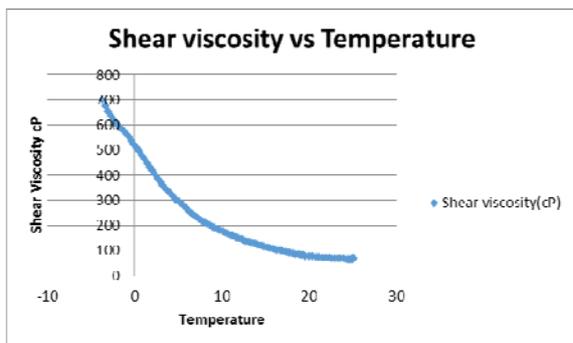
<sup>2</sup>ENEAS, Faculty of Engineering, University of Porto, Portugal.

Viscosity as a measure of the resistance to flow or shear, is a fundamental characteristic property of all the matter and produces some of the most important parameters required in the planning and design of technological processes.

ENEAS (European Network for Environmental Assessment and Services) acquired recently a last generation rotational rheometer, intended to study the mechanical properties of liquids, emulsions and suspensions. This Kinexus™ pro+ rheometer has dual-action capabilities for both shear and vertical testing and also enables pioneering Standard Operating Procedure driven rheological testing.

Olive oil, a very praised fat obtained from olives, and the most important ingredient of the so famous Mediterranean diet, is a rich substance in fat acids, oleic acid, linoleic acid and vitamins A and E. It is commonly used in cooking, cosmetics and others. For some countries that have a large export and consumption of olive oil it is fundamental to characterize and understand the rheological behavior of this liquid. It is a very interesting way to ascertain the genuine character of olive oil as also its origin. The ultimate goal of our work is the devise of a series of procedures intended to define DOC (denominação de origem controlada =“Controlled designation of origin”) olive oils.

On a first approach the shear viscosity of several brands of olive oil was studied using temperatures between -3°C e 30°C, using a rotational rheometer [1] (Kinexus Pro+, Malvern™). This equipment gives us the opportunity to control at the same time the temperature range and the shear stresses used. The comparisons between our results and those in literature allowed us to obtain already some new information about olive oil. Namely, we are already able through this non expensive and expedite test to separate between legitimate olive oil and some poor quality brands that correspond to the mixture of several alimentary oils [4].



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# Hydrogen generation from catalytic hydrolysis of sodium borohydride for portable applications: new challenges

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It is well-known that a proton exchange membrane (PEM) fuel cell is an attractive alternative power source for portable applications. However, the performance of PEM fuel cell depends on the supply of purity of the fuel – hydrogen (H<sub>2</sub>). Bearing in mind a H<sub>2</sub> releasing on demand, chemical hydrides are pointed as the chosen *hydrogen carrier* since they have shown attractive features such as safe handling, high hydrogen densities and fast kinetics of hydrogen release. Sodium borohydride (NaBH<sub>4</sub>), with a H<sub>2</sub> content of 10.8 wt%, is the finest example. It reacts with water to generate molecular H<sub>2</sub> from catalytic hydrolysis reaction presented by Eq. (1), where  $x$  represents the hydration factor. [1]



According to Ferreira et al. (2012) [2], the reactor geometry has an important role on the reaction performance. Thus, a portable batch mini-reactor, with an ovoid optimized geometry, was designed to generate and storage H<sub>2</sub> from the NaBH<sub>4</sub> hydrolysis reaction. The novel geometry not only provides innovate insights to establish an H<sub>2</sub> based economy for portable applications, but also brings out new challenges: (i) outstanding catalyst – the demand for a catalyst with high durability, high reusability and lower cost that induces the generation of fast H<sub>2</sub> with negligible induction times; (ii) refueling – investigation of the capability of reuse a constant amount of catalyst during successive loadings of fuel without any treatment and minor loss of activity; (iii) storage in the liquid phase – improve the affinity of H<sub>2</sub> storage in liquid phase through solubility effects by changing the polarity of the remaining solution from the addition of small quantity of additives (green polymers, ionic liquids, surfactants, etc.) in order to increase the non-polar H<sub>2</sub> electrolyte interactions; (iv) recyclability of the reaction by-products – implement and validate alternative solutions for sodium metaborates conversion into NaBH<sub>4</sub> with economical potential; (v) reaction mechanism – development of an adequate kinetic model for H<sub>2</sub> generation from NaBH<sub>4</sub> catalytic hydrolysis reaction under moderate pressure.

The present work is committed in exploring these subjects based on experimental NaBH<sub>4</sub> hydrolysis kinetic data.

Acknowledgements:

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# Microalgal biofilms: the effect of surface physicochemical properties

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Microalgal harvesting is one of the major limitations of microalgal production systems. Currently applied harvesting methods may be time-consuming, expensive or toxic to the biomass. To overcome these problems, microalgal growth in biofilms can be a solution, as the cells grow attached to a surface allowing a simple and efficient harvesting of biomass. According to the literature, biofilm development depends on the type of surface and their physicochemical properties [1]. Accordingly, surface hydrophobicity of different microalgae and cyanobacteria and different materials was determined through contact angle measurements. From these results, the free energy of adhesion between the studied microorganisms and surfaces was predicted and these results were validated through adhesion assays using the cyanobacterium *Synechocystis salina*. Polystyrene (PS), AISI 316 stainless steel (SS), polyvinyl chloride (PVC), poly (methylmethacrylate) (PMMA), glass (G) and copper (Cu) were used as adhesion surfaces. For biofilm formation assay, two different media were used: a synthetic effluent (SE) and a test medium described in OECD legislation. The presence or absence of cells on the coupons was calculated as the CFU cm<sup>-2</sup>.

According to the literature, lower free energy of adhesion ( $\Delta G_{\text{adhesion}}$ ) values indicate higher affinity between the surfaces and, consequently, higher ability for biofilm formation. This hypothesis was confirmed through the adhesion assays, since higher concentrations of attached cells were determined in the surfaces presenting lower  $\Delta G_{\text{adhesion}}$  values (Table 1). Copper was the only exception, because through the  $\Delta G_{\text{adhesion}}$  value, it was expected the attachment of cells to the coupons. However, several studies have referred the mechanism of toxicity of copper to algae, which help to explain the obtained results [2].

**Table 1.** Free energy of adhesion,  $\Delta G_{\text{adhesion}}$ , (mJ m<sup>-2</sup>), and CFU cm<sup>-2</sup> between *S. salina* and the six studied surfaces when immersed in water. The means  $\pm$  SD for two replicates are given

Surface	$\Delta G_{\text{adhesion}}$ (mJ m <sup>-2</sup> )	CFU cm <sup>-2</sup>	
		SE	OECD
PS	30.0 $\pm$ 10.5	210 $\pm$ 9	233 $\pm$ 14
SS 316	23.5 $\pm$ 3.7	393 $\pm$ 5	286 $\pm$ 6
PMMA	18.7 $\pm$ 0.7	10 $\pm$ 14	300 $\pm$ 7
PVC	6.15 $\pm$ 9.7	8 $\pm$ 4	478 $\pm$ 10
G	3.2 $\pm$ 5.3	138 $\pm$ 16	452 $\pm$ 19
Cu	-11.9 $\pm$ 1.4	0 $\pm$ 0	0 $\pm$ 0

Concluding, the physicochemical properties of a surface can be used to predict microalgal adhesion to surfaces. There was a good correlation between the free energy of adhesion and the number of colony forming units.

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# Bioethanol from Brewers' Spent Grain: Pentose Fermentation

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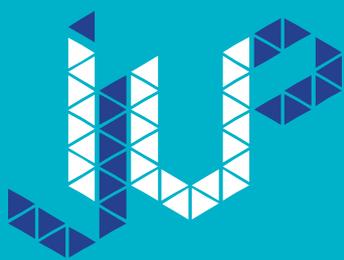
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Lignocellulosic biomass is one of the most promising feedstock alternatives for liquid biofuels (Caetano et al., 2014). The production process involves several steps including the biomass pre-treatment, hydrolysis of carbohydrates, fermentation of simple sugars into ethanol, and distillation for product recovery [1]. BSG represent around 85 % of the total by-products generated by the brewing industry [2]. Its chemical composition varies depending on the barley variety and harvest time, and on the brewing process conditions. On a dry weight basis, BSG contains about 40 - 50 % polysaccharides (consisting of 15 - 18 % cellulose, 24 - 31 % hemicellulose and 2 - 3 % starch) and 30 % or more proteins [3]. So far, the main destination for BSG has been cattle feeding, due to its high content of protein and fiber [1]. However this application has limited market value and other uses are under research, such as bioethanol production. Thus, this work aims to perform a preliminary optimization of the fermentation of brewers' spent grain (BSG) sugars to bioethanol, focusing on the pentose sugars. Firstly, it was conducted the acid pre-treatment and enzymatic hydrolysis for studying different reaction times and amounts of enzymes in order to convert cellulose and hemicelluloses into simple sugars. The greatest amount of total sugars achieved experimentally is 5.56 g/ 25 g of dry BSG, corresponding to a sugars maximum conversion of 22.24 % from a BSG sample with about 6 % cellulose and about 40 % hemicelluloses. This was obtained by sequentially adding the acids HCl and HNO<sub>3</sub> with 1 wt% of concentration to BSG to perform the pre-treatment, simultaneously with 1.0657 g of Glucanex 100g and 2.0 mL of Ultraflo L for the hydrolysis. Secondly, it was studied the fermentation step, using both synthetic medium and BSG hydrolyzate, and the yeasts *Pichia stipitis* NCYC 1541 (*P. stipitis*) and *Kluyveromyces marxianus* NYCY 2791 (*K. marxianus*). Results show that the fermentation efficiency of all sugars in the synthetic media is higher than 80 % for both yeasts, but in the BSG hydrolyzate it is just 45.10 % for *P. stipitis* and 36.58 % for *K. marxianus* for a 72 h fermentation time at a 30 °C temperature. The theoretical ethanol yield from BSG hydrolyzates is 0.27 and 0.19 g ethanol/ g of sugars for respectively, *P. stipitis* and *K. marxianus*, but the actual ethanol yield obtained in this work is 0.0856 and 0.0308 g ethanol/ g of sugars, respectively that is three times smaller than the theoretical yield for *P. stipitis* and six times smaller for *K. marxianus*, which can be attributed to the presence of inhibitors resulting from the previous steps.

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**A2** **CHEMISTRY III**  
PARALLEL ORAL SESSIONS III

# Computational Study of Chemical Reactions inside Nanoreactors

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Nanoreactors (NR) have been widely studied in recent decades and the results obtained so far have been promising regarding their applicability in several areas, particularly in the development of drugs and industrial processes. Among these NR, carbon nanotubes (CNT) emerged and, as such, the interest by the scientific community in these materials has been growing exponentially ever since its discovery [1].

Chemical species, under spatial and chemical confinement, inside CNT may show dramatic changes in their physical and chemical properties when compared to gas phase conditions. Thus, the effect of confinement of a variety of molecules, as well as, the study of chemical reactions, has been extensively studied [2].

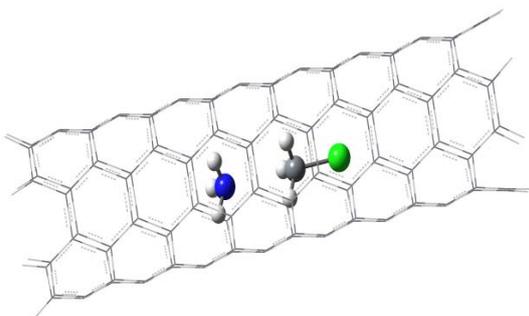


Figure 1 - NR with  $S_N2$  Menshutkin reactants.

This work aims at studying the structural and energetic features of chemical reactions when confined inside different single walled carbon nanotubes (SWCNT). The system is based on a computational methodology where the reaction system is treated at a rigorous quantum density functional (DFT) level, using the program package Gaussian 09.

The effect of the chirality and diameter of several SWCNT on the conformational and energy profile of  $S_N2$  Menshutkin reaction [3] (Fig. 1) was carried out as first approach.

The preliminary results show a strong polarization effect of the *armchair* SWCNT on the reaction, by decreasing the energy barrier and endothermicity, as observed previously in aqueous medium [4]. In addition, the results suggest as a general trend that the values of the two energetic parameters are proportional to the diameter of these SWCNT.

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# Self-organization in amino acid-based surfactants: from ribbons to nanotubes

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Surfactants with chiral headgroups, such as amino acid-based surfactants, often self-assemble in water into a wealth of complex nanostructures due to the combined effect of their hydrophobic/hydrophilic balance (packing parameter), chirality and headgroup charge and H-bonding interactions.[1,2] Thus, besides forming micelles, vesicles and liquid crystalline phases, they have the possibility of forming extended discrete assemblies such as fibers, ribbons and nanotubes. Parameters such as temperature, concentration and pH clearly influence the thermodynamic stability of a given structure over the others.[3] These aggregates with a high  $d/L$  aspect ratio have high potential as gelators and as stimuli-sensitive nanocarriers for biomolecules.[1-2] In this work, we present a detailed microstructural investigation of the aggregates formed by double-chained anionic lysine-based surfactants, designated as 8Lys16, 12Lys12 and 12Lys16.[3] All the surfactants below their chain melting temperature self-organize into tube-like “crystalline” structures, comprising twisted ribbons, coiled ribbons and nanotubes, which eventually induce gelation of the aqueous dispersion. Combined data from phase contrast light microscopy, TEM, SEM and AFM were used to obtain a consistent picture of the morphologies formed. The long-chained compound, 12Lys16, has the particularity of spontaneously forming stable vesicles once it solubilizes around 50 °C, while the other surfactants form micelles.[3] The effects of the chain length mismatch on the type of structures (size distributions and polydispersity) formed and their evolution towards equilibrium will be presented and interpreted at molecular level.

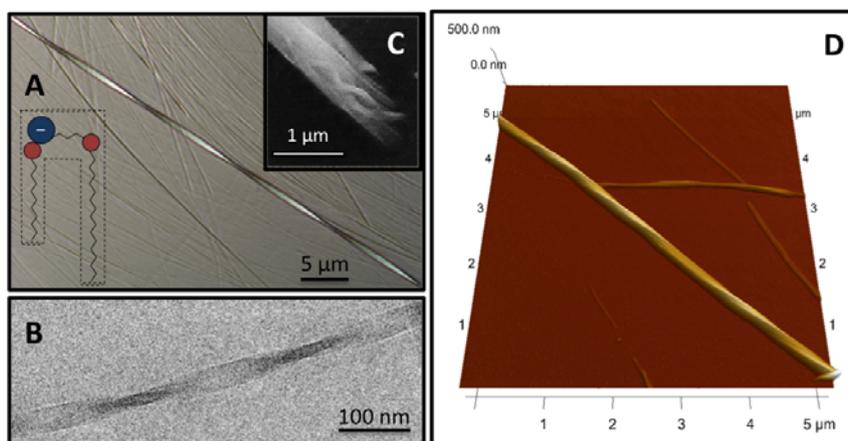


Figure: Twisted ribbons from 8Lys16 showing different length scales for the helical pitch, as observed by: A) phase contrast light microscopy; B) cryo-TEM; c) cryo-SEM; D) AFM.

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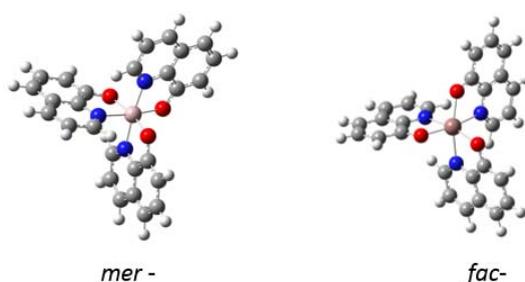
# Tris(8-Hydroxyquinolinato)M(III): Exploring The Molecular and Solid State Properties

**R.J.S Taveira,<sup>1</sup> C.F.R.A.C. Lima,<sup>1,2</sup> J.C.S. Costa,<sup>1</sup> L.M.N.B.F Santos<sup>1</sup>**

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Tris(8-Hydroxyquinolinato)M(III) derivatives belong to the list of the most used electroluminescent/electron transport materials in the production of Organic Light Emitting Diodes (OLEDs). These organometallic compounds have the common abbreviation of  $Mq_3$ , where M is the trivalent coordinated metal (e.g. Al(III), Ga(III) and In(III)) and q is the ligand, which can be 8-hydroxyquinoline or some derivative.<sup>[1]</sup> Depending on the substituents in 8-hydroxyquinoline, the  $Mq_3$  compounds may adopt *mer*- or *fac*- molecular configurations. This work focuses on evaluating how different substituents and metals influence the *mer*-/*fac*- isomerism in this class of compounds, and how their preferred molecular structure affects solid phase stability (e.g. existence of polymorphism and solid-solid transitions).



**Fig. 1-** *Mer*- and *fac*- conformations in Tris(8-Hydroxyquinolinato)M(III) derivatives

In this work, several  $Mq_3$  derivatives were synthesized using different metals and substituted 8-hydroxyquinolines, and characterized by UV-Vis, Fluorescence and NMR spectroscopy and DSC. We aim to explore the influence of small structural changes at the molecular level on the solid phase properties of Tris(8-hydroxyquinolinato)M(III) derivatives, and contribute for understanding and improving the applicability and efficiency of this class of compounds as organic semiconductors.<sup>[2]</sup>

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# PREPARATION AND CHARACTERIZATION OF NOVEL HYBRID Co<sub>4</sub>POM@CARBON-BASED NANOMATERIALS

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Carbon nanomaterials such as carbon nanotubes and graphene, have attracted considerable interests in several fields, owing to their unique physical, chemical and electrochemical properties [1,2]. The chemical functionalization through the non-covalent modification allows maintaining the structural integrity and electronic properties of carbon nanomaterials and immobilized species. Among the many compounds that can be immobilized on carbon nanomaterials are the polyoxometalates (POMs), which are a group of metal-oxygen clusters with a variety of structures, composition and properties [3,4]. These include the ability to reversibly attract and release a large number of electrons under marginal structural rearrangement producing the mixed-valence coloured species which makes them suitable materials for different applications [4]. Polyoxometalates have shown a promising way to modulate the outstanding properties of graphene flakes (GF) and carbon nanotubes (CNTs) for applications related to electrocatalysis [5] and in fuel cells technology with focus on cathodic oxygen reduction reaction (ORR) [1, 6].

In this work we present the immobilization of tetrabutylammonium salt of [Co<sub>4</sub>(H<sub>2</sub>O)<sub>2</sub>(PW<sub>9</sub>O<sub>34</sub>)<sub>2</sub>]<sup>10-</sup> (Co<sub>4</sub>POM) into four different carbon based nanomaterials: graphene flakes (GF), nitrogen-doped few layer graphene (N-FLG), single-walled carbon nanotubes (SWCNTs) and nitrogen-doped carbon nanotubes (N-CNT). All these compounds were fully characterized by several techniques such as FT-IR spectroscopy, XPS spectroscopy and cyclic voltammetry confirming the incorporation of Co<sub>4</sub>POM in the different carbon based nanomaterials.

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# Microwave-assisted bromination of *meso*-tetraphenylporphyrin

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The electronic properties and coordination chemistry displayed by porphyrins makes them attractive compounds for applications in photodynamic therapy (PDT) and diagnosis, as models of naturally occurring compounds, as oxidation catalysis and as chromophores in molecular recognition and materials science[1]. The importance of these applications has stimulated intense efforts in developing efficient strategies for the preparation of porphyrin derivatives having a variety of different substituents, both in *meso*- and  $\beta$ -pyrrolic positions of the macrocycle.

In this work we proceeded to the microwave-assisted synthesis of  $\beta$ -bromo-*meso*-tetraphenylporphyrin (**1**, Fig. 1) by bromination of *meso*-tetraphenylporphyrin (**2**, Fig. 1) using *N*-bromosuccinimide in  $\text{CHCl}_3$ . The results obtained in microwave heating will be compared with those obtained under classical oil-bath heating conditions[2]. We will also present the optimized conditions for the preparation of di-brominated derivatives. The  $\beta$ -bromo-*meso*-tetraphenylporphyrin (**1**, Fig. 1) and its zinc complex (**3**, Fig.1) were both characterized by NMR, absorption and emission spectroscopies and mass spectrometry.

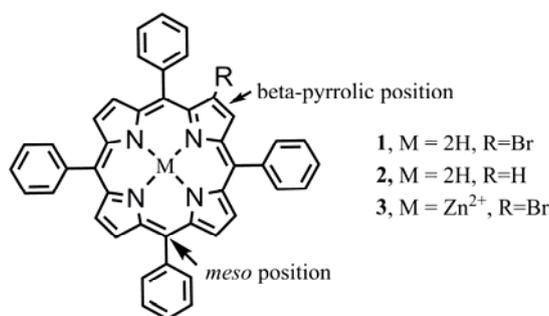


Fig. 1. Structure of *meso*-tetraphenylporphyrin and brominated derivatives.

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# Going forward with Menshutkin chemical reaction inside functionalized carbon nanotubes

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A rolled-up graphene sheet gives rise to the so-called carbon nanotubes.<sup>[1]</sup> Their diameters are in the nanometer range and structurally they can be *chiral*, *zig-zag* or *armchair* depending on the  $(n,m)$  indices. Also, their electrical, optical and mechanical properties have attracted the attention of the scientific world in several research and application fields.

In 2002, Halls and Schlegel studied, at DFT level, the energetics of the Menshutkin reaction inside finite *zig-zag* carbon nanotubes (8,0) and (9,0) terminated with hydrogen atoms.<sup>[2]</sup> They found a decrease in the activation energy due to the confinement of the reaction coordinates in a non-polar medium.

The aim of this work was to take the confinement ability of these carbon nanomaterials a step forward by functionalizing its dangling bonds with different electronegative species and perform a computational study at DFT level. It was found a decrease in the energy barrier, despite the DFT functional used and the type of chemical functional group, which resulted from a polarization effect of the sidewall of the material (Fig. 1).

These results may disclosure some advantages on using carbon nanotubes in industrial processes. Our future work focus on the effect of some other properties such as diameter and chirality.

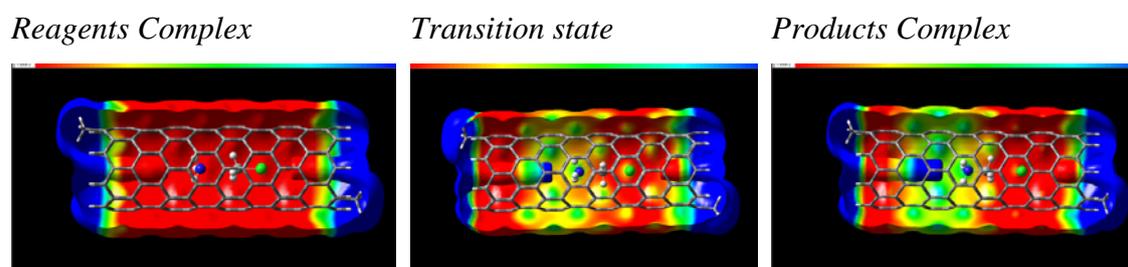
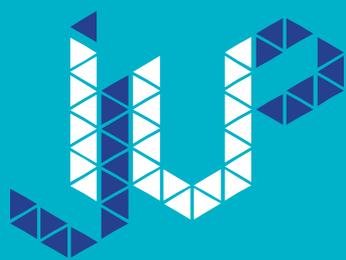


Fig. 1 Potential energy surface of Menshutkin reaction coordinates inside (8,0) CNT functionalized with one methyl group on each side calculated with M06-2x DFT functional. Negative electrostatic potential in red and positive in blue.

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ENCONTRO INVESTIGAÇÃO JOVEM  
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**A3** **BIOLOGICAL SCIENCES III**  
PARALLEL ORAL SESSIONS III

## Acute and subacute functional effects of relaxin-2 on human mammary artery

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Relaxin is a peptide hormone classically associated with important adaptive changes that occur in the reproductive system during pregnancy [1]. Recently, several studies have explored its cardiovascular effects and established relaxin as a new investigational drug in heart failure [2]. Nevertheless, its effects on human vascular territory still remain to evaluate. This research intends to assess the functional effects of relaxin-2 on human mammary artery (HMA) vasoreactivity.

HMA samples from 13 patients submitted to coronary revascularization (10 male patients, ages 67 to 83 years) were dissected, divided into small rings and underwent one of two experimental protocols: acute incubation (n=8 rings) or subacute incubation (n=18) with relaxin-2. In the acute incubation protocol, the vascular rings were mounted in a myograph bath system containing Krebs solution (pH 7.4±0.1) and normalized to the transmural pressure of 100 mmHg. Vascular viability was evaluated by incubation with KCl (0.1 M). Following phenylephrine-induced maximal vasoconstriction ( $10^{-5}$ M), the rings were exposed to increasing concentrations of relaxin-2 ( $10^{-10}$  –  $10^{-7}$ M) or vehicle. In the subacute protocol, the rings were incubated in DMEM culture medium with relaxin-2 ( $10^{-7}$ M) or vehicle for 24h and were mounted, normalized and had their viability determined by the same method. Following phenylephrine-induced maximal vasoconstriction ( $10^{-9}$  –  $10^{-5}$  M), the rings were incubated with increasing concentrations of acetylcholine ( $10^{-9}$  –  $10^{-5}$  M).

No differences in the active tension of the rings acutely incubated either with relaxin-2 or vehicle were observed. Following subacute incubation, there were also no differences in the vascular viability or the phenylephrine vasoconstrictor effect between groups. However, the subacutely relaxin-2-treated rings showed a statistically significant improved relaxation in response to acetylcholine (59.1±6.1%) when compared to the vehicle-treated rings (46.2±6.2%, p<0.01). No differences between male and female patients were observed.

In conclusion, acute treatment with relaxin-2 does not influence HMA vasoreactivity. Subacute incubation with relaxin improves acetylcholine-induced vasodilatation, with no accompanying significant changes in the vasoconstrictor response. These results suggest that relaxin may contribute to an improvement in the HMA endothelial function, a crucial vascular territory in coronary revascularization surgery.

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# Restoring the heartbeat: Tracking cardiomyocyte proliferation

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The heart is the first organ to become fully functional in the vertebrate embryo and it is composed by cardiomyocytes (CMs) and other cell types (e.g. endothelial cells and fibroblasts), mainly derived from the cardiogenic mesoderm during embryonic gastrulation (murine embryonic day 6.5) [1]. Mammalian cardiogenesis involves the formation of a linear tube, already composed by synchronously beating CMs, which undergoes rightward-looping and subsequent cardiac remodeling, forming the primitive cardiac chambers [2]. During embryogenesis, heart growth occurs due to intensive proliferation of mononucleated diploid CMs. Shortly after birth, CMs undergo terminal differentiation (at around postnatal day 5), comprising gradual augmentation of the sarcomeres complexity and a drastic morphological rearrangement, from polygonal to rod shape [3]. This event correlates with CMs' cell cycle withdrawal and cytokinesis blockage, culminating, according to the species, in binucleation or polyploidy, and ceasing of proliferation ability. Afterwards, cardiac growth results exclusively from cell volume increase. Hence, the adult mammalian heart has been considered a post-mitotic organ, devoid of intrinsic proliferative capacity. Over the last years, reports of CMs renewal in the adult murine and human heart have contradicted this longstanding dogma [4]. However, the magnitude of cellular renewal remains elusive due to the tissue's cellular complexity, difficulties in isolating adult CMs and to the unavailability of specific cardiomyocytic surface markers.

In this work, we have implemented a novel protocol to isolate and identify murine CMs along ontogeny. Using this protocol and combining immunohistochemistry and imaging flow cytometry techniques, we intend to perform a detailed characterization of the morphometrics, nuclear dynamics and cell cycle status of CMs throughout mammalian ontogeny. The data obtained thus far will be presented. The knowledge acquired in the scope of this study will allow a better comprehension of the dynamics of CM development and maturation and is thus of potential therapeutic interest.

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# Characterization of the neonatal immune response to *Escherichia coli* infection

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Sepsis remains a leading cause of mortality among neonates. *Escherichia coli* is the most common cause of Gram-negative neonatal sepsis and meningitis, with a high case fatality rate (up to 40%). Moreover, many of the survivors could develop severe neurological defects. The current intrapartum antibiotic prophylaxis leads to a great health concern, once it can promote the emergence of resistant bacterial strains and alters the first microbiota colonization. The knowledge of how the neonatal immune system responds to bacterial infection and the mechanisms underlying its pathogenicity is imperative for the development of new strategies. Therefore, the aim of the present study was the characterization of the innate immune response to *E. coli* infection using a mouse model of neonatal infection. For that purpose, BALB/c mice were subcutaneously infected, two days after birth, with a lethal dose of the *E. coli* strain K1, a serotype associated with neonatal sepsis and meningitis. At several times post infection, several organs were collected *post mortem* to evaluate the colonization. Moreover, at early times after infection, the levels of pro and anti-inflammatory cytokines, chemokines and cell recruitment were also quantified.

The obtained results showed a rapid colonization of the liver and lungs of infected animals, as early as 1 hour after infection, and a late colonization of spleen and brain 12 and 18 hours post-infection, respectively. In parallel with colonization, it was observed an early IL-6 production after infection that increases over time, in the liver and lungs, while IL-1 $\beta$  production only increased at later time points. The individual blocking of both cytokines signaling did not alter the outcome of the infection. The levels of TNF- $\alpha$  and anti-inflammatory cytokines IL-10 and IL-27 did not change over time in these organs. Moreover, we observed a high influx of neutrophils in lungs and liver shortly after infection which, despite the high levels of the chemokines MIP-2, KC and MCP-1, decreased with time to values below control.

In conclusion, our results indicate that *E. coli* rapidly colonizes the host and the innate immune response developed is not efficient to combat the bacterium, probably due to the unique features of the newborn immune system. This kind of study could help in the identification of new potential targets to attenuate/prevent the disease caused by this pathogen.

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## **Effect of taurine supplementation of low-fishmeal based diets in white seabream (*Diplodus sargus*) juveniles**

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White seabream is a new aquaculture species whose production depends on fishmeal based diets. However, the current increased demand, price and limited availability of fishmeal (FM) lead to an urgent need to replace it by alternative ingredients such as plant feedstuffs. Besides difference in macro-nutrients, FM contains several non-protein nitrogen compounds that are little or no present in plant feedstuffs. This is the case of Taurine (Tau) highly presented in FM but devoid of plant feedstuffs consequently may become conditionally essential in low-FM diets. Thus, the objective of this study was to evaluate the effect of Tau supplementation in low FM diets for white seabream.

A growth trial was conducted for 10 weeks at 22 °C. Four isoproteic (36% crude protein) and isolipidic (18% crude lipids) diets were formulated. Diets were divided into two groups, one with 5% FM (corresponding to 3.5% of total dietary protein) and another with 15% FM (corresponding to 10.5% of total protein dietary) and a mixture of plant protein feedstuffs (soybean, corn gluten, pea protein concentrated and wheat meal), supplemented or not with 1% of Tau. All diets were adequately supplemented with L-methionine to restore its essential amino acid profile. Triplicate groups of 15 white seabream juveniles (mean initial body weight  $\pm 58$ g) were feed with these diets. Fish were fed to apparent satiation, by hand, two times a day, six days a week.

Growth performance, feed intake and efficiency of feed utilization were not significantly affected by dietary FM level. However, irrespectively the dietary FM level, Tau supplementation significantly increased growth performance and feed utilization efficiency. At the end of growth trial, dietary treatments had little influence on whole-body composition. Nitrogen intake was similar for all treatments, while nitrogen retention (express as g/Kg body weight/day or % nitrogen intake) was significantly improved by both the increase of FM level and Tau supplementation. Five hours after the first meal, plasma protein, cholesterol, triglycerides or lactate did not differ among treatments, but Tau supplementation significantly decreased plasma glucose levels.

In conclusion, reduction of dietary FM level from 15 to 5% did not affect growth performance and feed utilization of white seabream juveniles. However, for both FM levels concomitant supplementation with 1% of Tau significantly increased growth and feed utilization efficiency, suggesting that Tau was conditionally limiting in 15%FM and 5%FM diets. Tau significantly reduce glycemia probably by increased cellular glucose up-take.

This work was partially funded by the Project AQUAIMPROV (ref.NORTE-07-0124-FEDER-000038), co-financed by North Portugal Regional Operational Program (ON.2-O Novo Norte), under the National Strategic Reference Framework (NSRF), through the European Regional Development Fund (ERDF).

# Evaluation of the recently launched Rapid CARB Blue kit for detection of carbapenemase-producing Gram negative bacteria

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Blue-Carba test is a recently proposed method for detection of carbapenemase production, based on the colour change of a pH indicator (bromothymol blue) resulting from imipenem hydrolysis by a carbapenemase. We aimed to evaluate the performance of the Rapid CARB Blue kit, recently launched by ROSCO Diagnostica, in comparison with the in-house Blue-Carba test. Furthermore, a modified protocol for the in-house Blue-Carba test is proposed for testing bacteria from CLED agar.

Seventy-five isolates from different *Enterobacteriaceae* (*E. coli*, *K. pneumoniae*, *E. cloacae*), *Pseudomonas* spp. (*P. aeruginosa*, *P. pseudoalcaligenes*) and *Acinetobacter* spp. (*A. baumannii*, *A. pittii*, *A. haemolyticus*) species producing a wide diversity of carbapenemases from KPC (KPC-2, -3; n=11), VIM (VIM-1, -2, -34 ; n=15), NDM-1 (n=9), IMP-5 (n=3), SPM-1 (n=1), OXA-23 (n=8), OXA-40 (n=8), OXA-48 (n=8), OXA-58 (n=7), OXA-72 (n=1), GES-6 (n=1), GES-14 (n=1) and OXA-48+NDM-1 (n=2) types were included. In addition, thirty non-carbapenemase producers (including extended-spectrum  $\beta$ -lactamase- and/or AmpC-producing isolates) with or without alterations in outer membrane permeability were tested. Carbapenemase-production was confirmed by phenotypic methods, PCR and sequencing and/or spectrophotometric assays. Both tests (in-house and kit) were performed according to the respective recommendations. The sensitivity, specificity and positive and negative predictive values of the tests were calculated.

All carbapenemase producers were detected by Blue-Carba test, which showed 100% sensitivity and 100% specificity. The Rapid CARB Blue kit revealed a high sensitivity (93.3%) and specificity (100%), and positive and negative predictive values were 100% and 85.3%, respectively. False negative results were obtained only for two *P. aeruginosa* isolates producing VIM-2 or GES-6 and three *A. baumannii* producing OXA-23, OXA-40 or GES-14. The modified protocol used for testing bacterial suspensions from CLED displayed a very good performance in detecting carbapenemase-producing *Enterobacteriaceae* or *Pseudomonas* sp., whilst variable for carbapenemase-producing *Acinetobacter* spp. (88.6% vs 32% sensibility, respectively). This modification can be highly useful to decrease inter-operator variability associated with the amount and homogeneity of the inocula, and also to increase compatibility with routine microbiology workflows.

In conclusion, we report high sensitivity (93.3%) and specificity (100%) for the detection of carbapenemase producers by Rapid CARB Blue kit. Both test variants are reliable and can be easily adapted to routine microbiology laboratories for quick detection of carbapenemase-producing Gram negative bacteria.



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**A4** **BIOMEDICINE III**  
PARALLEL ORAL SESSIONS III

## Acute brain and peripheral organ toxicity of MDMA to adolescent rats following a human relevant binge dose.

**A. Teixeira-Gomes**<sup>1</sup>, **V.M. Costa**<sup>1</sup>, **R. Feio-Azevedo**<sup>1</sup>, **J.A. Duarte**<sup>2</sup>, **M. Duarte-Araújo**<sup>3</sup>, **E. Fernandes**<sup>4</sup>, **M.L. Bastos**<sup>1</sup>, **F. Carvalho**<sup>1</sup>, and **J.P. Capela**<sup>1,5</sup>

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3,4-Methylenedioxymethamphetamine (MDMA or “ecstasy”) is a psychoactive drug used in recreational settings by teenagers and young adults. Despite years of research on MDMA toxicity, the consequences from MDMA exposure during adolescence require additional research [1]. Thus, we aimed to assess the acute brain and peripheral organ toxicity in an adolescent rat model after exposure to a MDMA binge dose regimen, to mimic drug abuse by human adolescents.

Adolescent male Wistar rats (postnatal day 40) received three 5 mg/kg doses of MDMA, intraperitoneally, every 2h, while control animals received saline [2]. The temperature of each animal was monitored for 7h after the first dose and the animals were sacrificed 24h later. Brain areas (cerebellum, hippocampus, frontal cortex and striatum) and the liver, heart and kidneys were collected.

Significant hyperthermia was observed in the MDMA-treated group after the second and third doses. ATP content was significantly decreased in the frontal cortex of MDMA-treated rats. In all brain areas, no other differences among groups were found regarding glutathione, quinoproteins and protein carbonylation levels. In the peripheral organs, histological examination revealed significant morphological tissue alterations including vascular congestion after MDMA, but no signs of severe apoptosis or necrosis, corroborated by absence of increase in plasma biomarkers and tissue caspases. Moreover, the formation quinoproteins was significantly increased in the liver of MDMA-treated rats. In the liver, heart and kidneys no changes were found in glutathione, protein carbonylation or ATP levels following MDMA exposure.

These results suggest that the MDMA binge scheme used in adolescent animals promoted hyperthermia, acute morphological signs of organ tissue damage and energetic imbalance in the frontal cortex, without oxidative stress in the brain.

Acknowledgments:

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# Chemical composition of “Krokodil”: revealing a new street drug

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Homemade drugs are becoming a real threat over the last few years, since they are replacing the conventional psychoactive substances [1]. “Krokodil”, whose psychoactive substance is believed to be desomorphine, is one of these new emerging homemade drugs and is being used as a cheap substitute to heroin, mainly in Eastern Europe. This drug is responsible for a wide range of clinical signs, including gangrene and open ulcers in the skin of the addicts [2]. “Krokodil” is made using commercial materials easily available and the synthesis is performed “at home” with no controlled or sanitary conditions [3]. Therefore, the chemical composition of street “Krokodil” must be complex, presenting a mixture with a large number of compounds.

In this study, we intended to prepare “Krokodil” using the “street” materials and to contribute to a better knowledge of this drug by establishing its chemical profile. Therefore, a large number of samples were synthesized mimicking a “street” method and their TLC, GC-MS, and HPLC-DAD profiles were recorded. It was verified that dozens of substances are present and the relative amount of desomorphine varies from sample to sample. All synthesized samples were fractionated by flash column chromatography and each fraction is being analyzed by preparative HPLC. The isolate compounds will be structurally elucidated by spectroscopic methods.

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# EXPLORING THE SYNTHESIS OF PLG PEPTIDOMIMETICS WITH POTENTIAL NEUROPROTECTIVE ACTIVITY BASED ON PICOLINIC ACID SCAFFOLD

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The tripeptide L-prolyl-L-leucylglycinamide (PLG, Fig. 1) is naturally present in the human body from the C-terminus cleavage of hormone oxytocin.[1] It is known that PLG show biological activity in neuronal transmission by positive alosteric modulation of D<sub>2</sub> dopaminergic receptors, denoting to have substantial therapeutic activity in Parkinson's disease. 2-picolinic acid (PIC) plays an important neuroprotective role since it protects neuronal damage against inductors of neurotoxicity in the brain.[2] Bearing this in mind we embarked in a synthetic program in order to achieve structure-related PLG prolinomimetics derivatives based on PIC scaffold (Fig. 1).

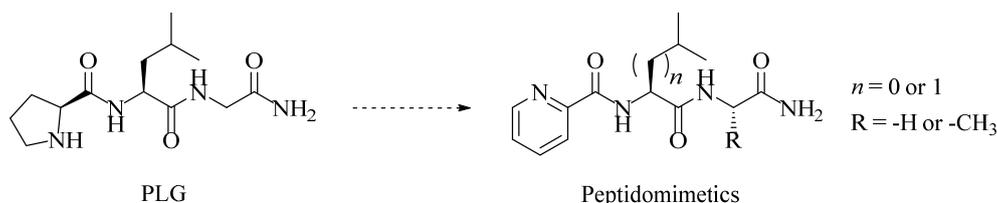


Fig. 1. PLG structure and the peptidomimetics based in PIC scaffold proposed to synthesize.

The methodology employed to achieve peptidomimetics are listed below:

- 1) Peptide coupling between PIC and the appropriated amino acid in C-protected form (methyl ester) using TBTU as coupling reagent to afford the desired dipeptides;
- 2) Alkaline hydrolysis using LiOH in a mixture of MeOH/H<sub>2</sub>O followed by dropwise addition of 1M H<sub>2</sub>SO<sub>4</sub>, affording the corresponding carboxylic acid;
- 3) Peptide coupling in the same conditions mentioned in 1) with the appropriated amino acids in C-protected form (methyl ester) to achieve the corresponding tripeptides;
- 4) Conversion of methyl ester into primary amide by treatment of gaseous NH<sub>3</sub> in methanolic solution of tripeptides made in 3), affording the final peptidomimetics.

Biological assays of the compounds will be held at University of Santiago de Compostela to assess both the neuroprotective potential against neuronal H<sub>2</sub>O<sub>2</sub> and glutamate injury as well as the modulation potential of dopaminergic D<sub>2</sub> receptors.

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# Neonatal Cardiac Regeneration: Fact or Fiction?

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Cardiovascular diseases lead the ranking of causes of death worldwide. This has been the ground for an increasing number of studies on the characterization of heart's response to injury and on the development of new therapies to functionally restore the damaged myocardium. In 2011, work by Sadek and colleagues, using the mouse as a model-system, demonstrated that the developmental window for mammalian heart regeneration extends to a short period after birth. Hearts of 1 day old mice (P1) after surgical apex resection progressively re-established the amputated region with negligible fibrosis. However, when the same procedure was performed at P7, fibrosis worsened and the hearts failed to regenerate [1]. Nevertheless, in 2014, controversy was settled when Andersen and colleagues, while attempting to establish the neonatal apex resected model, reported a complete absence of cardiac regeneration [2]. Overall, the concept of “regeneration” has become frail and unclear in the neonate as injury outcome is dependent on small variations in the surgical procedure and the formation of a fibrotic scar has been reported by independent laboratories. The herein work represents an independent evaluation on the regenerative potential of murine hearts. Special attention was given to the role of cardiac fibroblasts and of the extracellular matrix (ECM), known regulators of cardiomyocytic behavior. We demonstrate that neonatal heart response to injury involves the recruitment of inflammatory cells, fibroblasts activation, ECM production and neovascularization. Moreover, our results point towards a partial re-establishment of the removed tissue, via proliferation of resident cardiomyocytes. Despite the formation of scarring tissue (inner core of the injured area) and incomplete histological restoration, resected hearts remained functionally competent at 21 days post-injury. Altogether, we propose an explanatory model for the biological events following neonatal apex resection (Fig.1). Overall, our data lends credibility on the use of neonatal cardiac apex resection model to study the activation of regenerative mechanisms, despite no complete regeneration is observed.

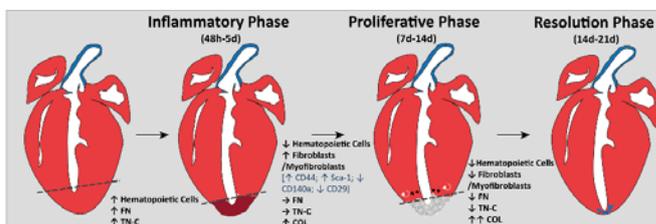


Fig.1 - Model proposed for the biological mechanisms underlying neonatal cardiac injury.

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# Synthesis of curcumin derivatives with potential to inhibit P-glycoprotein expression

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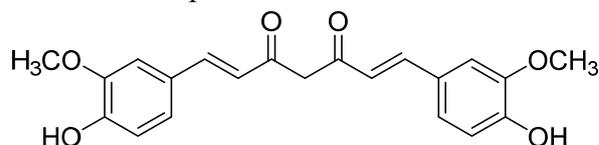
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Curcumin (Fig. 1) is a secondary metabolite isolated from the turmeric of *Curcuma longa* [1]. Many molecular targets have been identified for this compound, hence corroborating its diverse biological and cellular activities. Particularly, curcumin has been shown to act as a multidrug resistance (MDR) modulator in various cancer cell models by downregulating the *mdr1* gene expression and, therefore, decreasing the cellular levels of P-glycoprotein (P-gp) [2]. Thus, the structure of curcumin may represent an important basis for the development of effective therapeutic agents to overcome the problem of MDR in cancer.



**Fig. 1.** Structure of curcumin

However, curcumin has low chemical stability, which severely limits its application. The objective of the present work is to synthesize several stable curcumin derivatives with P-gp inhibitory activity.

The present results indicate that we were able to synthesize seven curcumin analogues expected to be more stable than curcumin. The structure elucidation of the derivatives was performed by <sup>1</sup>H and <sup>13</sup>C NMR. Stability and photostability studies are currently being conducted, by comparing curcumin with the dienone analogue. The main focus of the biological tests of such derivatives will be regarding their P-gp inhibitory activity in a P-gp overexpressing leukemia cell line.

**Acknowledgements:** This research was developed under the project CEQUIMED-PEst-OE/SAU/UI4040/2014 and was partially supported by ERDF through the COMPETE and national funds through FCT, under the project PEst-C/MAR/LA0015/2013.

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# The ADP-sensitive P2Y<sub>1</sub> purinoceptor favours intracellular calcium activation and growth of rat cardiac fibroblasts

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Cardiac fibroblasts (CF) have a broad range of functions, including electric processing and excitation/contraction coupling in collaboration with cardiomyocytes, besides their most known participation in wound repair and cardiac remodelling [1]. CF exhibit remarkable plasticity, undergoing dynamic phenotypic alterations in response to microenvironment changes [2]. Most frequent pathological changes in heart microenvironment are secondary to ischemia and reperfusion, which result in huge accumulation of ATP and UTP released from damaged cells [3]. The extent of the activity mediated by ATP may be influenced by membrane-bound ectonucleotidases, which rapidly metabolize ATP to ADP [4] leading to subsequent activation of ADP-sensitive P2Y receptors, namely P2Y<sub>1</sub>, P2Y<sub>12</sub> and P2Y<sub>13</sub> [3].

The phenotype and the expression of P2 purinoceptors in cultured CF from adult Wistar rats was confirmed by immunofluorescence confocal microscopy. Intracellular Ca<sup>2+</sup> ([Ca<sup>2+</sup>]<sub>i</sub>) oscillations were monitored in the time-lapse mode using a microplate reader (Sinergy HT, BioTek) after loading the cells with the fluorescent Ca<sup>2+</sup> dye, Fluo-4NW (2.5 μM, 45 min at 37°C). Results are expressed as a percentage of [Ca<sup>2+</sup>]<sub>i</sub> triggered by the calcium ionophore, ionomycin (5 μM). CF proliferation/viability was monitored using the MTT assay. Type I collagen production was evaluated using the Sirius Red assay.

Cultured CF from adult rats exhibit a myofibroblast phenotype characterized by positive immunoreactivity against discoidin domain receptor 2 (DDR2) and α-smooth muscle actin (α-SMA). These cells exhibit immunoreactivity against ADP-sensitive P2Y<sub>1</sub>, P2Y<sub>12</sub> and P2Y<sub>13</sub> receptors, but the P2Y<sub>12</sub> and P2Y<sub>13</sub> staining tend to decrease during differentiation of CF from culture day 7 to 21 while the P2Y<sub>1</sub> reactivity remains fairly constant throughout this period. Incubation of 7-day CF cultures with ADP (30 and 100 μM) concentration-dependently increased [Ca<sup>2+</sup>]<sub>i</sub>. ADP (10-300 μM) and its enzymatically stable analogue ADPβS (30 and 100 μM) increased cells growth and type I collagen production in a concentration-dependent manner. CF differentiate faster into stellate-shaped (activated) myofibroblasts when the incubation media was supplemented with ADP (100 μM) for 28 days. ADPβS-induced proliferation of rat CF was attenuated (*P*<0.05) by the selective P2Y<sub>1</sub> receptor antagonist, MRS2179 (0.3 μM, n=5), but not upon blocking P2Y<sub>12</sub> and P2Y<sub>13</sub> receptors with AR-C66096 (0.1 μM, n=6) and MRS2211 (10 μM, n=6), respectively.

Data suggest that the ATP metabolite, ADP controls [Ca<sup>2+</sup>]<sub>i</sub> transients, growth and type I collagen production by CF from adult rats. Although one cannot discount the participation of other ADP-sensitive P2 purinoceptors, the P2Y<sub>1</sub> receptor may be the subtype predominantly involved in myofibroblast activation and growth in the remodeling adult heart.

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**A5** **PSYCHOLOGY &  
EDUCATION SCIENCES II**  
PARALLEL ORAL SESSIONS III

# **Student Life: Senses and Knowledge Autonomous Work: Meanings and Impact on Global Student Training**

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Over the last two decades the Higher Education system underwent many changes and adaptations. The new academic reality, which encourages research, highlighted the importance of students developing a sense of responsibility and autonomy. University students are no longer seen as being strictly dependent on their professor to attain knowledge. Thus, autonomous work has become part of their skills.

Given the fact that this is a fairly recent phenomenon, we established as our object of study the ideas and concepts of autonomous work by the students of the University of Porto. Therefore, we resorted to the testimony of 28 students (14 female and 14 male), from seven different schools of this University, which expressed themselves through semi-structured interviews. Preceding the direct contact with students, a careful analysis of the existing literature was carried out.

After that process, it became clear that students have various conceptions as to what they understand as autonomous work. Students understand autonomy as a "time to work alone or on their own will," which meets the definition of Berbel (2011), who argues that "autonomy means the faculty to govern oneself; the right or faculty to be governed by ones own laws; moral or intellectual freedom or independence." Students also had the idea that the Bologna process arose from "(...) the need to adapt curricula to the demands and characteristics of the labour market, to boost students, faculty and staff mobility, and (...) to make higher education attractive in the global market" (Sobrinho, 2007:112). They showed they are attentive to the changes regarding the time dedicated to self-study, and the importance of class attendance. Finally, we also realized that there are students that see the university as a venue to build their own knowledge and learning.

**Acknowledgements:** This study is the result of a semester long class, and all the students of the class participated.

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# Rethink citizenship: school theater groups to psychosocial development.

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This research intends to reflect and analyze the importance of education through art [1-2], focusing on theater, implemented as a predictor for the construction of citizenship.

The educational theatre, proposed as the object in this research aims to research how education in art, focused on theater and dramatic expression, may have a favorable impact on building inclusive identities in high schools. Thus, refer to schools as a place of excellence in the implementation of teaching, contemplating the non-formal dimension of education that ensures the acquisition and developments in the dominion of vital psychosocial tools [3-4].

Theater and arts education will be analyzed as well as notions of citizenship, through the aid of interviews to students and teachers that integrate school theater groups in order to understand how they value of the dynamics that take place in the non-formal learnings [5].

We will find that spaces for citizenship are becoming scarces in middle school, starting with the extinction of subjects such as “Project Area” and” Civic Education”. The current teaching curricular plans required for teachers, leads towards a lower willingness to implant such projects (of citizenship). However, it was found, during the investigation, that defending the importance of theater promoting citizenship and even in their own personal and social development, lying in the opinion of students and teachers, a willingness to work on projects that promote - directly or indirectly - citizenship which is not always possible due to ministerial enforcement (compliance of a program, the suppression of subjects or even an educational policy that extols particular content type over another).

**Keywords:** *Citizenship; Intervention with young; Education through art; Educational theater..*

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# What can Specialized Artistic Schools teach us? Youths' narratives on schooling effects of secondary education

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In Portugal, compulsory education implies nine years of elementary school followed by three years of secondary education for which students can choose a major to specialize in. Among other possibilities, the public system offers courses on sciences, humanities, languages, technologies and arts. Such diversity of paths concerning secondary school has been encouraged since the National Law for the Educational System release in 1985, a document that established a new moment for education in the country and a step towards liberal theories of modernization [1]. Considering this scenario, we developed a master's dissertation aimed at debating the schooling effects of a specialized artistic secondary institution from the students' point of view.

To meet our research's goals, six biographical interviews [2] were performed with students enrolled in different years of a specialized artistic secondary program from a school located at North Portugal. These interviews privileged a comprehensive look at educational trajectories, helping to set biographically the importance attributed by students to their secondary school experience. Age group ranged between 16 and 18.

Data analysis was carried out through a narrative perspective within the use of content analysis techniques [3]. Results were grouped into three main areas: school, family, and future, in a way that the specialized artistic secondary experience crossed all domains. Data interpretation pointed out that students recognize the importance of school and expect it to break with hierarchical systems through dynamics of informality and innovation. For them, family's involvement in educational trajectories is essential and they welcome the support and participation of various members into their projects. In terms of future, these art-based students depict gloomy expectations because they believe in a depreciation of the artistic work in the country.

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# Subjective Time Experience in Film Viewing: An Integrative Approach

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The present study sought to critically conceptualize the subjective temporal experience within the fields of Psychology and Film Studies as an interdisciplinary and integrative approach. It is assumed that film has in its genesis the need for a representation of *movement, duration, succession* and *continuity* from which it is assumed as *a temporal art*: “*In watching a film, the spectator submits to a programed temporal form*” (Bordwell, 1985, p. 74) [1,4]. It is assumed that our mind processes the aesthetic visual ‘information’ in the same way it processes *reality*, using the same cognitive and affective mechanisms, though in a *simulated reality status* (Grodal, 1999) [2]. This is proposed to occur in a PECMA flow (Perception-Emotion-Cognition-Motor Action) following the brains’ basic architecture, and we organize our world experience in a *canonical narrative* (semantic and temporally logical narrative structure (Grodal, 2009) [3,6]. Some types of film, specifically, “Art-Films” (Bordwell, 1985) have a different structure (*non-canonical*) which activates a *saturated mode* of functioning, thus producing a different *time experience*, since our *time experience* seems to be altered by our emotions: “time flies by when we’re having fun” (Droit-Volet & Meck, 2007) [5]. Deriving from Grodal (2009) we propose that time experience when viewing a film is not independent from our cognitive and affective processing of the film but there are other variables that should be taken into account, such as: i) the chronologic order of the events, as in the processing of “objective time”; ii) the structure of the narrative; iii) the *montage* by which the director can manipulate time representation in the diegetic world and our time experience in the viewing act; iv) frequency which is linked to our *inner clock*; v) the experience of duration to the spectator; vi) subjectivity of the narrative; vii) memory by which we associate stimulus and evoke events; viii) emotions, related to the activation of affective states in the experience of viewing a film; ix) the role of sound and music in subjective time experience; x) the expertise of the cinephile spectator (Santos, A.) [7].

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**IV** PARALLEL  
ORAL  
SESSIONS IV



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**A1** **AGRO FOOD I**  
PARALLEL ORAL SESSIONS IV

## Evaluation of corporal superficial temperature and internal face of thigh temperature of Murrah buffalos using infrared thermography

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The infrared thermography is a promising toll for evaluating the response of animals to the environment and differentiating between genetic groups [1]. Therefore, the aim of this research was to estimate the corporal superficial temperature and temperature of internal face of thigh of 07 buffalas of Murrah breed during the heat tolerance test, in farm located in Pires do Rio city, Goiás state, Brazil. Both temperatures, considering in this study, was obtained using the thermographic camera of FLIR model E-5, with automatic calibration. The thermography images obtained during the heat tolerance test were processed by the use of the Flir tools *software*. The statistical analysis was realized by the use of the Sisvar, 5.1. version. The means were compared by the Tukey test ( $P < 0.05$ ). Could be observed significative difference ( $P < 0.05$ ) between the corporal superficial temperature measured at 01:00 p.m. hours (32.35 °C) and 02:00 p.m. hours (33.20 °C). Occurred increase of 0.85 °C when the animals were exposed, during 01 hour, at the sun. It was verified significative difference ( $P < 0.05$ ) between the corporal superficial measured at 02:00 p.m. (33.20 °C) and 03:00 p.m. (32.45 °C). In this case occurred decrease of 0.75 °C when the animals were taken off the sun and remained in the shadow during 01 hour. The average superficial temperature considering the three times was of 32.66 °C. This value is less than the 34.10  $\pm$ 0,20 °C find by [2] in buffalas kept in silvipastoril system in Oriental Amazônia, considering the rainy period, in vespertine turn. The temperature of of internal face of thigh measured at 01:00 p.m., 02:00 p.m. and 03:00 p.m. was 31.92 $\pm$ 0.49, 32.50 $\pm$ 0.48 and 31.92 $\pm$ 0.48, respectively, and no occurred significative difference ( $P > 0.05$ ) among these means. After the data interpretation could be conclude that the animals does not show evidence of thermic stress during the realization of the heat tolerance test.

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# Modelling evapotranspiration of soilless cut roses cv 'Red Naomi' based on climatic and crop parameters

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Evapotranspiration (ET) is the combined process of evaporation from soil or substrate and plant surfaces and transpiration from plants. Since ET is the main process affecting irrigation requirements of crops, its precise estimation is a key issue in agriculture management. The aim goal of this study was to develop a model for estimating daily ET using climatological and crop parameters for soilless cut roses cv 'Red Naomi'.

The study was carried out in a commercial (Floralves) glass greenhouse, located in Vila do Conde, in the Northwest of Portugal, where cut roses of cv. Red Naomi are grown in a substrate (coir) cultivation system. Between July and October of 2014, weighting lysimeters were used to determine and record the daily ET of 12 sampling units, distributed equitably among 4 sectors of the greenhouse. Each sampling unit was constituted by a substrate bag with four plants. Data of greenhouse air temperature and relative humidity were also recorded on a daily basis. Additionally, the number of leaflets of the bended stems and stem leaf area were determined periodically. A multiple stepwise regression technique was applied to fit the model for the daily ET estimation. A model validation using external data (n=9) and a cross-validation (LOO-“leave-one-out”) process were applied to test the robustness of the model.

The selected model, based on the variables daily relative humidity, stem leaf area and number of leaflets of the bended stems was able to explain 90% ( $R^2 = 0.90$ ;  $n = 33$ ,  $p < 0.000$ ) of the daily ET variability. When the observed daily ET values were plotted against the estimated values, the slope of the identity line was 0.99 and the coefficient of determination was 88%, showing that the model presents high accuracy and precision. The indicators of the residual analysis showed the good performance of the model, with the mean relative error (MRE) between the observed and estimated data equal to 10%, and the mean absolute error (MAE) and the root mean square error (RMSE) equal to  $0.27 \text{ mmd}^{-1}$  and  $0.46 \text{ mmd}^{-1}$ , respectively. The results of MAE, MRE and RMSE obtained for the model validation procedures (both with external data and the cross-validation) were very similar to the ones obtained in the calibration process which indicates model robustness.

In conclusion, the model is able to estimate daily ET with accuracy and precision. When compared to other models it is more expeditious and user-friendly since it requires less and more accessible input parameters. The model's simplicity and flexibility are of great advantage for its practical use in irrigation requirements of soilless cut roses.

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## **Influence of planting density in yield and quality in Touriga Franca, in the Douro Demarcated Region**

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The Douro Region has about 57% of its vineyard area planted in slopes greater than 30%. Slopes of this order don't allow vertical planting, being only feasible to install in narrow terraces, according to the legislation. A major disadvantage of mountain viticulture, such as the Douro region, is low planting density, which leads to lower yields.

Looking for a solution for the low yield problem, an experiment was developed in cooperation with a private company, *The Fladgate Partnership Wines, S.A.* in "Quinta de Santo António", located in Pinhão. For this experiment were used Touriga Franca grapevines trained in vertical shooting position (VSP), grafted onto rootstock Richter 110.

To study the density of planting grapevines, it was compared two different planting distances: 0.80 m in the planting line and 1.20 m in the planting line.

The grapevines distanced 0.80 m proved to be a better solution, with a larger bud number left at pruning and thus a larger number of shoots and inflorescences per meter of hedge.

Leaf area, canopy size and the resulting exposed leaf surface did not change as the planting density was decreased. The same occurred with the quality parameters of maturation in the controls done before harvest.

As for the yield, significant differences were observed, favoring the 0.80 m planting distance. In this distance was observed a greater number of bunches per linear meter, greater weight of the bunches, as well as a yield per linear meter 41% higher, when compared to the grapevines with 1.20 m planting distance, while there was no quality decrease.

These values have confirmed, in a mature vineyard, the results obtained in previous studies on the same vineyard, showing the decrease of planting distance as a valid option in the Douro Region.

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## **MAChoice: interactive WEB tool to agricultural machinery management**

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Machinery operating and ownership costs, although sometimes overlooked, are often more than half of total crop production costs in many crop systems. This fact, coupled with the need for timeless in cultural operations, comfort needs and environmental impacts, implies that agricultural machinery management is of prime importance to efficiency and profitability. Technological developments have resulted in larger, more complex and more expensive machines being made available. However, to maximise the contribution of these machines to agricultural production, it is essential that they be used effectively so as to take advantage of their engineering refinements.

The purchase of an agricultural machine involves considerable cash outlay and represents commitment to a particular crop system. Therefore, prior to investing in an agricultural machine, thorough analysis of the economics the potential benefits of owning and operating the machine should be undertaken. In particular the following 2 question facing concerned growers need to be answered: i) On what crop area (or volume of work) could the machine ownership and operation be economically justified? ii) What difference in economic performance can be expected between alternative machinery systems.

However, the complexities of crop production, make it difficult to specify a common cut-off point between profitable and unprofitable machine operation. Any effort to observe the machine's performance under all possible crop systems operating conditions would be very costly if not impractical. The feasible solution under such circumstances is to synthesise crop operations by means of computer simulations techniques. Using physical and financial information previously collected from in-field studies of the machine's performance, cost and break-even area (or work volume) formulations could be derived.

Therefore, MAChoice, an agricultural machinery management web tool, was developed. This tool is a machinery ownership and operating costs estimator adapted from [1]. The MAChoice web site was developed in Javascript, SQL, PHP programing languages and can be accessed freely at [www.fc.up.pt/MAChoice](http://www.fc.up.pt/MAChoice) by every known browser.

The information provided by MAChoice may be helpful for a broad set of users, ranging from small farmers to producers' associations or even machinery manufacturers in many situations such as: (i) determine the break-even point; (ii) help the decision between purchasing or renting equipment, (iii) compare alternative crop systems, (iv) selection between different machine models, (v) oportune equipment replacing and (vi) prediction of fuel and repair costs and cash flows.

By evaluating the economic soundness of several alternating machinery systems, it is expected that the MAChoice provides agriculture sector with a frame of reference in adjusting to changing technology inherent in modern agriculture.

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## Evolution of phenolic and anthocyanins in port wine

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Port wine is one of the Portuguese gastronomy flagship products, its color being one of the traits that characterizes it and the target of great attention not only by experts of the wine area, as well as scientists, interested in understanding which compounds give such quality. Closely associated with this feature are the phenolic compounds, particularly anthocyanins, as responsible not only for it as well as allies to the antioxidant activity. The evolution from bright red to dark red and brown colors due to oxidation-reduction reactions from phenols, anthocyanins and their derivatives is throughout all the aging process is a relevant issue that needs to be study more in deep.

The samples analyzed in this study were collected at seven important stages of winemaking, during the vintage of 2013, in Quinta da Roeda vineyards, as part of a batch of grapes divided between the two types of methods; the traditional ran in granite lagares by treaders and the mechanical, using technology to imitate the traditional.

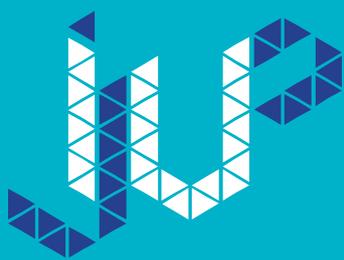
Anthocyanins were analyzed by HPLC/Diode Array <sup>[1]</sup>; malvidina was the most prevalent although other anthocyanins were also identified. During wine fermentation the anthocyanin content increased, presenting a decrease after fortification with spirit and during 6 months aging in stainless steel vats. The same profile was also observed for total phenolics performed by the 96-well microplate Folin-Ciocalteu procedure using carbonate buffer as alkaline reagent applied with some modifications <sup>[2]</sup>. The results of both methods were statistically treated with an ANOVA two way to verify the effects of production method (namely, traditional ran in granite lagares by treaders and the mechanical process) and also the effect of Port wine production (fermentation, fortification and aging). No significant differences were observed for both effects ( $p < 0,05$ ).

The formation of new anthocyanins during wine aging needs to be studied in detail by LC-MS/MS. Furthermore, as this wine was only studied for a period of six months it would be appropriate to continue to study its evolution until bottling.

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**A2** **SPORT SCIENCES**  
PARALLEL ORAL SESSIONS IV

## **SPORT THAT MAKES THE FUTSAL SPORTS TRAINING SOCCER FIELD ATHLETE**

**William Guerra**

FADEUP

The article aims to demonstrate the contribution that futsal offers sports training in the field of soccer athlete. The sample consisted of 60 athletes of the categories under 15, under 17 and under 20 an elite club of Brazilian football. For data collection was applied an interview. He rose to 90% of respondents athletes practiced futsal; with respect to average indoor soccer practice, there was a significant result the Under 17, with a mean of 4.5 years compared to an average of 2.9 years the Under 15 and 3.1 years in the U20 category; to the level that was practiced futsal, most with 70.4% participated in championships organized by the federations of each state; only 25.9% played at school level and only 3.7% for leisure. It was concluded that although many of the participants has started in futsal, most, competed in a federated level and the importance of futsal football is not scientifically proven, but there is a large field of evidence that has been evaluating the actual contribution athletes who started in futsal for the current football.

**Keywords:** futsal; football; training of athletes

# Assessment of Food Regime Related to Energy Expenditure in Italian Canoeists

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Canoeing and kayaking require specific nutritional strategies to support both training and competition necessities. Advanced athletes are subjected to high intensity and training volumes for most of the period of training, then the calorie intake must be sufficient to support the phases of recovery and adaptation. A low content of muscle glycogen before an exercise limits the high intensity performance, then the daily intake of carbohydrates should be distributed through all phases of training and competition. According to Stellingwerff et. Al. [1] the time, the type and amount of protein influences recovery and adaptation after training. The correct monitoring of energy expenditure and the different training intensity in canoe and kayak athletes are important to ensure an adequate energy supply with adequate intake of micronutrients in relation to the specific demands of the training. The purpose of the study is to assess a correct diet in relation to energy expenditure in Italian canoeists and if the quantity and time of intake of protein and carbohydrates are suitable for the type of training. 15 male athletes, with international experience, will be monitored for a 5-days period during a given training stage. Firstly, anthropometric characteristics and BIA (bioelectrical impedance analysis) will be analyzed. Sense Wear Armband (SWA) allow us to evaluate energy expenditure [3] in terms of: Basal metabolic rate (estimated by the energy expenditure during the sleep period between 2 and 3 pm) and its relationship with lean body mass (FM) and cell body mass (BCM); Total and mean energy expenditure (kcal); Energy expenditure during whole training stage and training session (kcal); Training session Intensity (MET's). Those values will be compared to: total and daily energy intake; total and daily carbohydrate intake (quantity, quality and timing of intake); Total and daily protein intake (quantity, quality and timing of intake). For each athlete, the correct energy intake compared to energy expenditure will be determined from adequate intake of carbohydrates and protein during the time and training loads. These results show whether the athletes will be able to understand and satisfy their nutritional needs.

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# Biomechanical Parameters correlated to Running Economy in Long-distance Runners

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In the last decades, the running practice has become widespread not only as a competitive sport but also as a recreational practice. Further, running long distance, such as half-marathon and marathon races, has become progressively a trend in recent years. Successful distance running performance, particularly in long distance events, also depends on the interaction of several physiological factors, including a low energy cost to run at a particular speed. This factor is one form of running economy (RE) [1]. RE is defined as the energy required to run at a given submaximal velocity, and is typically determined by measuring the steady-state oxygen consumption (VO<sub>2</sub>) at the submaximal running speed [2-3-4]. Furthermore, RE showed a strong relation with long-distance running performance [5-6-7] and to be a better predictor of performance than VO<sub>2</sub>max in elite runners with similar VO<sub>2</sub>max values [3]. Several biomechanical factors appear to influence RE in highly trained or elite runners and changes in running mechanics could lead a runner to use less energy at any given speed is advantageous to performance [3]. Running implies the conversion of muscular forces into complex movement patterns, involving all the major joints, thus, exists a logical relationship between running technique and economy of movement, since performing mechanical patterns without non-productive movements and applying forces of appropriate magnitude in the right directions with precise timing will result in the lesser energy consumption at a given running speed [8]. For this reasons, it is obvious that, during running, various biomechanical factors contribute to performance by influencing RE [9]. The purpose of this study is to find a relationship between a better RE and biomechanical aspects of long-distance running. Twenty long-distance runners with an experience of a minimum one year will perform three sub-maximal stages on a treadmill with force plate (AMTI Tandem Treadmill) during a video recording with high-resolution cameras for motion capture (Qualysis). Biomechanical parameters (stance phase; flight phase; cadence; hip, knee, ankle ROM during the different phases of running; vertical and lateral displacement of Center of Mass (COM); stride duration; contact and flight time) are analyzed to find a relationship with RE.

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## Motor Deficit in school 3-10 anos the São Paulo public education

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**ABSTRACT:** Several studies have demonstrated the existence of a positive association between the domain in the execution of motor skills during childhood and physical activity throughout life. In cross-sectional design, the objective of this study was to measure and compare the performance of 529 children outboard for 3 to 10 years with unfavorable socioeconomic status of a public school in the eastern region of São Paulo. The Test of Gross Motor Development was used (TGMD-2) and compared the performance of girls and boys in different age groups by 2-way ANOVA test [2 (gender) x 4 (age)], with post-hoc Tukey when needed. The results showed motor deficits with more than 53.2% of the female sample and 49% of the male profile much lower than expected. Overall, the girls had better locomotor performance, and the boys manipulative performance. In both sexes, there was no significant evolution of engine performance with increasing age, indicating a possible low quality of environmental stimuli that lead to the acquisition and mastery of motor skills.

**KEYWORDS:** motor competence; childhood; development.

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## **Anthropometric measurements and physical activity in an elderly population of Autonomous Region of Madeira**

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On the latest years, there has been an increase on the number of people over 65 years. Consequently there has been an increase of studies that focus this population and tries to understand their changes, characteristics and risk factors so we can create scales of risk on this age group.

With aging, there are changes in body composition that have great influence in the comfort of the elderly and can put their own health in question. Decreased muscle mass and bone mass and increased fat mass are view frequently on this population, contributing to the weakness.

Adding to these changes, increasing sedentary lifestyle has a major impact on health and well - being of elder persons.

This research tries to contribute to the deepening of the existing knowledge about the elderly and tries to characterize and establish a relationship between anthropometric measurements and physical activity in an elderly population.

The aim of this study was to evaluate a representative sample of the population of Autonomous Region of Madeira over the age of 65. In total 1181 individuals of all counties and parishes in the region were surveyed. Data collection was conducted between February and August of 2013.

According to the objectives, a questionnaire was developed which included questions related to sociodemographic characteristics, anthropometric data, clinicopathological background, assessing nutritional risk, among others.

The parameters that showed influence on anthropometric values in this population were gender, age, education, the place of residence and type of physical activity performed. The average weight was 72,2 kg and the average BMI was 29,4 kg/m<sup>2</sup>, values higher than found in other studies in Portugal.

Gender and physical activity reveled to influence body composition of old people. Women and inactive persons had higher values for the parameters used to assess fat mass and lower values for the ones that measure lean mass. Porto Santo was the county with the higher BMI and body fat percentage. On the other hand, in Santa Cruz, the body fat percentage was the lowest and values of muscle mass were the highest.

In conclusion, older people in Madeira Archipelago are at risk of developing metabolic complications associated with obesity because they have showed values above the cutoff. In this study, we showed the relevance of measure anthropometric values and physical activity in elderly population.



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**A3** **BIOLOGICAL SCIENCES IV**  
PARALLEL ORAL SESSIONS IV

# Analysis of water quality of reservoirs from Cávado River's basin

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Reservoirs are artificial lentic water bodies formed by the construction of dams [1]. The sudden interruption of the normal flow of the river has major consequences to the ecosystem and the quality of the water. Reservoirs are subjected to bigger inputs of nutrients and water-level fluctuations than the natural lakes, thus they are more susceptible to eutrophication and disturbance in the stability of the biological communities [2].

With the increase in human dependence on reservoir's for water supply, there is an urgent need to create tools and methods to evaluate the quality of the water and the ecosystem stability. Concerning this, documents such as the Water Framework Directive (WFD), which provides guidelines to evaluate the ecological status of different types of water bodies [2], have been used to assess water quality in reservoirs. Therefore, the main aim of this study is to evaluate the water quality of four reservoirs (Venda Nova, Alto Cávado, Alto Rabagão and Paradela) belonging to Cávado River's hydrographic basin, using physical and chemical and biological indicators.

To perform this objective, reservoirs were sampled every month during nine months (between March and November). Samples of water, zooplankton and phytoplankton were collected and brought to the laboratory for further analyses. Physical and chemical parameters (pH, BOD5, total phosphorus, nitrates, TSS, chlorophyll *a* and turbidity) were evaluated according to standard protocols. Zooplankton samples were analysed and identification of specimens of cladocerans and copepods was carried out.

The first results obtained showed that chlorophyll *a*, TSS and dissolved O<sub>2</sub> were the parameters that presented more variation through the sampled period. A seasonal variation was also observed with high differences between the hot and cold months. Venda Nova was the reservoir with higher variation on most parameters analysed, while Paradela was the reservoir which kept a higher stability through the sampling period. Concerning the TSI (Trophic State Index) and using the chlorophyll *a* values, Venda Nova was the only one reaching the classification of hypertrophic, Paradela was classified as oligotrophic, and Alto Rabagão and Alto Cávado were classified as mesotrophic ecosystems.

**Keywords:** Reservoirs, Zooplankton, Water quality, Water Framework Directive, Ecological Status

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## **The role of salinity on estuarine ammonia oxidising activity**

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Estuaries are the interfaces between freshwater and marine environments being extremely dynamic zones. The mixing of freshwater and saltwater along with the geomorphology, winds and tidal heights creates physical-chemical gradients that are accompanied by shifts in the resident microbial communities. In addition, anthropogenic impacts keep driving alterations to estuarine systems. Nitrogen input is one consequence of these anthropogenic actions and the fate of excess nitrogen in estuaries is determined by the microbial-driven nitrogen cycle. In this study we evaluate the influence of salinity changes into nitrification process in Douro estuarine sediments. Results revealed that microbial communities adapted to distinct estuarine salinity regimes show different responses in terms of nitrification activity when facing salinity changes. In addition, our findings demonstrated that salinity is an important variable in controlling the selection of the group of organisms (Archaea or Bacteria ammonia oxidizers) that mediated the nitrification processes in estuarine systems. We believe that results from this study were critical to understand the dynamics of the nitrification processes under a gradient of salinities, providing better insights on how nitrifier communities respond to salinity changes.

## Charcoal analysis as an ecological interpretation method for *Pistacia lentiscus* distribution in Sabor valley, Northeastern Portugal

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Archaeobotany is the study of plant remains from archaeological sites in order to better understand the environmental context of past societies and how their environment was exploited and modified.

Recent archaeological excavations in the Sabor valley provided the largest assemblages yet of archaeobotanical remains in Portugal. This material was collected in several archaeological sites of different chronologies, covering the last 25 000 years.

Among these were samples that yielded charcoal from a wide set of taxa, which included two species of the genus *Pistacia*: *Pistacia terebinthus* and *Pistacia lentiscus*. Most samples containing this genus came from Iron Age and Roman contexts, from the 4<sup>th</sup> century BC to the 4<sup>th</sup> century AD.

In the present *P. terebinthus* is found in Trás-os-Montes region and is frequent in sub-serial communities on the first stage of degradation of oak forests. On the other hand, *P. lentiscus* is found presently only in South and Central Portugal. It is not common in continental areas with harsh winters such as those found in Sabor valley.

Only a single *P. lentiscus* tree is known in the region and it can be found near the abandoned village of Cilhades (Felgar, Torre de Moncorvo). The tree is well known by the local population and there is a legend concerning its presence there. Nevertheless, charcoal fragments from *P. lentiscus* were found in four archaeological sites in the Sabor valley: Castelinho (4<sup>th</sup> century to 1<sup>st</sup> century BC), Olival da Santa (2<sup>nd</sup> century AD), Crestelos (4<sup>th</sup> century BC to 3<sup>rd</sup> century AD) and Quinta do Medal (17<sup>th</sup> to 19<sup>th</sup> century). Although *P. lentiscus* charcoals are not very frequent in the soil samples that were analyzed, it is clear from its presence in these several sites along the valley that its distribution in the past was quite different from the one existing today.

Thus, this presentation will address the question of the distribution of *Pistacia lentiscus* in Sabor valley from the Iron Age until the present day.

## Development of a methodology based on potentiometric detection for the determination of iodine in urine samples

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Iodine is a nutrient involved in vital biochemical reactions, namely in protein synthesis and enzyme activity in vertebrates. Being a component of thyroid hormones, its deficiency may lead to hypothyroidism, and physical and mental retardation in humans. There is a notorious iodine deficiency in many parts of the world, including Portugal, constituting a serious public health issue. The main sources of iodine in the human diet are marine fish, shellfish, marine algae and sea salt. Other potential sources of iodine are enriched processed foods like bread, margarine and milk. However, Universal Salt Iodization was approved worldwide as a way to fight iodine deficiency [1], and a tool to achieve the required daily iodine input. On the other hand, monitoring iodine in urine is an expedite way to control iodine deficiency in humans. Among current methods used for iodine determination, several detection systems are available, namely spectrophotometric, potentiometric, and ICP-MS. Since they may turn out to be cumbersome and expensive to operate, there is a need to design user-friendly approaches. Therefore, this work aimed to develop a flow analysis [2] method exploring the potentiometric detection. Previously reported work [3], proved the efficiency of this approach in complex sample matrices. In fact, the use of an iodide selective electrode ensures a minimization of sample interferences, namely colour and suspended solids. To improve the detection limit, urine samples were spiked prior to analyses and conditioning of the determination performed in-line. Finally, the accuracy of the developed method was assessed using certified CDC sample standards and then, effectively applied to urine samples.

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## Development of an analytical method by HS-SPME-GC/MS for volatile carbonyl biomarkers discovery in urines from patients with prostate cancer

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Prostate cancer (PCa) is one of the most commonly diagnosed neoplasia in men, being its diagnosis a big challenge. Identification of cancer biomarkers through non-invasive diagnostic methods is an important tool for an early detection and management of diseases. For this reason, diagnostic strategies based on metabolomic profile has recently been applied to prostate cancer.

For the investigation of potential urinary volatile carbonyl metabolites, an simple analytical approach consisting of headspace-solid phase microextraction (HS-SPME) sampling coupled with gas chromatography-ion trap/mass spectrometry (GC-IT/MS) with a prior step of derivatization with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) was developed. A central composite design (CCD) was used in order to optimize conditions that affect the efficiency of the SPME sampling such as the time and temperature of extraction and incubation, as well as the PFBHA amount. The SPME fiber used was divinylbenzene/polydimethylsiloxane (DVB/PDMS). The applicability of the optimized method was then tested in a pilot targeted analysis of urine samples obtained from 15 patients with prostate cancer (PCa) and 15 healthy individuals. Target chemometrics analyses (principal component analysis and partial least squares discriminant analysis) revealed a group separation with good PLS-DA scores (R2X: 0.308; R2Y: 0.64; Q2: 0.465). Monte Carlo Cross Validation, reflecting a good sensitivity (92%) and specificity (93%) with a classification rate of 92.4%, validated the robustness of these models. The target results showed good capability to differentiate PCa patients from healthy individuals being notice differences between urines from cancer patients and healthy controls. In patients, four compounds (2-propenal, 2-propanone, 2-cyclohexanone and an unknown compound) were significantly increased ( $p < 0.05$ ). The target analytical method for 27 carbonyl compounds, including aldehydes, dialdehydes, and ketones, was validated with regard to the linearity, repeatability, inter and intra-day precision and accuracy.

Overall, the methodology developed is suitable for a fast and reliable determination of these compounds in urines and provides a potential non-invasive tool for an early prostate cancer diagnosis.



ENCONTRO INVESTIGAÇÃO JOVEM  
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**A4** **BIOMEDICINE IV**  
PARALLEL ORAL SESSIONS IV

## Potential of new steroidal aromatase inhibitors in breast cancer: anti-proliferative effects and cell death mechanisms

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Estrogens are key players in hormone-dependent (ER+) breast cancer progression. Tumor growth can be blocked by the use of aromatase inhibitors (AIs), which inhibit the enzyme aromatase preventing estrogen biosynthesis. Although AIs are used for treatment of postmenopausal women with ER+ breast tumors, there are some drawbacks to overcome, such as the development of endocrine resistance and bone loss. In the past few years, several steroidal compounds were designed and studied by our group, with structural modifications in androstenedione, the aromatase substrate, in order to obtain superior AIs [1]. The present work focus on the biological evaluation and anti-tumor mechanisms of newly synthesized steroidal compounds (**49**, **50**, **51** and **52**). These compounds are potent AIs on breast cancer cells that overexpress aromatase (MCF-7aro). Moreover, cell viability assays demonstrated their anti-proliferative capacity. Presently, we intend to explore the mechanisms related to the anti-proliferative effects of the AIs, by studying cell cycle progression and the occurrence of cell death, namely necrosis by LDH assay and apoptosis by evaluating cell morphology and caspase-7 activity. Autophagy involvement was also assessed by using the autophagic inhibitor 3-methyladenine (3-MA).

None of the compounds induced necrotic effects. However, all were able to induce cell cycle retention in G0/G1 phase, together with morphological alterations, mainly chromatin condensation, as observed by Giemsa and Höechst staining. Moreover, preliminary results indicate an increase in caspase-7 activity, suggesting an apoptotic process. In addition, results with 3-MA showed that autophagy may also be a cell death mechanism for AI **49** and a pro-survival process for AIs **50** and **51**.

In conclusion, the new compounds are potent AIs that induce anti-proliferative effects in breast cancer cells mainly through cell cycle arrest and cell death. This work will contribute to elucidate the tumor suppression activity of new molecules for potential use in breast cancer treatment.

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## Targeting Cdc20 protein to increase sensitivity of colorectal cancer cells to paclitaxel

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In normal cells the accurate segregation is monitored by the spindle assembly checkpoint (SAC) that is responsible for arresting cells in mitosis in case of errors in chromosome attachment to the mitotic spindle.[1] In chemotherapy treatments, many cancers exhibit resistance to antimetabolic agents as paclitaxel. These agents are responsible for an alteration in cell microtubules that will arrest cells in mitosis, due to SAC activation, but paclitaxel-resistant cancer cells overcome the arrest and to continue their proliferation.[2] In view of that, it has been suggested that the SAC can be used as a new target in anticancer treatment by targeting proteins involved in its molecular pathway.

The aim of this work is to deplete by RNA interference (RNAi) the SAC target protein Cdc20 in colorectal cancer cells with the intention of sensitize these cells to paclitaxel action. For that, we assessed the cell viability by MTT assay in cells depleted of Cdc20 in the absence and presence of different concentrations of paclitaxel. Our results show that the combination of Cdc20-RNAi with paclitaxel decreases the viability of cells when compared to Cdc20-RNAi-only or paclitaxel-only treated cells. In conclusion, the Cdc20 depletion sensitizes the colorectal cancer cells to paclitaxel thereby pointing to its therapeutic potential.

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# Establishment of a 3D model of EMT/MET-induction using molecularly-designed ECM-like hydrogels

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## Introduction

Epithelial to mesenchymal transition (EMT) is a biologic process that allows a polarized epithelial cell to undergo biochemical changes that enable it to assume a mesenchymal cell phenotype. EMT has been associated with a concomitant decrease of epithelial markers (e.g. E-cadherin) and increase of mesenchymal markers (e.g.  $\alpha$ -SMA), as well as enhanced migratory and invasion capacity, resistance to apoptosis and increased production of extracellular matrix (ECM) components. EMT-cells may undergo a reverse process, mesenchymal to epithelial transition (MET), through which they are able to recover the epithelial phenotype. In some cases, EMT reversion may not be fully accomplished, giving rise to a metastable phenotype characterized by a simultaneous expression of epithelial and mesenchymal features. Several reports suggested that EMT/MET may have a role during cancer progression and metastasis establishment. To elucidate this hypothesis, different EMT models have been implemented using traditional 2D systems. However, such models lack the influence of a proper 3D ECM-like structure that could better mimic the *in vivo* microenvironment, and closely reproduce the EMT transcriptional program. The central aim of work was the establishment of a 3D *in vitro* model of TGF- $\beta$ 1-driven EMT/MET induction in EpH4 epithelial cells cultured on an artificial alginate matrices with tuneable properties. In addition, we aimed to further characterize the 2D *in vitro* model of TGF- $\beta$ 1-driven EMT/MET induction previously established in the group.

## Materials and Methods

EpH4 cells were collected at different stages of EMT/MET processes in order to scrutinise the expression of several epithelial and mesenchymal classical markers both at RNA and protein levels. For that purpose, real-time PCR, immunofluorescence, cytometry and zymography assays were performed. In addition, we measured the metabolic activity of these cells and we characterized the spheroids obtained in the 3D system, both in size and in number.

## Results and Discussion

Our results demonstrated that the 2D EMT-derived cells have enhanced stem-like features (enrichment of CD29<sup>+</sup>/CD44<sup>+</sup> subpopulation) and produce increased levels of MMP9. Regarding the 3D EMT/MET model, our findings showed a TGF- $\beta$ 1-mediated EMT program characterized by E-cadherin impairment and increased mesenchymal markers, both at RNA and protein level (e.g. Vimentin and  $\alpha$ -SMA). Moreover, the removal of TGF- $\beta$ 1 stimulus enabled the establishment of a metastable phenotype with concomitant E-cadherin presence at cell membrane and  $\alpha$ -SMA expression in the cell cytoplasm. In conclusion, we created a 3D *in vitro* TGF- $\beta$ 1-mediated EMT/MET system that makes use of RGD-modified alginate hydrogels that provide a well-defined and tuneable environment. The insights gained with this work, may ultimately be useful for the establishment of a high-throughput system for the study of cancer progression and metastasis.

## Expression of TGF-beta in different adipose tissue depots is singularly regulated by a high-fat diet and energy restriction

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**Introduction:** Transforming growth factor beta (TGF- $\beta$ ) contributes to the pathogenesis of obesity, playing an important role in adipogenesis, cytokines release and inflammation. In fact, adipose tissue of obese rats and humans seems to express higher levels of TGF- $\beta$  that activates both SMAD-dependent and independent pathways – the latter include ERK1/2, Akt and MAPK p38-dependent pathways. It is our aim to evaluate the effect of a high-fat diet and energy restriction on TGF- $\beta$  expression levels and to study TGF- $\beta$ -related signalling pathways activation on different adipose tissue depots.

**Methods:** Subcutaneous, epididymal, mesenteric and retroperitoneal adipose tissue of 12 months aged rats, submitted to a (1) high-fat diet (HF; 45% of energy from fat), (2) energy restriction (ER; ingestion of 75% of the feed given to controls), or (3) control diet (4% of energy from fat) were collected. TGF- $\beta$  expression was evaluated by real-time PCR. Activation of SMAD2/3, ERK1/2 and Akt signalling pathways was assessed by Western-blotting.

**Results:** Concerning retroperitoneal adipose tissue, HF- and ER-fed rats showed a higher expression of TGF- $\beta$ , ERK1/2 and Akt compared to controls. However, ER-fed rats showed a lower Smad2/3 expression when compared to controls and HF-fed rats. Curiously, in epididymal and mesenteric adipose tissues, ER-fed rats presented a lower expression of ERK1/2 and Akt, respectively. No significant differences were observed in subcutaneous adipose tissue in rats submitted to different diets concerning TGF- $\beta$  mRNA expression or ERK1/2, Akt, or Smad3 protein expression.

**Conclusions:** The effect of diet on TGF- $\beta$  expression and signalling profile is differently felt across different subtypes of adipose tissue. Among them, the retroperitoneal adipose tissue seems to have a more diet-dependent response.

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## Preparation of pH-sensitive nanoparticles for site-specific delivery of dapsone

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Dapsone (DAP) is a bactericidal agent used in the treatment of leprosy, caused by *Mycobacterium leprae*. Despite its therapeutic potential, DAP has low solubility, which may result in low therapeutic index and a high microbial resistance. Recently, new approaches have been applied in order to increase DAP solubility, however, supersaturation in the medium, *per se*, does not necessarily lead to increased bioavailability. For this purpose, it is necessary to maintain the supersaturation throughout the gastrointestinal tract, especially in the drug absorption site. Accordingly, the development of pH-sensitive systems of DAP appears to be a good strategy for the modulation of its absorption, leading to an increase of the drug time duration, which may help to overpass the antimicrobial resistance. The pH-sensitive polymeric nanoparticle (NP) is an effective carrier for oral drug delivery, which not only possess the advantages of nanoparticle including improvement of intracellular penetration and retention time, and controlling the release of the drug, but also has the properties of protecting drug from degradation in the acid environment [1]. In addition, enteric nanoparticle has been used to enhance the drug absorption. Eudragit<sup>®</sup> L 100 (EL100) is anionic copolymerization product of methacrylic acid and methyl methacrylate, which is pH-dependent and soluble above pH 6 medium, being commonly used for the preparation of enteric solid dosage forms [2]. The aim of this work was to develop suitable EL100 NP loaded with DAP for its pH-sensitive controlled release. The NP was synthesized by the single emulsion technique. Several variables were evaluated as: amount of polymer, volume of organic solvent, concentration of surfactant, volume of aqueous phase and amount of drug. The NP was evaluated measuring the average size, zeta potential, polydispersity index and encapsulation efficiency. Furthermore, the release kinetic of NP was carried out at different pHs.

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## Sustained release matrix tablets: formulation and *in vitro* evaluation

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Extended release dosage forms are widely used to minimize the frequency of dosage form administration, thus improving patient compliance, and to optimize the blood plasma concentration of the active pharmaceutical ingredient [1]. Matrix systems are commonly classified in three groups, namely, hydrophilic, inert and lipophilic [2].

The purpose of this study was to investigate the influence of the type of matrix, Kollidon<sup>®</sup> SR (inert) and Carbopol<sup>®</sup> 71G NF (hydrophilic), and fillers, Tablettose<sup>®</sup> 80 (soluble) and Avicel<sup>®</sup> PH-102 (insoluble), as well as their concentrations, on the release profile of acetylsalicylic acid (ASA), used as a model of a poorly soluble drug, from matrix tablets.

Eighteen formulations of tablets, with a target weight of 400 mg ( $\pm$  20 mg), were developed by direct compression using an alternative machine (Korsch 9048-71) [3]. Afterwards, the uniformity of weight (mean  $\pm$  standard deviation (SD), n = 10), hardness (mean  $\pm$  SD, n = 10), and friability (n = 10, 25 rpm/4 min) were evaluated in the obtained tablets [3]. The *in vitro* drug release studies (mean  $\pm$  SD, n = 3) were performed during 8h using a dissolution apparatus (Sotax, model AT7) according to the Portuguese Pharmacopoeia 9 [4]. The ASA concentration was determined by UV-VIS spectrophotometry ( $\lambda_{\text{max}}$  = 265.0 nm;  $y = 3.2319x - 0.0071$ ;  $R^2 = 0.9998$ ) [4].

Matrix tablets with suitable physical properties (weight uniformity, hardness and friability) were produced by direct compression. However, the tablets obtained with Tablettose<sup>®</sup> 80 (fragmentable excipient) showed higher friability and lower hardness than the tablets prepared with Avicel<sup>®</sup> PH-102 (plastic excipient). The ASA dissolution rate is influenced by the type of matrix in the tablet, i.e., the percentage of ASA released is greater from Kollidon<sup>®</sup> SR than Carbopol<sup>®</sup> 71G NF. When the concentration of the matrix was increased, a reduction in the amount of ASA released was noticed. The fillers did affect the drug release profile, namely, Avicel<sup>®</sup> PH-102 (insoluble filler presenting also disintegrating properties), which contributed to a faster ASA release than Tablettose<sup>®</sup> 80. Additionally, the tablets obtained from mixtures of diluents (50:50) presented an ASA release profile similar to the ones obtained with Avicel<sup>®</sup> PH-102 and showed better physical characteristics (hardness and friability) than tablets containing only Tablettose<sup>®</sup> 80.

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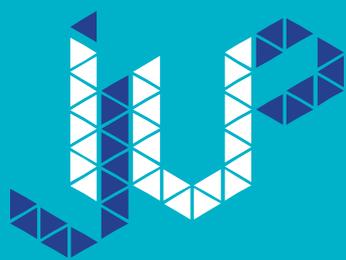
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**V** PARALLEL  
ORAL  
SESSIONS V



ENCONTRO INVESTIGAÇÃO JOVEM  
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**A1** **AGRO FOOD II**  
PARALLEL ORAL SESSIONS V

## Effect of different CATA ballots on the overall acceptance of lemongrass (*Cymbopogon citratus*) infusion.

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Check-all-that-apply (CATA) questions are a descriptive technique in which panellists have to choose from a ballot with a list of terms those that better describe samples. The aim of the present work is to study the effect of applying different CATA ballots on the overall acceptance of lemongrass (*Cymbopogon citratus*) tea.

For this purpose, sensory (list of 84 sensory attributes divided into four dimensions: appearance, odor, texture and flavor) and emotional (list of 29 emotions) profiles CATA ballots [1] were used by two panels of 75 naïve panelists. For each panel, four infusions were prepared following a factorial plan according to type of cut and area of the plant. The infusions were prepared with selected leaves of organic *Cymbopogon citratus* plant, using 4.5g of dried leaves infused in 1.5L of natural mineral water for 8.5 minutes at 99°C [2]. For each panel, samples were presented in a sequential monadic mode, to compensate for order effects [3], with each infusion being evaluated for overall acceptance (on a 9-point scale), followed by the evaluation of the sensory or of the emotional profile. Acceptance data was analyzed following a 5-factor ANOVA (type of ballot, type of cut, area of the plant, order of presentation and panelists as a random factor).

Results show that the order of presentation is the most significant fixed factor ( $p < 0.0005$ ), with the first sample presenting an average acceptance of  $0.40 \pm 0.07$  points above all the other three presentation orders. The type of ballot had a low impact ( $p = 0.062$ ) on the overall acceptance of the infusions: data obtained with the emotional CATA was slightly higher ( $7.84 \pm 0.08$ ) than data obtained with the sensory CATA ( $7.60 \pm 0.08$ ).

Framing of the overall acceptance with different profiles has no significant impact, at 95 % confidence level, on the acceptance itself, and clearly below the impact of the well know first-order carry-over effect.

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# Development and validation of an HPLC-DAD-FL method for the determination of food supplements adulteration with undeclared phosphodiesterase type-5 inhibitor drugs

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The consumption of food supplements has been increasing in developed countries. However, regulations and guidelines for this type of products reveal several gaps, and do not guarantee an efficient quality control, allowing for the possibility of intentional adulteration. Supplements used for improvement of male sexual performance are among the most popular food supplements. One of the major concerns in these products is the possible adulteration with synthetic drugs used for the treatment of erectile dysfunction, namely phosphodiesterase type-5 (PDE-5) inhibitor drugs, such as sildenafil, vardenafil and tadalafil. The side effects of these compounds and possible interactions with other drugs are well documented, thus its illegal addition to food supplements could pose a serious risk for consumers with known health constraints [1]. In the last years, the presence of this type of drugs have been detected by FDA in the US and reported in food supplements commercialized in Asia and the EU. Recently, Portuguese legal authorities reported the apprehension of some food supplements due to the presence of illegal PDE-5 inhibitor drugs.

In this work, an high performance liquid chromatography (HPLC) based method was developed and validated for the detection of four PDE-5 inhibitors, namely sildenafil, vardenafil, tadalafil and yohimbine, in three sexual performance enhancement supplements. The analyses were performed by HPLC-DAD-FL in a JASCO chromatograph following the conditions of a previously published method [2]. A YMC-Triart C18 analytical column (3 µm, 250 × 4.6 mm) was used, together with (A) 50 mM Ammonium acetate; (B) acetonitrile/ methanol (50:50) as eluents. A simple liquid-liquid extraction with sonication using acetonitrile/methanol (50:50) was used in all samples. To validate the proposed methodology, the limits of detection (LOD) and quantification (LOQ), linearity range, intra- and inter-day precision and accuracy of the method were determined, showing high reproducibility scores and adequate recoveries for the tested compounds. One of the analyzed supplements showed the illegal addition of sildenafil.

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## Development of a polymerase chain reaction assay for the specific detection of *Lupinus* spp. in processed foods

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Lupine flour has been regarded with increasing interest owing to its nutritional value and functional/technological properties. It is used in the formulation of a wide variety of processed foods (e.g. bread, cookies, pasta, chips, muffins) as an alternative source of proteins to gluten-containing cereals, milk or soybean [1]. However, lupine is also classified as an allergenic ingredient known to cause mild to potentially life-threatening immune reactions in sensitised/allergic individuals [2]. In the European Union, lupine and other 13 groups of allergenic ingredients must be declared and highlighted from the rest of the list of ingredients in the label of pre-packaged foods (Directive 2007/68/EC, Regulation (EU) 1169/2011), independently of their amounts. To verify labelling compliance, to help food industry in allergen management and to safeguard the health of sensitised individuals, the development of new methodologies for the traceability of allergenic ingredients is of major importance [3].

This study aimed at developing a qualitative polymerase chain reaction (PCR) assay for the detection of lupine allergens in processed foods. For this purpose, model mixtures of rice spiked with known amounts of lupine were prepared ranging from 10% down to 0.0001% ( $n=10$ ). Different *Lupinus* spp. (*L. albus*, *L. luteus*, *L. angustifolius* and *L. mutabilis*) and other plant/animal species were tested for genus specificity and cross-reactivity, respectively. A total of twenty-seven samples of processed foods were also acquired at local markets. DNA was extracted using the NucleoSpin Food kit with minor alterations as described by Costa et al. [4]. Primers targeting the sequences encoding the allergenic proteins Lup a 4/Lup 1 4 were specifically designed to amplify *Lupinus* spp. The optimisation of qualitative PCR allowed a relative sensitivity for lupine in rice material down to the level of 0.0005% (5 mg/kg), with an absolute detection of 0.2 pg of lupine DNA. The proposed method was successfully applied to the commercial samples, detecting lupine as an ingredient or at trace levels in 5 or 10 tested food products, respectively. In general, PCR results were in good agreement with product labels, although in one sample, in which lupine was declared as an ingredient, it was not detected.

**Acknowledgments:** This work was supported by FCT grant no. PEst-C/EQB/LA0006/2013. Joana Costa and Telmo J. R. Fernandes are grateful to FCT grants (SFRH/BPD/102404/2014 and SFRH/BD/93711/2013, respectively) financed by POPH-QREN (subsidised by FSE and MCTES). The authors are grateful for the kind supply of samples from Bank of Plant Germplasm of Tucson, USA.

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## Real-time PCR detection and quantification of genetically modified soybean with the event GTS40-3-2 in food

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Soybean is the main genetically modified (GM) crop for food and feed, totalising more than 90 million hectares, which corresponds to approximately 82% of the total soybean production in 2014 [1]. The soybean line GTS40-3-2 authorised in the European Union (EU), as well as in several other countries, it was developed by Monsanto and marketed under the name of Roundup Ready™ (RR) to allow glyphosate tolerance, the main ingredient of RR herbicide [2]. Since the GMO were introduced in the food chain, several concerns, raised by both the general public and by the scientific community, were issued regarding their safety. For this reason, in EU, under the Regulations (EC) No. 1829/2003 and 1830/2003 regarding authorisation and traceability of food and feed derived from GMO, the labelling of food products containing more than 0.9% authorised GM material is mandatory. To comply with legislation, it became essential to verify labelling statements, using appropriate analytical methodologies. For GMO testing, the methods of choice are based on DNA analysis by means of polymerase chain reaction (PCR) due to their demonstrated effectiveness. The higher thermal stability of the DNA molecules, when compared to proteins, it is an advantage to analyse processed foods. The technique of real-time PCR is the most suitable tool for GMO quantification.

The objective of this study was to detect and quantify the presence of RR event in soy-based foods available in the market, with different levels of processing. Four experimental phases were then performed: (i) DNA extraction; (ii) lectin gene amplification; (iii) detection of RR soybean; and (iv) quantification of RR soybean event. Event-specific primers targeting RR soybean were used to amplify an expected fragment of 106 bp using certified reference materials containing 0, 0.1, 1 and 5%. For the confirmation and quantification of the event, real-time PCR technique with hydrolysis fluorescent probes was used. Quantification of GMO content was performed using the double calibration curve method. The results showed that in 6 out of 190 tested samples, RR soybean was detected by PCR, which were further confirmed by real-time PCR. The quantitative analysis showed that one sample contained 22.7%, while the others ranged 0.01-0.37%, emphasising the effective presence of GMO in food and the need for verifying labelling compliance.

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# CYNARA SCOLYMUS CLUSTERING IN PLANT FOOD SUPPLEMENTS BY HIGH RESOLUTION MELTING ANALYSIS

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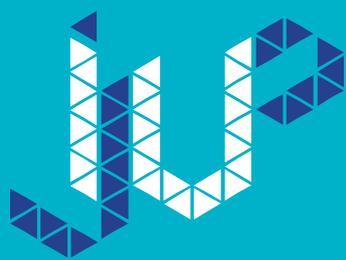
Plant food supplements (PFS) have been regarded with special interest since they are concentrated sources of nutrients or other substances with a nutritional or physiological effect, contributing to a better homeostatic balance. Weight-loss PFS, one of the most consumed PFS, may include *Cynara scolymus* (artichoke) as an ingredient due to its antioxidant, diuretic, choleric and hepatoprotective properties [1]. Considered as foods under the EU Directive 2002/46/EC, the PFS are not usually subjected to any safety assessment prior to their commercialisation. This can lead to adulteration issues, such as accidental swap of plants or deliberate substitution of higher cost botanicals by other similar, but cheaper species. Thus, to ensure consumer's safety, the development of analytical methods for the correct identification of different plant species in PFS has become essential. Until now, DNA-based methods have been reported as highly appropriate tools for plant authentication [2]. The aim of this study was to discriminate *C. scolymus* from other *Cynara* spp. using real-time polymerase chain reaction (PCR) coupled to high resolution melting (HRM) analysis.

For this purpose, different *Cynara* species (*C. scolymus*, *C. syriaca*, *C. cardunculus* and *C. humilis*) were obtained from Portuguese and French Germplasm Banks. A total of 8 PFS tablets for weight-loss containing artichoke were acquired at local herbal stores. DNA from plant material and PFS was extracted using the commercial *NucleoSpin Plant II* kit. Former to DNA extraction, PFS were pre-treated with a phosphate buffer 1M (pH8, 15% ethanol) to enhance the purity and quality of the extracts. The specificity and sensitivity of the designed primers targeting *C. scolymus* were assayed by qualitative PCR and real-time PCR with HRM analysis. The application of the specific PCR assay was successful in the detection of *Cynara* spp. in some of the PFS samples. The results of real-time PCR with HRM analysis showed that different *Cynara* spp. were included in three distinct clusters with a level of confidence above 99.4%, thus discriminating artichoke from other *Cynara* species. The proposed HRM analysis allowed confirming the unequivocal presence of *C. scolymus* in the tested PFS with high level of confidence (>98.8%). To our knowledge, this is the first successful attempt for the rapid discrimination of *C. scolymus* in PFS.

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ENCONTRO INVESTIGAÇÃO JOVEM  
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**A2 HUMANITIES &  
SOCIAL SCIENCES**  
PARALLEL ORAL SESSIONS V

## **Urban gardens in the Municipalities of Oporto: Typologies and Spatial Patterns.**

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The concept of urban agriculture has been increasingly driven by the recognition of their value to the population and environmental quality in cities.

This work aims to contribute to the knowledge of the types and territorial patterns of urban agriculture in the municipality of Porto and identify priority areas for its enhancement. First we identify and characterize the different types of cultivated areas using three methodological processes: interpretation of high resolution satellite images; field surveys; interviews with urban farmers. Secondly we analyze the relationship between natural / sociodemographic characteristics and spatial patterns defined by the cultivated areas, and defined priority areas of intervention for the enhancement of urban agriculture.

The results show the current state of urban agriculture in Porto, which may be useful both to act on the already involved population's needs as to enhance new agents and new urban gardens.

## **Media narratives on violence: framings of the urban violence in print media from Amazon of Pará, Brazil**

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The coverage of violence and public security in the Amazon region, specifically Pará has media frameworks that pinpoints the denial of a debate on the complex social problems that involves violence, especially in the context of Brazil. The narratives are built emphasizing the act of the crime itself and the subjects that are involved, coupled with the superficiality and general lack of pertinent information [1]. Therefore, this paper aims to understand how media narratives of the local print media frame the phenomena of violence, denying the debate on public security in the region.

The research analysed six months of the Police Section from newspaper editions that circulate in the region, namely the *Diário do Pará e O Liberal*, during 2012 and the *Amazônia Jornal* in 2013. The Narrative Analysis of Journalism was the qualitative method used to understand the cultural aspects and journalistic codes present in the elaboration of reports on the events [2]. In addition, the method of Frame Analysis was used to understand the different frames that the narratives build for each event, providing interpretations of the social phenomena [3].

We found differences in the narratives and the frames, in addition to common occurrences among the three newspapers, when treating violence as a commodity for consumption as well as for political purposes.

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# Coworking and incubation organizations: similarities and differences between two emerging work settings

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Since the industrial revolution, the world of work has suffered countless changes. The more recent information technology revolution offered a background for the emergency of new work formats during the beginning years of the 21<sup>st</sup> century [1]. This paper aimed to analyze, compare and contrast two of those examples: coworking [2] and incubation [3] organizations.

The methodology of this investigation consisted in content analysis [4] that relied on the study of news, statistics and reports produced about this theme. This strategy intended to accomplish an examination of the socioeconomic context for the emergence and growth of these settings and to distinguish the assumptions that compose the *modus operandi* of these work environments.

Both emerged in the USA, albeit at different times and places: incubation on 1959 in New York and coworking on 2005 in Los Angeles. Although they seem to have experienced an exponential growth worldwide after the financial crisis of 2007/2008, coworking and incubation present different options to independent or start-up workers.

On the one hand coworking organizations offer an extremely flexible setting, one in which coworkers are able to swiftly enter or leave these spaces. They are usually small, with a reduced managing team, with circa 20 places available for coworkers. The focus relies on the promotion of knowledge exchange and creation of bonds between coworkers. On the other hand, incubation organizations typically assume a large dimension, offering a wide range of services and experts to the incubatees that can vary from economic and strategic guidance, to partnership and product development. Also, it usually entails a stable and lasting relationship with incubatees, frequently lasting several years and having a post-incubation accompaniment.

Two main conclusions derive from this comparative study. Firstly, both settings gained traction and were fueled on account of the financial crises of 2007/2008 and are mainly focused in urban areas that have a strong creative and information knowledge based economy. Secondly, incubation organizations have a balanced scheme of capitals according to Pierre Bourdieu's typology [5], while coworking spaces focus on developing social and cultural capital with its members, in spite of its flexible configuration.

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# Obstruent Devoicing of Northern Mozambican Portuguese: An autosegmental analysis

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Portuguese is undergoing a large set of change and variation processes – diatopically, diastratically and diaphasically motivated [1] – which, by the one hand, show the vitality of this language, and, by the other hand, contribute to take its different dialects apart. A phenomenon that clearly shows this change may be observed, at the phonetic level, in the production of obstruents (stops and fricatives) by speakers of the northern-most dialects of Mozambican Portuguese (henceforth: NMP, Northern Mozambican Portuguese).

Indeed, NMP is characterized by the loss of [+voiced], with the subsequent activation of [-voiced] ([+voiced, -syll] → [-voiced]), when obstruents are produced [2]. Such devoicing may be attributed to the lack of [+voiced] in Emakhuwa, the mother tongue of most speakers of NMP from the districts of Niassa, Cabo-Delgado, Nampula and Zambezia (ca. 5.307.378 speakers) [3]. Therefore, we could assume that obstruent devoicing would trigger two major phonological consequences: i. The reduction of the segment inventory of NMP (erasing voiced stops /b, d, g/ and, arguably, some voiced fricatives, such as /z, ʒ/); ii. The perceptual processing of context-dependent utterances.

For the study of this change process affecting NMP, we will assume the basic tenets of Goldsmith's autosegmental phonology, since this model allows us the integration of phonological phenomena not restricted to the segmental tier [4]. Hopefully, we will attain a 'natural' analysis for the phonetic dimension of this variation, too [5].

The *corpus* upon which our observation will be based consists of spoken samples of 30 adult L2 speakers of NMP, from the cities of Cuamba (Niassa district) and Nampula (Nampula district) who have Emakhuwa as their L1.

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## **The Role of Higher Education in Table Responsibility Linguistics in Mozambique : A reflection on the teaching of Portuguese language and communication of speakers**

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This article presents some reflective elements around the role of Higher Education in Mozambique face linguistic responsibility to the Portuguese Language Teaching. The current reality of the Portuguese, according to the data obtained from the Portuguese corpus Oral Maputo (CPOM) consisting of Mozambique in the NIED (National Institute for Educational Development) systematized in search of books, Moçambula varieties corpus of African Portuguese consisting in Linguistics Center, University of Lisbon (CLUL), and some newspapers online News of Mozambique, in particular, "The Journal @ Verdade", seem to show a tendency, on the one hand, to position itself as a non-autonomous variety, but semantically interesting linguistic specificities if compare with those of the European Portuguese (hereinafter PE) among other varieties of Portuguese (Portuguese of Brazil, Cape Verde, Guinea-Bissau, São Tomé and Príncipe, Angola and Mozambique). On the other hand, there is more and more interesting linguistic coexistence between the Mozambican Portuguese variety (hereinafter PM) and the Bantu languages in Mozambique. The reflection that we focus in this text is limited to the effect that there is a need for universities to invest in linguistic responsibility and no exclusion of this when talking about the development in Mozambique. The Institutions of Higher Education in Mozambique, mostly perform their teaching duties, research and extension using natural languages in many cases the current official language, Portuguese. It is therefore a challenge of these invest in the development of inclusive teaching materials to the Mozambican reality of the Portuguese.

Keywords: Higher Education, Linguistics Responsibility, Teaching Portuguese, Sustainable Development.

## ***Foreign Policy Analysis* and the history of Portuguese Foreign Relations: how does assessing continuity and change disproves (some) Realist myths?**

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Throughout the history of Portugal, its foreign policy has always faced different and multiple models of international insertion: it is an Atlantic country, but also European (although peripheral), with its shared land border with a single country (Spain). Most studies in History of International Relations refer systematically that its foreign policy has always been determined by a geopolitical dilemma: choosing between Europe and becoming a continental nation, or preferring the Atlantic and fitting as a maritime one.

We have reviewed and employed the literature and tools provided by the *Foreign Policy Analysis* (a subfield of International Relations), and in particular, studies of the change (and continuity) in foreign policy, aimed at looking broadly at the main international guidelines of Portugal, the structure of its foreign policy both in its early history as in the recent one (including the imperial policy, establishment of the Republic, Estado Novo, and during the democratic regime).

We will identify the main moments in history leading to change in the international orientation of the country, and how, in the main events of the country's foreign relations, three essential elements, identified by the theory as ever-present in a crisis/rupture situation, were combined: change of the essential structural conditions, strategic political leadership, and some sort of crisis. Additionally, as we assess continuity and change in Portuguese foreign policy, we have determined that despite being, up to a certain level, essentially structural in nature and led by some geopolitical factors, there is a great degree of the decision-making process which could be more accurately attributed to other elements, such as internal politics, ideology, as well as the role of pressure groups and the public opinion. Consequently, looking into the diplomatic history of Portugal, we were able to disprove some myths of the realist theory of International Relations, as: the unitary perspective of the state, acting in accordance with the objective goals it defines; that its actions follow a national interest, rationally defined and decided upon; that foreign policy is mostly defined by the international setting, with a non-existing role of national politics; that "high-politics" (military and security issues) is more important to the state's foreign policy than "low-politics" (economics or social affairs).

In conclusion, this empirical work has not only provided significant proof of the noteworthy continuity of Portuguese foreign policy, but also gave us a greater understanding on how and why that country's foreign relations change over time. Additionally, and perhaps more considerably, the observed diplomatic orientation of the state, and a detailed landscape of internal and international events, lead us to believe that there may be no verifiable evidence to support a number of realist claims on how foreign policy is made.

## **The Evolution of the Right/Left Binomial**

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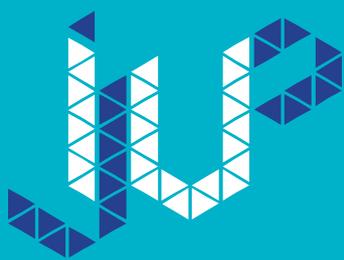
This paper is based on several works that illustrate the specificities of the different ideological groups and their evolutional path, analysing their characteristics (separatly or comparatively), whilst analysing their importance and impact on the societies of each time.

To investigate the historical developpement of the opposite ideological groups, I analysed several texts on the subject, using them alongside specific statistic data about the political situation.

Throughout this text I consider the right and left groups, but also their sub-divisions (for example: marxism, social-democracy, liberalism, fascism, and so on) analysing its specificities and comparing them based on the information from the books, concluding if this opposition is still accurate and if the ideologies changed (and if so, why).

During the research, I verified that this dichotomy was born during the French Revolution, but is reminiscent from the first half of the XVIIIth centurie. The two ideological groups have followed since parallel evolutional paths and, naturally, have had several changes that either approached, either apparted one from another. I also remarked that after the 1960's, we have seen a diversification of the political panoram, and so this opposition became an actual multi-leveled reality (with the appearence of the identitary mouvements).

All in all, I concluded that even though it seemed a bit outdated, this binomial is still pretty much alive and playing a determinant role in todays political-ideological reality, and ultimately influencing and beeing influenced by the society.



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**A3** **BIOLOGICAL SCIENCES V**  
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## Identification of intermediate and off-pathway species during amyloid fibril formation of $\alpha$ -synuclein

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Increasing evidence suggests pre-fibrillar species as important pathogenic agents in neurodegenerative and prionopathies pathogenesis. The current work aims at the characterization of intermediate and off-pathway species during amyloid fibril formation of  $\alpha$ -synuclein, an abundant brain protein whose aggregation is associated to Parkinson Disease (PD). The preliminary conclusions resulting from the analysis of  $\alpha$ -synuclein aggregation kinetics are subsequently validated using direct morphological and analytical assays. The first part of this work presents the analysis of in vitro progress curves obtained using thioflavin T (ThT) fluorescence and turbidity techniques, while in the second part the results from Gradient Gel Electrophoresis, Transmission Electron Microscopy (TEM) and Circular Dichroism (CD) techniques are discussed. Besides contributing for a better understanding of the molecular basis of PD, this study envisages the validation of new screening tools targeting fibrillar and non-fibrillar species.

# Identification of novel molecular players involved in the control of glial cell migration in *Drosophila melanogaster*

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The generation and maintenance of a functional complex nervous system relies on an intricate interaction between neurons and glial cells and the precise positioning of glia in neuronal substrates [1]. As glial cells are generally born distant from the place where they settle, molecular cues are important to direct its migration. Low numbers of glial cells in their target sites is often associated with neurological disorders, however high numbers can also lead to the development of glioblastomas, the 3<sup>th</sup> most lethal brain tumor [2]. Therefore, new insights in the control of glial cell migration can offer new opportunities to develop effective cancer therapies as well as help to dissect signalling pathways involved in developmental biology. The aim of this project is to find novel molecular players involved in the autonomous and non-autonomous control of glial cell migration. For that we use the *Drosophila melanogaster* larvae as a model system where we can down or up-regulate genes involved in cell migration, adhesion and/or tissue development. In order to study genes required autonomously for glia cell migration, we are manipulating gene expression in glial cells whose cytoplasmatic membranes were marked with a red fluorescent protein, allowing us to detect differences in glial cell numbers and glia migration in peripheral nerves. To unveil new non-autonomous cues we are using the visual system, since glial cells migrate from the CNS to the developing eye. During this process, glial cell migration has to be accurately synchronized with photoreceptor differentiation [3], making the developing retina a perfect system to search for cues sent from the eye epithelia to regulate glia development.

We identified a novel autonomous player in glial cell migration and, interestingly, we showed that a protein already described as a controller of tissue growth also regulates glial cell migration in a non-autonomous manner. When development of photoreceptor progenitors and precursors is disturbed by downregulating this protein, glia migrates beyond its normal borders (glia overmigration). We will present a genetic and cellular characterization of the mechanisms associated to these novel aspects of glia migration control.

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## ***Mytilus galloprovincialis* exposed to the neurotoxin BMAA: enzymatic response and glutamate receptor expression**

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BMAA is a putative neurotoxin that in marine environments has been shown to find its way from the phytoplankton first producers (e.g. cyanobacteria and diatoms) to higher trophic levels such as marine invertebrates, and fish. In vitro, BMAA has been shown to act as a glutamate receptor agonist and induce excitotoxic effects. Effects upon glutamate receptors have not been yet tested in marine organisms, despite the fact that these organisms have been shown to be able to accumulate BMAA. This work aimed at testing the hypothesis that exposure to BMAA results in changes in the expression of glutamate receptors as well as in antioxidant enzyme activity in the Mediterranean mussel *M. galloprovincialis*. This organism was chosen for being an important component of estuarine and marine food webs, and because being a sessile filter feeder, it may be over-exposed to phytoplankton toxins.

*M. galloprovincialis* were exposed to 1 mg L<sup>-1</sup> of BMAA standard in seawater. Animals were collected after 48h of exposure, and subsequently depurated for 24h. Gills and digestive gland of exposed and unexposed animals were separated for total enzymatic analysis (Acetylcholinesterase (AChE) and Glutathione S-transferase (GST)) and total RNA extraction. Two transcripts, termed GLU4 and GLU5 were selected from a catalogue of *M. galloprovincialis* Expressed Sequence Tag (ESTs). Relative expression of the transcripts was assessed by qPCR, using the elongation factor alpha-1 (EF-1) gene as endogenous control.

GST in gills showed an increased activity during exposure and depuration, and digestive gland showed an increased activity during exposure to BMAA. AChE in gills decreased its activity during exposure to BMAA, but no effects could be seen for the digestive gland. For the gills both transcripts displayed a clear downregulation during the exposure period that was reversed after depuration. These results suggested that feedback mechanisms may be involved in an attempt to restore neuronal output to homeostatic levels. For the digestive gland the results were not conclusive.

These results indicate that an exposure to sublethal concentrations of BMAA during short periods of time (< 48h) can effect at a molecular level, which contributes to elucidating putative target and/or detoxification mechanisms

### Acknowledgments

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## **Peroxiredoxin 2, glutathione peroxidase and catalase in the membrane and cytosol of erythrocytes under oxidative stress induced by H<sub>2</sub>O<sub>2</sub>**

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The cytoplasmatic enzymes, peroxiredoxin 2 (Prx2), glutathione peroxidase (GPx) and catalase (Cat) are responsible for H<sub>2</sub>O<sub>2</sub> scavenging from erythrocytes, protecting the cells against oxidative stress damage, especially their hemoglobin (Hb) and the membrane constituents. In a previous work (1), we showed that cytosolic Prx2 binds to the erythrocyte membrane when erythrocytes are under H<sub>2</sub>O<sub>2</sub> mediated oxidative stress. In the present study, our aim was to evaluate the changes of Prx2, GPx and Cat in the cytosol and membrane of erythrocytes under H<sub>2</sub>O<sub>2</sub>-induced oxidative stress. We incubated erythrocytes for 30 minutes at 37°C, with increasing concentrations of H<sub>2</sub>O<sub>2</sub> (0, 5, 10, 20, 40 and 80 µM), in 5 different assay conditions (5 independent experiments each): A) no enzyme inhibition, B) Prx2 and GPx inhibition, C) Cat inhibition, D) Cat, GPx and Prx2 inhibition and E) Cat, GPx and Prx2 inhibition after prevention of methemoglobin (metHb) formation. Afterwards, erythrocytes were processed to obtain membrane and cytosol fractions. The cytosol and membranes were used to perform immunoblots for Prx2, Cat and GPx. The oxidative stress markers, lipoperoxidation (LPO) and membrane-bound hemoglobin (MBH) were also evaluated, in the membrane. Cat never linked the erythrocyte membrane and its cytosol amount was unchanged, however we showed the binding of GPx and Prx2 to the membrane when the three enzymes were inhibited with or without metHb formation (conditions D and E); when only Cat was inhibited (condition C) GPx bound the membrane, but not Prx2. In the cytosol, the decrease of Prx2 monomer was followed by an increase of the dimer form and by Prx2 linkage to the membrane, and, also, GPx decreased in the cytosol as it bound to the membrane. Moreover, in conditions C, D and E the oxidative markers, LPO and MBH, increased with H<sub>2</sub>O<sub>2</sub> concentration. In fact, whenever Cat was active (conditions A and B) there were no significant changes in LPO and MBH and no enzymes bound to the membrane, which led us to confirm that active Cat is capable of scavenging high H<sub>2</sub>O<sub>2</sub> levels and protect the cell from oxidative damage; however, when it becomes impaired, Prx2 and GPx bind to the membrane in response to H<sub>2</sub>O<sub>2</sub> induced oxidative stress. The role of the binding of these cytosolic enzymes to the membrane is still unknown: it might be for membrane protection against oxidative stress or, another possibility is that Prx2/GPx suffer structural and/or functional damage and then bind to the membrane.

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# Phosphorylation of the Estrogen Receptor Alpha in Breast Cancer: The Hunt for the Elusive Kinase

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**Background:** The emergence and progress of breast cancer is strongly associated with elevated levels of steroid hormones that act upon tumour cells through their respective receptors. The overexpression of the nuclear Estrogen Receptor  $\alpha$  (ER $\alpha$ ) plays a key role in breast malignancy. Recently, phosphorylation of the Threonine-594 (T594) residue of ER $\alpha$  C-terminal region has been reported as crucial for receptor's activity and protein-protein interaction. However, the identity of the responsible kinase remains unveiled.

**Aims:** To identify the kinase responsible for ER $\alpha$  T594 C-terminal phosphorylation.

**Materials & Methods:** Two parallel sets of experiments were conducted. **1<sup>st</sup> Set:** Hydrophobic Interaction-Fast Protein Liquid Chromatography (HI-FPLC) was performed on fresh cell extracts of HEK293 cell line. Eluted fractions were incubated, at 37°C, with magnesium, natural ER $\alpha$  ligand 17 $\beta$ -Estradiol (E<sub>2</sub>), ATP and ER $\alpha$  Ligand-Binding Domain (LBD), purified from previously transformed TOP10 *E. coli* cells. Incubation products were immunoblotted with antibodies against both total and T594 phosphorylated ER $\alpha$ . **2<sup>nd</sup> Set:** PJ69 yeast strain expressing either wild-type (wt) ER $\alpha$  LBD or its T594A mutated version, which cannot be phosphorylated at the 594 position, were grown in the presence or absence of E<sub>2</sub> and ER $\alpha$  competitive antagonist and 4-Hydroxytamoxifen (4-HOTAM). Cell extracts from treated yeast cultures were immunoblotted as described above.

**Results:** **1<sup>st</sup> Set:** ER $\alpha$  T594 phosphorylation was detected in FPLC lately eluted fractions. **2<sup>nd</sup> Set:** Differential wt ER $\alpha$  T594 phosphorylation in yeast cells showed to be ligand-dependent. Phosphorylation on ER $\alpha$  T594A was not detected. While both E<sub>2</sub> acted as a powerful phosphorylation trigger, 4-HOTAM prevented ER $\alpha$  phosphorylation.

**Discussion:** **1<sup>st</sup> Set:** Detection of kinase activity in FPLC lately eluted fractions provides valuable insights into the enzyme's isoelectric point and its ability to establish strong ionic interactions. Moreover, mass spectrometry can now be performed on these fractions to identify the kinase. **2<sup>nd</sup> Set:** ER $\alpha$  phosphorylation in yeast further narrows down the number of kinase candidates, since the yeast and human kinome greatly, but not totally, overlap. Additionally, differential phosphorylation of ER $\alpha$  T594 appears to be ligand-dependent. Upon E<sub>2</sub> binding, conformational changes in ER $\alpha$  expose T594 to phosphorylation, similarly to what happens, at great extent, in breast cancer cells due to ER $\alpha$  overexpression. On the other hand, ER $\alpha$  structure upon 4-HOTAM binding makes the T594 residue sterically inaccessible, preventing receptor phosphorylation and subsequent activation.

**Conclusion:** Here, a novel molecular mechanism linking ER $\alpha$  T594 phosphorylation and receptor differential conformation upon ligand binding is proposed. Furthermore, new insights into the identity of the enzyme responsible for the ER $\alpha$  T594 phosphorylation indicate this kinase as a promising molecular target in breast cancer therapy.



**ENCONTRO INVESTIGAÇÃO JOVEM  
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**A4 BIOMEDICINE V**  
PARALLEL ORAL SESSIONS V

## Design and synthesis of novel antimicrobial agents inspired on phytochemicals

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Nowadays, one of the major challenges for industrial, environmental and biomedical sectors is bacterial growth, particularly when it occurs in the form of a biofilm (sessile state), a phenomenon that causes increased bacterial resistance [1]. Bacterial multidrug resistance to antibiotics is a worldwide concern and so the development of new active products and alternative strategies for biofilm control is an emergent issue. Thus, intensive efforts have been made to look for new antimicrobials from diverse sources such as phytochemicals due to their chemical diversity and versatility, being one of the most inspiring systems [2]. In this context, the outcome of caffeic acid, a phytochemical found in plants, in the control of planktonic bacterial growth of *Escherichia coli* and *Staphylococcus aureus* was evaluated.

A series of alkyl esters derivatives of caffeic acid, with different alkyl side chain length, were synthesized and tested. Caffeic acid esters were found to be effective antimicrobial agents against both planktonic and sessile cells of *E. coli* and *S. aureus*. The data gathered from the assays of bacterial growth and adhesion inhibition allow to establish a structure-activity trend that is essentially related with the lipophilicity of the systems: the selected Gram-positive bacterium (*S. aureus*) was more affected by more lipophilic compounds (longer alkyl side chain) while medium length alkyl side chain compounds were more effective in the selected Gram-negative bacterium (*E. coli*). These compounds work as membrane permeabilizers, as they induced irreversible membrane alterations and caused local rupture or pore formation in the cell membranes and consequent cell death.

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## **L-proline improves nitric oxide availability and attenuates the blood pressure rise in angiotensin II-infused rats**

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L-proline (Pro) exerts a dual role on redox regulation, acting as an antioxidant or as a prooxidant. Antioxidant protective effects of Pro appear to involve the upregulation and stabilization of antioxidant enzymes, scavenging of reactive oxygen species (ROS), metal chelation, balance of intracellular redox homeostasis and improvement of cell resistance to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). On the other hand, ROS generated during Pro metabolism have been shown to induce signalling pathways involved in cell protection. Pro has also the putative ability to increase the production of nitric oxide (NO) due to the interrelationship of Pro cycle with the L-arginine-urea cycle. Reduced NO availability and increased production of ROS, such as H<sub>2</sub>O<sub>2</sub>, contribute to the pathogenesis of angiotensin II (Ang II)-induced hypertension. Given the proposed protective effects of Pro, the aim of this study was to evaluate its effectiveness in the prevention of Ang II-induced hypertension.

Pro (2 g/kg/day) was administered in the drinking water, from day 0 to day 21, to male Sprague Dawley rats. Hypertension was induced by s.c. infusion of Ang II (200 ng/kg/min) from day 7 to day 21. Systolic blood pressure (SBP) was measured by the tail-cuff method. Urinary biomarkers of oxidative stress (H<sub>2</sub>O<sub>2</sub> and isoprostanes) and NO availability (nitrate and nitrite) were evaluated using commercial kits. Plasma levels of Pro and L-arginine were analyzed by HPLC. Creatinine, urea, sodium, potassium and chloride were evaluated in the plasma and/or 24h urine using an automated biochemical analyzer.

Pro supplementation significantly increased systemic Pro levels in both sham and Ang II-treated rats. It also induced a tendential rise in plasma L-arginine concentration, as well as a significant increase in plasma urea levels, in Ang II-infused animals. Treatment with Pro significantly attenuated the Ang II-induced rise in SBP (day 21: 134±2 mmHg vs 165±9 mmHg, p<0.001) but did not alter SBP in sham rats. Pro supplementation significantly increased urinary H<sub>2</sub>O<sub>2</sub> in Ang II-infused animals (1.91±0.83 micromol/kg/day vs 0.20±0.04 micromol/kg/day, p<0.05) but tendentially attenuated the excretion of isoprostanes in the same group (63.88±3.43 ng/kg/day vs 80.67±9.00 ng/kg/day). It also markedly increased the urinary excretion of nitrate and nitrite (1.22±0.08 micromol/kg/day vs 0.56±0.15 micromol/kg/day, p<0.01) and the fractional excretion of sodium (0.46±0.03% vs 0.31±0.02%, p<0.001) or chloride (0.93±0.03% vs 0.68±0.04%, p<0.01) in Ang II-infused rats.

We can conclude that Pro supplementation attenuates the development of Ang II-induced hypertension. This antihypertensive effect appears to be related with an increased synthesis of NO, which is known to have vasodilator and natriuretic properties, and that probably results from the interrelationship of Pro cycle with the cycle responsible for L-arginine production.

# **Exercise and obesity. The influence of voluntary physical activity and endurance training in adipose tissue mitochondria functionality**

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**Introduction:** Obesity-related modulation in visceral adipose tissue (VAT) mitochondria has been reported. However, the role of exercise against the modifications induced by high-fat diets (HFD) on VAT mitochondria is poorly understood. We aimed to analyze the influence of voluntary physical activity (VPA) and endurance training (ET) on markers of mitochondrial function and biogenesis in VAT from rats fed with a HFD-induced obesity.

**Experimental description:** male-Sprague-Dawley rats were randomly assigned into SED, VPA and ET groups fed with two isocaloric diets, a standard and HFD (35% or 70% fat-derived Kcal, respectively) as follows: SED35, VPA35, ET35, SED70, VPA70 and ET70. VPA-animals had free access to voluntary running wheel throughout the entire protocol. After 9-wks of hypercaloric diet regimens, ET-animals were submitted to 8-wks ET maintaining dietary treatments. The epididymal fat pad was excised and used to determine the protein expression of subunits of the oxidative phosphorylation system (OXPHOS), mitochondrial transcription factor A (TFAM), Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1- $\alpha$ ), adenine nucleotide translocator (ANT) and sirtuin 3 (SIRT3)

**Results:** The animals submitted to HFD showed a decrease in protein expression of the subunits of complex IV and V, PGC1- $\alpha$  and TFAM. Chronic VPA did not induce significant changes in the subunits of the ETC. The program of ET increased the protein levels of the complex IV in animals subjected to both diets, and the V complex, only in animals subjected to the standard diet. Diet, VPA and ET did not induce significant changes in protein levels of ANT. We didn't verified significant changes in protein content of PGC1- $\alpha$  and TFAM after 17 weeks of VPA. ET reversed the protein expression of PGC1- $\alpha$  and TFAM decrease induced by HFD. Diet and VPA did not induce significant changes in protein content of SIRT3. ET promoted a reduction in the protein content of SIRT3 in animals subjected to a HFD.

**Conclusions:** The results of our study suggest that consumption of a HFD may lead to an impairment of mitochondrial function and biogenesis. In contrast to the initial hypothesis, the VPA did not showed a preventive effect against the deleterious effects associated with an HFD. ET seems to induce an increase in mitochondrial function and biogenesis in animals subjected to a HFD.

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## Therapeutic targeting of the mitotic checkpoint to increase sensitivity of glioblastoma cells to anti-mitotic agents

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Many cancers are treated with chemotherapy, such as anti-mitotic agents. Anti-mitotic agents like paclitaxel arrest cells at mitosis by damaging their microtubules. However, paclitaxel-resistant cancer cells are still able to proceed with mitosis. The spindle assembly checkpoint (SAC) is the mechanism responsible for paclitaxel-mediated mitotic arrest [1]. In this study, we hypothesized that by depleting the SAC target Cdc20, we will sensitize glioblastoma cells to the action of paclitaxel.

To test our hypothesis, we depleted Cdc20 in two glioblastomas cell lines by transfection with siRNA. Then, we conducted a MTT assay to determine cell viability of Cdc20 depleted cells that were treated with different concentrations of paclitaxel (50 nM, 10 nM and 5 nM). Also, we assessed the ability of this combinatorial treatment to inhibit proliferation in a colony formation assay and to interfere with cell migration in a wound healing assay.

Our results show that cell viability is decreased when Cdc20 is silenced and that decrease is more pronounced when cells are also treated with paclitaxel at the different concentrations, especially at 10 nM. The colony formation assay showed that this treatment decreases the ability of forming colonies. The wound healing assay showed that the combination treatment of Cdc20 depletion and paclitaxel reduces the migration rate of glioblastoma cell lines.

Overall, the depletion of Cdc20 in glioblastoma cell lines seems to sensitize these cells to paclitaxel, highlighting the potential of Cdc20 targeting in glioblastoma therapy.

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## Identification of a novel iron chelator compound with promising antibacterial activity against Gram positive bacteria

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Antibiotic resistance spreads rapidly amongst bacteria in clinical settings, reducing the therapeutic options to treat infections. A wide diversity of iron chelators (alike bacterial siderophores) with variable chemical structures and affinity for iron(III) are described and seem to possess antibacterial activity in representative isolates from few bacterial species. Our goal is to evaluate the antibacterial activity of diverse iron chelator compounds derivatives of 3-hydroxy-4-pyridinone on different bacterial species, including with variable antibiotic resistance patterns.

We tested the commercially available iron chelator deferiprone (3-hydroxy-4-pyridinone) and its derivatives (n=8) functionalized or not with different rhodamines. Their activity was evaluated against reference *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* PAO, *Acinetobacter baumannii* ATCC19606, *Acinetobacter baylyi* ADP-1, *Enterococcus faecium* BM4105 and *Staphylococcus aureus* ATCC25913 strains by using the broth microdilution method (0.05-100 mg/L and 0.08–180 mg/L), according to CLSI guidelines. Bacterial suspensions in MH broth were prepared after incubation in MH agar, and experiments were performed at least in triplicate. Compounds exhibiting a promising minimal inhibitory concentration (MIC<10 mg/L) were further tested against a larger collection of Gram positive isolates (n=41, *Staphylococcus* spp., *Enterococcus* spp., *Listeria* spp. and *Bacillus* spp.) from different species, including antibiotic resistant bacteria. Minimum bactericidal concentrations (MBC) were determined by broth microdilution (1:5 in MH broth) followed by an incubation of 24h.

We found high MIC values (>80–180 mg/L) for most of the iron chelators evaluated for reference strains, although one compound (a bidentate iron chelator functionalized with carboxi-rhodamine) exhibited low MIC values (5.9 and 8.8 mg/L) for Gram positive strains. Further testing of this compound in a wide range of Gram positive bacteria revealed consistent MIC values (n=31, 12.2 mg/L), including in multidrug resistant strains. MBC values were 4-fold higher for most *Enterococcus* spp. isolates (24.4–48.8 mg/L). Lower MIC values (3.05-6.1 mg/L) were observed for *S. capitis* and *Bacillus* spp., all of them showing a 2-fold increase in MBC (12.2 mg/L).

Our study reveals a promising antibacterial activity for a novel 3-hydroxy-4-pyridinone iron chelator compound against different Gram-positive genera and species (including antibiotic resistant strains). The MBC values obtained for this iron chelator suggest a bactericidal mechanism of action and *in vitro* studies for evaluation of the toxicity of these compounds are in course.

## LEM2: a new small molecule inhibitor of the p73-MDM2 interaction

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The p53 family proteins (p53, p63, and p73) are transcription factors with central roles in the control of cell differentiation, proliferation and death, which make them key therapeutic targets in cancer [1]. The complex function of these proteins is tightly regulated by MDM2, a major negative regulator of wild-type p53 [1]. MDM2 also inhibits the antitumor activity of p73 (TAp73 form) in human cells [1].

In a previous work, the development of a yeast-based assay for a simpler and faster screening of p73-MDM2 interaction inhibitors demonstrated that the negative effect of human MDM2 on human p73-induced growth inhibition, cell cycle arrest, and p73-dependent transcriptional activity is conserved in *Saccharomyces cerevisiae* [2]. In the present work, the aforementioned approach was used to search for p73-MDM2 interaction inhibitors from a library of xanthone derivatives. Among the tested compounds, LEM2 was identified as a potential p73-MDM2 interaction inhibitor. The molecular mechanism of action of LEM2 has been validated in a p53-null human colon carcinoma tumor cell line (HCT116 p53<sup>-/-</sup>) expressing p73, but not p53 and p63. The results obtained showed that LEM2 had a potent growth inhibitory effect associated with G2/M-phase cell cycle arrest, apoptosis, reactive oxygen species generation and mitochondrial membrane potential dissipation. Moreover, LEM2 led to p73 stabilization, and increased the expression levels of p73 target genes. The disruption of the p73-MDM2 interaction by LEM2 was further confirmed by co-immunoprecipitation. Altogether, in this work, a new inhibitor of the p73-MDM2 interaction was identified, thus validating the effectiveness of the yeast-based p73-MDM2 assay to screen for inhibitors of this interaction. LEM2 may represent a potential anticancer agent to be explored in anticancer therapy.

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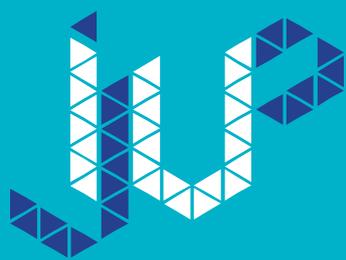
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**VI** PARALLEL  
ORAL  
SESSIONS VI



ENCONTRO INVESTIGAÇÃO JOVEM  
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**A1** **PSYCHOLOGY &  
EDUCATION SCIENCES III**  
PARALLEL ORAL SESSIONS VI

# **Entrepreneurial attitudes and motivations of children: An exploratory study**

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Entrepreneurship is associated to economic growth and to the development of the societies. Conventional wisdom convey that the acquisition of entrepreneurial attitudes and motivations early in life tend to have a positive influence in future entrepreneurial behavior. Despite such widespread argument very few scientific evidence exist that permit to corroborate such statement.

The purpose of this study is to understand which are, at the present, the main attitudes and motivations of children, to assess how these attitudes and motivations relate with other relevant variables, namely gender, age, grade, children's vocations and aspirations and parent's occupations, and to assess what is the influence of the education in children's attitudes and motivations.

In order to pursue this goal, we gathered primary data from children using an adaptation of the Entrepreneurial Attitude Survey. Four classes from the 3rd and 4th grade from a private school of Porto with a pro-entrepreneurial context were studied centered in the children.

Exploratory and econometric analyses evidence that distinct determinants impact differently on children's entrepreneurial attitudes – achievement, innovation, selfcontrol and self-esteem. Specifically, gender significantly influences innovation, with boys evidencing higher levels of innovation compared to their female counterparts. Older children and those enrolled in the 4th grade reveal lower achievement and personal control compared to 3rd grade and younger children. In general, children enrolled in the 3rd grade show higher levels of entrepreneurial attitudes than those enrolled in the 4th grade, with exception of innovation. This result seems to convey the idea that as individuals progress in their educational path, they lose part of their entrepreneurial attitudes. Although such a result should be taken with cautious given the limited number of children involved and the specific context of study, it highlights a potential risky side effect that education might have on the formation of entrepreneurs and the need to gather more evidence to properly analyze such issue.

# **Toward acceptance: Experiences of mothers and fathers in the face of their children's gender identity non-compliant with the biological sex**

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The starting point for this study was the reflection about the realities of people whose gender identity does not conform to biological sex. In a mostly heteronormative and transphobic society, transsexuality is an even atypical issue, that touches on fundamental aspects of identity with regard to gender and sexuality. Nowadays, the coming out to family happens more frequently and in more young ages. At the same time, the change in terms of perceiving transgender people as being absent within the family is noticeable and, currently, parental figures are increasingly taking part in the transition process. However, the literature that explores the familiar experiences toward the phenomenon is scarce (Blumer, Green, Knowles, & Williams, 2012).

Thus, this study, through semi-structured individual interviews, explores the experience of eight mothers and two fathers in the face of their children's gender identity non-compliant with the biological sex and the consequent transition process. Data were analyzed using thematic analysis method (Braun & Clarke, 2006). Eleven main themes emerged, six of which are exposed (Becoming aware; Adapting to the new name/pronoun; Adapting to physical changes; Fears; Interpretations and/or etiologies referring to gender identity that does not conform to the biological sex; and The health professional's role) which were a part and influence the way toward accepting the situation that every parent took.

The main conclusion was the idea that the paths toward acceptance were diverse and, for the most part, did not involve attributing a meaning of loss to the situation, contrary to that which the majority of literature suggests. Furthermore, all paths appeared to be taken for the sake of happiness and to minimize the child's suffering, revealing an extreme focus on the descendant's experience. Therefore some implications for clinical practice followed, like adapt clinical practices to family members, seeing transition process as a potential issue, of gender and sexuality, which all families might face. Finally, suggestions for future research were put forward.

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# **The production of autobiographical narratives in children with typical and atypical life paths: Structural coherence, productivity and themes**

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In the present study the cognitive-narrative model is taken as its theoretical mainframe, by which the existence of a narrative matrix is asserted, and where the structural coherence comes into play, defining how the various elements of the story come into place in respect to one another. Having into account that the narrative construction allows for the assignment of meaning to life experiences, within the narrative paradigm it can be explored how each child perceives her path.

This research aims to add knowledge about the production of life narratives by children. To this end, we focused on the analyses of structural coherence and productivity in autobiographical narratives of children with different paths of life, having in consideration spontaneous life narratives and life narratives told with an adult's support. The study also aims to explore the thematic content of the autobiographical narratives of children with different life trajectories and to test the impact of the scaffolding in the construction of life narratives, regarding coherence, productivity and thematic diversity. In order to do so, we used two narrative tasks from *Life Narrative Interview with Children* (Henriques, Ribeiro & Saraiva, 2009) that requiring previous specialized training. There were used three methods of analysis: a structural coherence analysis, using the *Structural Coherence Coding System* (Gonçalves, Henriques & Cardoso, 2006), analysis of productivity by the *Productivity Coding Manual* (Henriques, Silva & Teixeira, 2013) and an analysis of *emerging themes* in the narratives. All the methods were subject to specialized training and inter-judge agreement testing, with standards over 80% being obtained according to several measures. In our study we had as participants 50 children aged between 6 and 11 years old ( $M=8.88$ ;  $DP=1.77$ ) and with different paths of life: 20 who had always lived with their biological family; 20 living in institutional care; and 10 with adopted family.

Results show that almost all the children were able to produce spontaneous life narratives. All children were able to produce guided life narratives and the scaffolding provided by the adult led to a significant increment in the mean scores of structural coherence and the narrative's productivity, when compared to the spontaneous life narrative. Productivity, age and path of life came out as predictors of structural coherence in spontaneous life narratives, being that the first two also predicted structural coherence in the guided life narratives. The content analysis of narratives in respect to the child's context of care allowed to verify that children with an adverse life path produce narratives that are diverse in terms of thematic content and also integrate adverse life events into their narratives.

# Multiple Perspectives on Quality in Early Childhood Education Settings

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The aim of the current work was to examine multiple perspectives on early childhood and education care quality. Taking as conceptual framework the multiple perspectives proposed by Lilian Katz [1], we analyzed: i) the researcher's perspective, focused on quality of classroom interactions between the preschool teacher and children (process quality) and on the type of organization of the activities in which the target-children was participating; ii) the children's perspectives, induced by their behavioral engagement in classroom activities and their feelings about their school and preschool educator and iii) the preschool educators' perspectives, based on their perceptions about quality in early childhood and care settings. Twenty four children ( $M = 61$  months,  $SD = 10$ ) attending 12 early childhood classrooms in the metropolitan area of Porto, as well as their teachers, participated in this study. The classrooms served a high social risk population. We used the *Classroom Assessment Scoring System* [2] for evaluating the process quality in the classrooms; the *Classroom Observation Protocol* [3] for the observation of type and quality of behavioral engagement; the *Feelings About School* [4] to gauge children's feelings about their school and preschool teacher, and a semi structured interview for listening to teacher's perceptions about quality in early childhood education settings. The results revealed that: i) process quality showed positive associations with the time children spent in structured activities and negative associations with the time they spent in transitions and routines; ii) active engagement in academic activities occurred mostly during free play, and passive engagement was most commonly observed during structured activities; iii) children spent a quarter of their morning in an unproductive engagement, without academic value; iv) children's feelings about their school and preschool teacher were mostly positive, and were associated with quality of behavioral engagement; v) early childhood educator's valued topics consistent with observational data. These results suggest that during free play moments teachers have a unique opportunity to support children's learning. They also suggest that transitions and routines should be viewed as opportunities to promote a more productive engagement. At last, results also alert for the importance of investing in children's engagement in early childhood classrooms.

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# **The autobiography as possible methodology in a study of spirituality.**

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This paper presents an initial perspective, in which we sought to study the autobiographical method as a methodology for research on spirituality. This time, I try to understand if the above referenced method can contribute to studies that is something that is intrinsic to human beings and that part of a subjective view of each individual. To analyze what contribution the autobiographical method can provide for the study, I left a bibliographic research, in which pretedeu analyze some theorists who talk about the importance of this method and so realize if he may or may not contribute in later studies on spirituality.

In the autobiographical method is allowed a very particular attention to be designed and a great respect for processes of people [1]. Therefore the researcher is in line with the research subjects because the narratives they described are based on a self reflection and analysis of their memories and souvenirs, taking into account the learning acquired throughout your training process is required, either, social, political or cultural. Another intrinsic factor that aborgagem methodology is important given the subjectivity that should be seen as a value of knowledge, granted by the subjects, in addition to a qualitative domain, which is found by being one primordial factor, since we work with people and do not are manageable objects.

Therefore, we consider that the autobiographical method can be a methodology for spirituality in studies because contributes to a resumption of the subject to his inner world bringing the outside world the processes they beckon to be important to understand the formation process especially with regard spirituality. I make it clear that the concept of spirituality in which I want to address in future work should not be confused with religion, but something that is internal to each individual and that makes him have a descent as "better" person. Something as subtle, more exercises positividade to humans, making a more human person and ready to understand his neighbor.

All these contours that the autobiography grants, enables defy the inside of the subject in order to understand issues that are not often seen in the scientific means and when it is seen as something without much consideration or relevance. But at the same time we should look at the contributions that this method provides for studies dealing with human beings, because it is no methodology that will rem takes account of the study subjects its peculiarities.

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# Attachment, Personality and depression in the elderly - that relations?

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Quality of Attachment, Personality traits and levels of Depression play an important role in improving the prognosis and quality of life in old age. The main objective of this study is to explore the relationship between these three constructs, trying to understand how they articulate and the role of linking and Personality in the development of Depression. The sample consisted of 103 participants (30 male and 73 female) with a mean age of 73.82 (SD = 9.81).

In order to measure Attachment, we used a portuguese adaptation of the Late Adult Attachment Security, for Personality the NEO-FFI-20 and for Depression the GDS.

The results show positive associations between the Security, Extraversion, Openness to Experience and Conscientiousness and negative associations between Security, Neuroticism and Depression. Avoidance and Anxiety were negatively related with Extraversion. Anxiety was negatively associated with Conscientiousness.

Depression and Neuroticism was positively associated with Anxiety.

Regarding the effects of gender, it was found that women had higher levels of Depression and Neuroticism when compared to men. It was noticed also that Depression is predicted by Neuroticism, by Academic qualifications and the Extraversion trait of personality, in order of strength, in this sample of participants.

In this study it were also explored the psychometric properties of *Late Adult Attachment Security* for the Portuguese elderly population in a sample of 110 older adults ( $M = 72.26$  years,  $SD = 10.62$ ).

Overall the results highlight the importance of taking into account the quality of meaningful relationships and characteristics of Personality in psychological intervention and prevention of psychopathology in the elderly.



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**A2** **MATHS**  
PARALLEL ORAL SESSIONS VI

## Missing and unbalanced data in logistic regression: the study of silicosis within tuberculosis.

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The ultimate goal of this study consisted of the identification of the clinical and demographic factors that are able to distinguish patients with tuberculosis (TB) from those that also have Silicosis (SL). The provided data required special attention: apart from only having 200 individuals with silicosis in a database of over 16000 patients, there were several missing values in some key variables. As these questions may arise in similar studies, especially in those related with low prevalence diseases, the methodology used here may be of interest to the research community.

The first step in the analysis concerned the choice of the adequate explanatory variables. The presence of missing data and the severe unbalance between the SL and the TB classes hindered the possibility of using common supervised classification methods, such as logistic regression. Actually, very few such methods are able to handle missing data, except classification trees. Nevertheless, they are extremely biased by the unbalance of the given problem [1][2]. Hence, random oversampling the minority class was performed until a state of even balance. Then a classification tree was constructed and a level of importance of each variable was provided by the model.

Typically, in the biostatistics field, the regression logistic model is quite appreciated. We next investigated such a model, having the variables assigned with a predictive importance greater than zero as predictors. Naturally, the missing data and the unbalance of the response variable must again be dealt with. A new dataset was considered, consisting of 164 silicotics and 10432 non-silicotics (out of the initial 205 and 16354, respectively), all being complete observations. Then the following procedure was implemented: 1) oversample of the SL class until both classes were equally represented; 2) full-size random selection with replacement; 3) logistic regression with the obtained dataset and saving of the estimated coefficients; 4) repetition of the procedure 1000 times. While the first step ensured a balanced dataset, the others incorporated randomness within the SL class. Regression coefficients were obtained by averaging through the 1000 repetitions. Any such coefficient was said to be statistically significant if its values disregarding the adequate lower and upper percentiles did not include zero.

Interpretation of the results was finally performed.

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# The back-calculation method and its use in the estimation of the AIDS incidence rate in Portugal

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It is well-known that the Human Immunodeficiency Virus (HIV) causes progressive failure of the immune system which can evolve to the Acquire Immune Deficiency Syndrome (AIDS). The size of the affected population with HIV is a critical question asked by the scientific community. In this work, the back-calculation method is applied to the estimation of the 2000-2012 AIDS incidence rate in Portugal. Such estimates are crucial to understand the extent of transmission of the infection, evaluate intervention strategies and effectively plan new public health control measures.

The back-calculation method retro-projects notification data, assuming an incubation period from HIV infection to the onset of AIDS. However, because HIV infection is usually not immediately detected, good data on incubation times are only available for specific cohorts. Moreover, in Portugal, as in most countries, the epidemic is monitored by a surveillance system based on cases notification by physicians. It can take several months, and often years, until the diagnosed cases are notified.

For a fixed month, the number of newly infected individuals in the previous months was estimated from the number of individuals diagnosed at that month and from the distribution of the incubation time (taken to be the difference between the diagnosis date and the infection date). It was considered that the latter follows a Weibull or a Gamma distribution, by [1]. The number of new diagnoses and newly infected cases in any given month was assumed to follow a Poisson distribution. The method was based on a convolution equation adapted for the discrete case, [2]. The likelihood function was considered for the complete data, including the mean number of new infections per month as unknown parameters, which are to be estimated given the number of new diagnoses per month. Maximization of the complete likelihood used a combination of the expectation-maximization algorithm [3] and linear regression, in order to ensure smoothing. Results from the methodology were tested on simulated data.

Correction for the reporting delays was obtained by conditional likelihood estimation for count data assuming a Poisson distribution, as previous work in [4] and [5].

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# Structural breaks in heart rate variability time series

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The detection of structural breaks in signal processing and time series analysis is a relevant problem in various areas. A structural break is a change in a certain characteristic of the signal or time series, such as the process mean or variance.

The general aim of this study is to describe and evaluate different methods for modelling an univariate nonstationary heart rate variability (HRV) time series, by segmenting it into blocks which are fitted by stationary processes, considering multiple change points detection.

There are different methodologies for the detection of structural breaks in time series [1]. In this work we begin with the study of changes in mean (Fig. 1a) and the autoregressive (AR) coefficients (Fig. 1b) in piecewise AR processes, here illustrated for 3 segments of simulated data. We will evaluate these methodologies with 24-h of real heart rate variability (HRV) recordings, which present changes in both the conditional mean and variance [2].

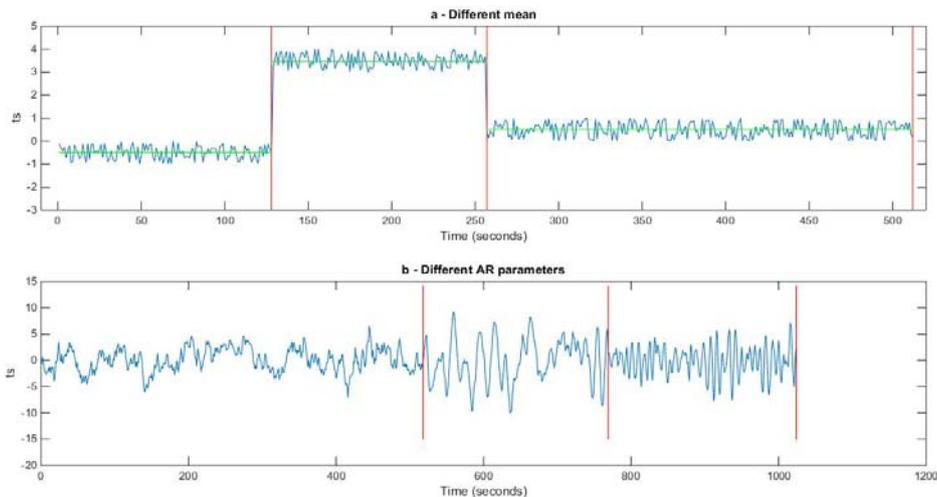


Fig. 1 Results with 2 realizations from piecewise AR processes

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# Math diagnostic tests at FCUP: a psychometric study

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A Mathematics diagnostic test has been performed at FCUP since 2008. The test is non-compulsory and has been directed essentially to first-year students from Mathematics, Computer Sciences and Physics.

This work assesses the quality of those tests, through modelling and calibration of the results.

An observed score in a test should reflect the candidates' knowledge on the evaluated domains, usually denoted by "ability". As the score can be influenced by several factors, such as illness, distraction or fatigue, what the examiners would really like to have is the (true) score that is free from these factors. The observed score can also be biased due to bad formulations or excess of difficulty (ease) of the test items. It is therefore important to estimate the probability of a correct response to an item as a function of the item characteristics (difficulty, discrimination and guessing) and of the ability level of the candidate. Denoting each individual by  $i$  and any given item by  $j$ , this probability is modelled by a logistic function of the form

$$P_{ij} = P(X_{ij} = 1 | \theta_i, a_j, b_j, c_j) = c_j + (1 - c_j) \frac{\exp[D a_j (\theta_i - b_j)]}{1 + \exp[D a_j (\theta_i - b_j)]}$$

where  $c_j$ ,  $D$ ,  $a_j$ ,  $b_j$  and  $\theta_i$  denote the lower asymptote (guessing parameter), a scaling constant, the item discrimination, the item difficulty and the individual ability, respectively.

Classic Test Theory (CTT) [1] and Item Response Theory (IRT) [2] are applied to evaluate the overall quality of the test, and in particular, the quality of the items formulation, namely through the estimates of the difficulty and discrimination parameters.

Finally, the calibration of the yearly results obtained so far is performed, allowing for the assessment of the evolution of the math ability taught at the portuguese secondary school over the last years.

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# Best-Response Dynamics in Hotelling model

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In 1929 Hotelling[1] presented a model for spacial competition between firms selling the same commodity. Since then the Hotelling model has become a very attractive framework for the study of duopoly markets, see for example [2-4]. In this model firms are competing in a two-staged game, in first place firms choose their location and after they choose their price. In this work we introduce a dynamic way for the price establishment in the second sub game, namely Best-Response Dynamics[5]. In particular, we fully characterize the set of prices with duopoly property, that is the set of prices where each firm has a non-empty market share. We show that if the Nash Price Equilibrium has duopoly property then is asymptotically stable and, in addition, the whole set of prices with duopoly property is forward invariant.

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# Agent-Based Models: Application to the Portuguese Demography

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Simulation is a modelling mechanism often employed to deal with phenomena with lacking data. In the case at hand, the study will revolve around the age specific growth of the Portuguese population, considering migrations [1,2].

A new modelling mechanism, denoted by Agent-Based Models (ABM) [3], is applied. In ABM, instead of directly modelling the phenomena, the interveners are modelled and their interactions rule the model evolution. The model starts with the Portuguese demographic and economic information from 2011 [4-6] and is run until 2014, considering three economic scenarios (recession, stability and prosperity).

We project that, independently from the economic scenario, there will be a reduction of the total Portuguese population. Nevertheless, the economic scenario will change the growth dynamics: for a situation of recession, the population will reduce almost linearly throughout the years; for a stable economy, the population will reduce almost linearly for 20 years, and afterwards will approximately stabilize; for a prosperous economy, the reduction will be slow and seemingly stabilize after 10 years. These variations are mostly due to migration, which is highly affected by the economic scenarios.

Additionally, and independently from the economic scenarios, the population will tend to get older and with a low percentage of young-aged individuals.

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**A3** **ENVIRONMENT I**  
PARALLEL ORAL SESSIONS VI

# Optimization of solid phase extraction of micropollutants from drinking waters

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The occurrence of micropollutants in the environment became a global concern, with special attention for emerging contaminants, continuously introduced into the environment and found at trace concentrations ( $\mu\text{g/L}$  or  $\text{ng/L}$ ) [1]. Some of them are not completely removed during domestic wastewater treatment being, therefore, discharged into receiving water bodies, some of them used as sources of drinking water. In this context, it is important to set up fast, sensitive and reliable analytical methods that enable the determination of a wide range of pharmaceuticals, pesticides and other pollutants in drinking waters at the low concentration levels.

The present work describes the development of an analytical method based on off-line solid phase extraction (SPE) followed by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS), for the fast and simultaneous determination of 39 pollutants in drinking water: 25 pharmaceuticals, 8 pesticides, 3 hormones, 1 industrial compound and 2 metabolites. Some of these pollutants were defined as priority substances in the Directive 2013/39/EU and included in the recent watch list of Commission Decision 2015/495/EU. UHPLC was optimized by testing different mobile phases consisting of methanol, ethanol or acetonitrile as organic phases, and ultrapure water, 10 mM of ammonium acetate or 0.1% of formic acid as aqueous phases. MS parameters such as desolvation and source temperature, nebulizing and drying gas flow and capillary voltage were tested. In order to evaluate the conditions of SPE that yield higher recoveries of the target compounds, several parameters were studied: type of cartridges, the extraction solvents, sample's pH and volume and the addition of substances for quelating and dechlorinating.

The optimized mobile phase for the majority of compounds was ethanol/water and the best conditions of MS were:  $2.5 \text{ L min}^{-1}$  and  $10 \text{ L min}^{-1}$  for nebulizing and drying gas flow, respectively, 0.5 kV for capillary voltage,  $450 \text{ }^\circ\text{C}$  for desolvation temperature and  $200 \text{ }^\circ\text{C}$  for source temperature. The higher recoveries for the most compounds (up to 98.7%), were achieved with Oasis® HLB, sample pH 3, ethanol as solvent, 250 mL of water samples and sodium thiosulfate as dechlorinating agent.

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# Integration of chemical and biological processes in winery wastewater treatment: effect of Fenton pre-oxidation and SBBR (Sequencing Batch Biofilm Reactor) operation

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Currently there is an increased interest in the development of technologies for treatment and reuse of wastewater. The integration of chemical and biological processes allows the removal or processing of products initially resistant to biodegradation [1]. Biological wastewater treatment is useful for degradation of wastewaters with high organic content, such as those coming from wineries, and the use of attached biomass systems like SBBR (Sequencing Batch Biofilm Reactors) seems an interesting possibility. However, the presence of compounds recalcitrant for the microorganisms frequently makes the complete treatment of winery wastewater impossible. Combination of a chemical treatment (Fenton pre-oxidation) followed by biological processes may prove useful in this situation [2], but the effects on reactor performance have not yet been evaluated.

This work aims to study the possibility of combining chemical and attached biological processes for winery wastewater treatment using a phased approach. In the first phase the colonization of two types of biofilm media (carriers), Bioflow 9 – BF9 and Bioflow 40 – BF40, was studied in two different situations: in the laboratory and in a reactor of a winery full scale plant. Results based on biofilm accumulation (as ST and SVT) showed that BF40 carriers colonized in the real winery were more suitable for further SBBR studies. The second phase consisted in the optimization of the  $[\text{Fe}^{2+}:\text{H}_2\text{O}_2]$  ratio and the iron concentration of the Fenton reaction for effluent pre-treatment. The best ratio found was [1:4] for 1g/L of  $\text{Fe}^{2+}$ , based on increased biodegradability, for input values above the initial wastewater concentration, an essential condition for proceeding to the biological treatment. The third stage is ongoing and comprises monitoring two SBBR reactors, one normally operated and the other feed with Fenton pre-treated effluent, in order to understand the advantages of integrating the two processes.

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# CHARACTERIZATION OF AUTOCHTHONOUS MICROORGANISMS ALONG THE NORTHERN PORTUGUESE COAST - POTENTIAL FOR HYDROCARBON DEGRADATION

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Oil spills are of main environmental concern, with tons lost annually by shipping accidents, pipelines losses, or other anthropogenic uses. The oil pollution and its derivatives are considered worldwide problems, threatening coasts and estuaries that are ecologically very important areas. Several methodologies have been proposed for the recovery of these ecosystems, including bioremediation that is considered an efficient and economic option. The foundation of this technique involves the use of biological agents, particularly microorganisms, to remediate organic contaminants in the environment. For proper implementation of this technique it is necessary to have prior knowledge about the capacity of the autochthonous microbial communities to biodegrade petroleum hydrocarbons.

The present study aimed to characterize the hydrocarbon degradation potential of autochthonous microorganisms collected along the northern Portuguese coast. Sediments were collected in 13 locations including sandy coastal beaches and Minho and Douro estuaries. These sediments were characterized in terms of total microbial abundance (by DAPI), hydrocarbon degrading microorganisms' abundance (HD by MPN) and microbial community structure (by ARISA). In addition, the Total Petroleum Hydrocarbons concentrations were also determined (by FTIR).

It was observed a clear differentiation of the microbial community structure between locations, with analysis of similarities (ANOSIM) revealing significant differences between the three zones (Coast, Douro and Minho). In addition, higher abundance of hydrocarbon degrading microorganisms was detected in the Douro estuary, which was also the location with higher levels of total petroleum hydrocarbons. Nevertheless, hydrocarbon degrading microorganisms were found in all collected sediments, despite the different degrees of petroleum hydrocarbon contamination.

Therefore, at the selected sites, the characterized autochthonous microbial communities have the potential to degrade hydrocarbons, being important to assess experimentally their ability for bioremediation of these pollutants.

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# Response of a salt marsh plant-microorganisms association to antibiotic contamination

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Polluted estuaries have been reported all over the world and their sediments can be considered both as sinks and sources of contaminants. Salt marsh plant-microorganisms association can be determinant in contaminants degradation because plants can enhance microbial degradation through specific microenvironments for pollutant-degrading microorganism. Thus, the aim of this study was to understand the response of salt marsh microorganisms associated with *Phragmites australis* to a veterinary antibiotic contamination, in controlled conditions. Plants and respective rhizosediment (combined or individually) were collected in an estuary and exposed for 7 days to enrofloxacin (ENR) under different nutritional conditions in sediment elutriates doped with the antibiotic. Both water and sediment were analyzed at the end of the experiment to i) assess ENR removal (by HPLC) and ii) evaluate the potential changes in terms of microbial community structure (evaluated by ARISA) and abundance (estimated by DAPI) in sediments. Results showed high ENR removal, which was highly dependent on the nutritional status of the system. No significant differences in microbial abundance among tested conditions were in general observed, although some changes in bacterial richness and diversity were detected in unplanted systems. Nevertheless, analysis of similarity showed statistically significant effect of both the presence of plant and type of treatment on the microbial community structure, and significant differences among all treatments tested. This study emphasizes the potential of salt marsh plant-microorganism association for antibiotic removal that, although highly dependent on their nutritional status, shows promising results for its application in the remediation of the environmental impact of these contaminants.

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ENCONTRO INVESTIGAÇÃO JOVEM  
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**A4** **BIOLOGICAL SCIENCES VI**  
PARALLEL ORAL SESSIONS VI

# ***In vitro* screening assay using the murine pre-adipocyte cell line 3T3L1 to study anti-obesogenic activities of chemical compounds**

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Obesity is a global health threat with several etiologies. Standard obesity treatment (e.g., bariatric surgery, healthy lifestyle promotion) may not be enough to treat obese people that represent more than half of the European Union population [1]. Model cell culture systems have been vital in obesity studies. The 3T3-L1 cell line [2] has been widely used for the study of bioactive natural compounds research, including research of anti-obesity properties of several natural compounds as fucosterol and phlorotannins from the brown algae *Ecklonia stolonifera* [3,4], meridianins from the tunicate *Aplidium meridianum* [5], and extracts from the dandelion *Taraxacum officinale* [6]. Cyanobacteria, known as blue-green algae and producer of cyanotoxins, have shown high content in secondary metabolites with relevant activity (e.g., antibiotic, antifouling, anticancer) [7]. We are currently exploring the chemical richness of these prokaryotes by testing cyanobacterial strains regarding their anti-obesogenic activity in (pre)adipocyte cells.

Various cyanobacterial strains were grown and their extracts were produced through simple vacuum filtration of their lyophilized mass suspended in a mixture of Dichloromethane and Methanol (2:1). These extracts were then fractionated using vacuum liquid chromatography. Bioactivity of the fractions was tested in the proliferation assay of preadipocyte cells. Proliferation can be assessed by the Sulforhodamine B (SRB) staining and the incorporation of Bromodeoxyuridine (BrdU) into the DNA. Furthermore, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT bioassay) delivers information about the cellular viability and metabolic activity, through the reduction of MTT to formazan by mitochondrial activity.

The most polar fraction (eluted with 100% MeOH) of the cyanobacterial strain *Planktothrix planctonica* has shown the strongest effect on cell proliferation (above 50% compared with the solvent control). The same was verified for the MTT assay, where the activity of mitochondrial enzymes was increased. After consequent sub-fractionation, purified fractions were obtained and the most polar fraction (fraction 7) showed once again pro-proliferative activity through SRB and MTT assay. Further column chromatography will allow us to purify the single compound responsible for this activity and enable us to elucidate its chemical structure.

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# Ecological interpretation of a *Pinus pinaster* Aiton archaeological assemblage from the Sabor valley, Northeastern Portugal

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The science of anthracology studies preserved carbonized wood from various contexts in order to infer taxonomic, ecological and ethnological information. When applied to archaeological remains it can allow us to infer elements about past cultures, climates and/or environments. In the case of the Sabor river valley, the particularly rich assemblage of charcoals from various archaeological sites of different chronologies offers us a unique opportunity to infer ecological data on several taxa. In the specific case of *Pinus pinaster*, the surprising amount of charcoal fragments, retrieved from 12 archaeological sites covering different chronologies, from the beginning of the Holocene to the 19<sup>th</sup> century, allows us to attempt a reconstruction of the evolution of the relative significance of the maritime pine to man and the environment across a large timespan.

In this study, charcoal fragments were obtained from archaeological samples proceeding from the archaeological intervention in the Sabor valley. They were then observed under a stereo- and reflected-light microscope. The three classic diagnostic sections (Cross-, tangential and radial) were performed and the fragments identified with the help of wood anatomy atlases (Schweingruber 1990 [1], Vernet 2007 [2]). Notable anatomical features and alterations (e.g. Reaction wood, tangential fissures.) were recorded, and the information stored in a digital database.

The distribution of *P. pinaster* remains allow us to observe that although there is a clear preponderance of roman sites from the 1<sup>st</sup> to the 3<sup>rd</sup> centuries AD (e.g. Olival da Santa and Cabeço da Grincha), *Pinus pinaster* is also frequent in Iron age (e.g. Crestelos and Castelinho) and Bronze Age (Foz da Ribeira do Medal and Terraço das Laranjeiras) sites, from the 2<sup>nd</sup> millennium BC to the 1<sup>st</sup> century BC. The oldest contexts with pine wood are those from the Mesolithic levels of Foz da Ribeira do Medal (8<sup>th</sup> millennium cal BC) and the most recent came from Quinta do Medal (17<sup>th</sup> to 19<sup>th</sup> centuries). The amount and context of these finds suggest that *P. pinaster* was autochthonous to the Sabor valley in the early Holocene. Moreover, it was already well established by the Iron Age. (5<sup>th</sup> to 1<sup>st</sup> century BC) These data together with previous information, suggest that the Sabor valley could have been a refugium for this species. In addition, its co-occurrence with *Cistus sp.* suggests that its expansion followed anthropic disturbance.

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# Interannual variability in early life history of *Pleuronectes platessa* (L.) in a cold-water nursery ground

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Plaice, *Pleuronectes platessa*, is a commercially exploited flatfish species in northern European waters. Despite numerous studies on plaice early life history, information for its northern range of distribution is still scarce.

In this study, the otolith microstructure of post-settlement plaice, sampled from an inshore nursery ground (Valosen, Bodø, Norway) between 2005 and 2006, was examined to analyse interannual variation in early life patterns of juvenile plaice. For that, 30 sagitta otoliths (15 of each year), from plaice ranging between 14 mm and 40 mm, were mounted in microscope slides using a thermoplastic cement (crystalbond™), with the concave side down. Otoliths were then hand-ground to the otolith core using sandpaper (3, 1 µm) and examined under transmitted light in a microscope linked to an image analysis system. This technique exposed the daily increments in the otoliths that once counted and characterized, allowed to determine hatch dates, larval duration, settlement dates and total age of the fish. A minimum of two counts per otolith was done to maintain rigorous results, and the average values were used.

A one-month difference in hatching times was found between 2005 and 2006, with most hatchings occurring during May in 2005 and June in 2006. Larval duration was however similar with an average of 39 (±9) days in both years. Settlement in 2005 occurred in June while in 2006 was mainly in July. The time between metamorphosis and completion of settlement was quite variable ranging from 5 to 23 d considering all fish analysed. Further work needs to be done including estimation of larval and post-settlement growth rates based on otolith increment widths in order to fully understand temporal variation in plaice early life dynamics.

## Sexual dimorphism of the brown shrimp *Crangon crangon*

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One of the most remarkable phenomena in the evolution of mating systems in living organisms is sequential hermaphroditism, commonly referred to as ‘sex change’ or sex reversal’. As the name suggests, sequential hermaphrodites naturally change their sex during their life: individuals reproduce initially as males and then change to females (protandry) or vice versa (protogyny). Many theoretical models have been proposed, mainly based on sexual allocation theories and the differential advantage of body size on the reproductive success of each sex: sex changers should increase their fitness becoming large males (in protogyny) or large females (in protandry). The brown shrimp *Crangon crangon* L. (Decapoda, Caridea) is an interesting candidate for studies of sex reversal. Its sexual mode of reproduction is still debated, being described as gonochoristic (fixed separate sexes) [1], but also as obligate protandric (male to female sex change) or facultative protandric (some but not all individuals change sex) [2]. This suggests a degree of sexual plasticity and implies that primary females, males and secondary females (resultant from male sex change) coexist in natural populations. Descriptions of the species sexual dimorphism, however, are not validated; moreover, no intermediate morphotypes have been described to date. Therefore, in this work the sexual distinctive features of *C. crangon* were analyzed applying Geometric Morphometric tools in order to objectively describe the sex differences and to depict possible intermediate morphotypes, including the correspondent to secondary females.

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# Toxic effects of endocrine disruptors on *Daphnia magna*

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In the past century the amount of chemicals released into water bodies has increased, particularly xenobiotics that possess endocrine disruption proprieties (e.g. bisphenol A and paracetamol). Endocrine disruptors are substances capable of mimicking hormones. These substances are also capable of inducing oxidative stress. Bisphenol A, is an important industrial prime matter [1], and has been extensively described and characterized as an endocrine disruptor. *In vitro* studies have shown that acetaminophen shows signs of endocrine disruption but *in vivo* studies this effect did not demonstrate such a clear correlation. The main goal of this study is to assess the effects of paracetamol and bisphenol A as endocrine disruptors. Additionally we pretend to evaluate the effects of oxidative stress induced by these xenobiotics. Acute toxicity assays were performed in order to assess EC<sub>50</sub> for both xenobiotics. In a second step a sub-chronical toxicity assay (7 days) was performed to evaluate reproductive endpoints. *D. magna* with less than 24 h were exposed to different xenobiotics concentrations (EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>40</sub>) defined according to EC<sub>50</sub>. After exposure biochemical markers were analyzed, including those focused on oxidative stress parameters (catalase (CAT), and glutathione-S-transferases (GSTs) activities); lipid peroxidation levels (TBARS levels). In order to perceive endocrine disruption effects a life-history of daphnids (fecundity, population growth, somatic growth and ecdysis) was evaluated.

Results of acute assays showed an EC<sub>50</sub> = 4.75 mg l<sup>-1</sup> for paracetamol; and EC<sub>50</sub> = 16.9 mg l<sup>-1</sup> for bisphenol A. According to these results a sub-chronic assay was performed with the calculated values of EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>40</sub> (corresponding to 3.7; 4.1, and 4.5 mg l<sup>-1</sup> for paracetamol exposure). Our results showed that paracetamol can induce an increase of anti-oxidant defense enzymes and an increase of lipid peroxidation, demonstrating that paracetamol induces oxidative stress. Development endpoints such as ecdysis and fecundity have not been significantly affected after paracetamol exposure.

Previous studies have demonstrated that exposure to paracetamol did not induce severe deleterious effects. In spite of that it causes significant alterations on the rate of population increase for high concentrations [2]. Bisphenol A, when present in high concentrations can be responsible for high mortality as well as affect physiological endpoints such as brood size, length of individuals, and number of neonates [1].

Overall, paracetamol shows possible signs of endocrine disruption, and significant biochemical alteration level (oxidative stress induction). In order to clarify this effect of acetaminophen the next step intends to expose *D. magna* to bisphenol A (12 mg l<sup>-1</sup>=EC<sub>10</sub>; 13.7 mg l<sup>-1</sup>=EC<sub>20</sub>; and 16.0 mg l<sup>-1</sup>=EC<sub>40</sub>), due to its well-known endocrine disruption proprieties.

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**VII** PARALLEL  
ORAL  
SESSIONS VII



ENCONTRO INVESTIGAÇÃO JOVEM  
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**A1** **ECONOMICS**  
PARALLEL ORAL SESSIONS VII

# **State-Owned Enterprises as key transforming agents in the world economy: a bibliometric study**

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**Abstract:** Over the past ten years, State-Owned Enterprises (SOEs) have been gaining great momentum in the global economy as key promoters of Outward Foreign Direct Investment (OFDI). The global financial crisis may have established the conditions for the fast-growing internationalization process of SOEs, particularly of those originating from emerging countries. In spite of being at the core of the international economic debate, the intervention of SOEs in OFDI has not been systematically studied, being the subject of only very recent and scattered research. This dissertation seeks to gather, as exhaustively as possible, relevant and updated data on this topic, aiming to bring a valuable contribution for the state-of-the-art knowledge of OFDI activity by SOEs.

Bibliometry constitutes a suitable and rigorous methodology to undertake a comprehensive empirical analysis of the relevant literature and assess the trends, patterns and characteristics of extant research. This study analyses publication, citation and co-citation patterns, providing a segmented analysis of the gathered information per country, academic journal, academic/scientific institution and author. The ultimate goal of the present paper is to draw up the overall scenario of the transforming activity of SOEs in the world economy, highlighting the core economic and geopolitical topics within the literature, and unveiling the most meaningful contributors and contributions to this debate.

**Keywords:** State-Owned Enterprise (SOE), Outward Foreign Direct Investment (OFDI), Emerging Countries, Ownership, Bibliometry

# Functional Structure of Public Expenditure and Economic Growth

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This research aims at understanding the importance of reallocation of public expenditure as an economic growth determinant in the short and long run. This subject appears to have a crucial importance in the current European context, given the rigor imposed to the public finances, and the slow recovery from one of the most important economic and financial downturns in the recent past. Re-allocation of public expenditure, while keeping constant the overall level of public expenditure, may contribute positively to enhance growth, in a context where high tax burden crucially limits expenditure efforts by the fiscal authorities.

We have developed an empirical application, for a sample of twenty-seven countries of the European Union, in the period between 1990 and 2012, in order to assess the relationship between different categories of public expenditure (classified by function) and the *per capita* output growth. The specifications deemed appropriate by the literature were taken into account in the estimation of this model. The estimation method chosen was Ordinary Least Squares with the fixed effects and White correction (Diagonal), to allow valid statistical inference.

Thus, we have estimated a growth equation based on a model of neoclassical type, considering public expenditure classified by functions, based on Classification of the Functions of Government, developed by OECD – COFOG, as the works of Afonso e Alegre (2008) and Ferreira *et al.* (2012). In the work done, we have considered either the variations in the COFOG components for each year, either their initial level. The relevance of the explanatory variables for our analysis refers to the five functional components of public expenditure that are most significant: General Public Services, Economic Affairs, Health, Education and Social Protection.

Our main results showed a positive impact on the real Gross Domestic Product (GDP) growth of public spending on Education, which contrasts with the negative impact of spending on Health, Social Protection and Economic Affairs, which is consistent with some of the main literature. There was no significance to expenditure in General Public Services, at the usual levels. Therefore, we have concluded that a redistribution of public spending towards Education, at the expense of other categories, may have some positive effects on the GDP growth of European economies. The accumulation of human capital, potentially more evenly, proves to be an engine of potential growth for the more developed countries.

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# Fiscal multipliers and fiscal rules: An application for the European Union

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The recent sovereign debt crisis had raised many questions about the use of discretionary stabilization policies, with particular relevance to fiscal policy orientation and its macroeconomic impacts, mainly in a context of high public indebtedness. This discussion is particularly relevant for the European Union (EU) case where, despite the set of rules imposed by the Stability and Growth Pact, it was not possible to ensure budgetary discipline on a broad set of countries.

This essay aims to understand how fiscal multipliers change in the presence of fiscal rules, especially in a high public debt environment. With this study, we aim to contribute to the existing literature in terms of: i) a critical review of the transmission mechanisms of fiscal policy, with particular emphasis to situations of high public debt, and ii) an empirical evaluation, applied to the EU, of the impact of different types of fiscal rules on the value of fiscal multipliers, especially in a context of high public debt-to-output ratios.

It was used a VAR methodology, for 26 EU countries and covering the period 1993-2008. The main set of variables included in the model were the real gross domestic product (GDP), as explained variable, and public spending, tax revenue and real interest rate on government debt (10 years), as explanatory variables. Moreover, it were added in the estimations a debt-to-GDP variable, as also were tested four dummy variables, representing each fiscal rule considered in our approach: expenditure rule, revenue rule, budget balance rule and debt rule.

The results obtained show that the effect on the expenditure-side multiplier reaches its maximum for public debt around 70-80% of GDP; above this threshold, the multiplier decreases, observing negative values when debt exceeds 110% of GDP. Fiscal rules, in turn, impact positively spending multipliers (except for public investment), while a positive effect is only verified for tax multipliers when a debt rule is used. It was also observed that the impact of fiscal rules on fiscal multipliers is positive when public debt is high, even though, if public debt levels rise above 105% of GDP, this effect decreases, which may predict a decrease of fiscal rules effectiveness, as debt increases to very high levels.

We thank João Loureiro for helpful comments and suggestions.

# The effect of globalization on wage inequality – an application to the European Union

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Our paper seeks to link the concepts of globalization and wage inequality, while comparing the different realities of developed and developing countries. It includes a special focus on the European Union in the empirical portion. We first review the available literature, then to test the foremost theoretical mechanisms by building and testing a panel data model on two samples of European Union countries (EU), one taking the place of the North<sup>1</sup> and the other taking the place of the South<sup>2</sup>, as they are defined in the mechanisms we examine.

As for our methodology, we used a general fixed effects panel data model, using the two samples of European Union countries we mentioned above. Our dependent variable was the Theil index for industrial wage inequality. Our independent variables were the following: trade openness level (to measure the effect of international trade on wage inequality), Foreign Direct Investment inflows and outflows (to measure the effect of international investment), Expenditure in Research and Development over Gross Domestic Product and Percentage of High-Tech Exports (to test the effect of technological development on wage inequality.) We controlled for labor institutions with the Labor Union Density variable, for the role of education using the Rate of Lower Secondary Education Completion and for general level of economic development with lnDP. We examined a period between 1970-2007.

We found that trade has the effect of enhancing inequality in the “North” countries of our EU sample, though we could not significantly conclude on its effect in the “South”. Foreign Direct Investment (FDI) Inflows have the effect of diminishing inequality in the “North”, while FDI outflows have the same effect in the “South”. We also tested the effect of technology on inequality using two variables: on the one hand, we found mixed evidence for how the share of High Tech Exports affects inequality; on the other hand, gross expenditure on Research and Development was, when significant, always positive for the “North” sample. By testing with a composite globalization index, we conclude that trade is dominant over FDI when it comes to which economic flow strongly affects inequality. Moreover, when we tested for the non-economic aspects of globalization, we found that both political and social aspects of it also cause wage inequality to increase.

We thank Rosa Forte for the helpful comments and suggestions.

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<sup>1</sup> Austria, Belgium, Denmark, Finland, France, Germany, Ireland, Italy, Luxembourg, Netherlands, Spain, Sweden and the United Kingdom.

<sup>2</sup> Bulgaria, Croatia, Cyprus, Czech Republic, Estonia, Greece, Hungary, Latvia, Lithuania, Malta, Poland, Portugal, Romania, Slovak Republic and Slovenia.

# Measuring competition and market power of the Iberian Electricity Market using concentration and market power estimators

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In recent years, the electricity sector has undergone a number of reforms, especially in its regulatory framework, through liberalization processes in the production and commercial segments of its value chain.

We also witness the integration of some national electricity markets in order to stimulate competition and consequently promote competitiveness in order to obtain significantly lower energy prices compared to the original situation of disaggregated national markets. Among these experiences we have the European cases of Nordpool, EPEX or MIBEL – Iberian Electricity Market.

Some intrinsic features to this industry, such as its high capital intensity or its inelastic short run demand, make possible the observation of a potential high market concentration levels and consequently a potential market power exercise from any supplier and arise as reasons that justify the analysis that we propose ourselves to do.

In this way, this study sought to infer the evolution of the competition in the upstream segment of MIBEL (2007-2014), through the calculation, analysis and interpretation of concentration indicators and market power estimators such as *Herfindahl-Hirschman Index*, *Pivotal Supplier Index* and *Residual Supplier Index*.

In order to achieve this purpose, two analysis' levels were considered. An aggregated one focused on the whole Iberian Market during the entire observed period, and another one, which both markets that compose the MIBEL were analysed separately, for the hours which a market *split* occurred. The aim of this alternative analysis' level was to clarify if the competition's scenario was different in each market for the hours which a price differential has occurred.

Our findings reveal that the MIBEL concentration levels decreased during the analysis' period, as well as never a supplier was assumed as pivotal – except in a few hours in 2007. Thus, we can assume that the MIBEL is competitive, as well as there was no place for a potential market power exercise.

In the hours which a price differential has occurred between both countries, the Spanish case is so much similar when compared with the Iberian Market, in an aggregated perspective. By the other hand, the Portuguese case is facing a serious state of high market concentration levels as well as a high potential of market power exercise from its pivotal supplier

For a further investigation, we raise the question of whether the observed trend of a potential increase in market's concentration levels at the end of the period under review, became in fact, a real threat concerning to a potential market power exercise from any electricity supplier.

# Transfer Pricing and Tax Havens for Attracting FDI

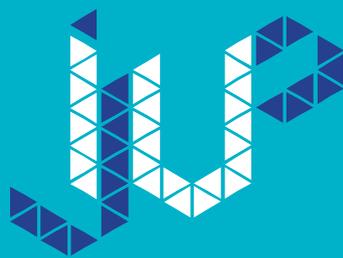
Darya Valeriivna Shangina\* & Pedro Cosme Costa Vieira\*\*

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**Abstract:** Enterprises seek profit maximization that comprises tax optimization which, within multinationals, includes the shifting of profits from the less to the most favorable countries using transfer pricing of intra-group transactions and tax havens as the “middle man”. Because FDI is an important engine of economic growth of, at least, the less developed countries and multilateral agreements impose limitations on the allocation of direct subsidies to businesses, in our research we explore the hypothesis that countries could use the relaxation of regulation on transfer pricing as a fiscal policy instrument for attracting FDI. Using a good composed of two parts, the brand and the hardware, and consumers with concave utility on the brand, we conclude that countries can use the relaxation of regulatory acceptance of transfer pricing to attract FDI when 1) the creation and development of the brand is an important part in the total cost of the good and 2) the increase in production costs of relocating the production from the most to the less developed country is not very large. Our results seem in accordance with empirical evidence.

**Keywords:** Transfer Pricing, Tax Havens, Foreign Direct Investment, Tax Incentives.

**Jel Codes:** K29, F21, H87, O23



ENCONTRO INVESTIGAÇÃO JOVEM  
DA UNIVERSIDADE DO PORTO

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**A2** **ASTRONOMY & PHYSICS I**  
PARALLEL ORAL SESSIONS VII

# Fine-structure constant constraints on dark energy

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The discovery of cosmic acceleration from measurements of luminosity distances of type Ia Supernovae [1,2] naturally led to the question of its nature, a debate that is still ongoing. The standard  $\Lambda$ CDM cosmological model is consistent with current observational data but its well-known fine-tuning problems led to the formulation of alternative models. Many alternatives are characterized by an additional scalar field (of which the Higgs field is an example) that drives the universe's accelerated expansion.

If this scalar field is coupled with the electromagnetic sector, an observable consequence arises – a spacetime variation of the fine-structure constant  $\alpha$ . Therefore, astrophysical measurements of  $\alpha$ , which can be made at least up to redshift  $z=4$ , allow us to probe the scalar field dynamics and therefore the nature of dark energy with a much larger redshift lever arm than other cosmological probes. While the potential of these measurements has already been demonstrated in the context of forthcoming facilities such as ESA's Euclid and ESO's E-ELT [3], here we show that currently available data already provides useful constraints.

Specifically, we show how astrophysical (high-resolution spectroscopy) and local atomic clock measurements constrain models where the same degree of freedom is responsible for dark energy and the  $\alpha$  variations. Through chi-square minimization techniques, limits were obtained for the dimensionless coupling constant  $\zeta$  and for the state equation  $w_0$  [4]. At the 95.4% confidence level and marginalizing over  $w_0$ , we found  $|\zeta| < 5 \times 10^{-6}$  with the atomic clocks data dominating. The next generation of ultra-stable high resolution spectrographs will allow significantly better constraints.

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# Optimization of ESPRESSO Fundamental Physics Tests

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ESPRESSO is a high-resolution-ultra-stable spectrograph for the VLT, whose commissioning is expected in late 2016. One of its key science drivers is to test the stability of fundamental couplings such as the fine structure constant with unprecedented accuracy and control of possible systematics.

In this talk we will describe the criteria for selecting an optimal target list for the Consortium's GTO and based on previous work [1-2], in which we have studied how astrophysical measurements of varying constants and Type Ia supernova measurements can constrain dark energy, I will present forecasts of the impact that this sample will have on fundamental physics and cosmology. In particular, I will discuss how these measurements will constrain the behavior of dark energy deep in the matter era (i.e., in the redshift range  $1.5 < z < 2.5$ ).

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# Domain Wall evolution and biased initial conditions

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Topological defects unavoidably form at symmetry-breaking phase transitions. It is of interest to study defect formation during phase transitions that occurred during the infancy of our Universe [1], as they might lead either to highly desirable cosmological and astrophysical consequences (such as contributing to the origin of large-scale structures) or to undesirable ones (in which case they can be used for excluding entire classes of models).

A specific type of topological defect (known as ‘domain wall’) occurs only if a discrete symmetry is broken. Among other phenomenology, they have recently been suggested as a source of axion dark matter. However, standard domain walls evolve into an attractor linear scaling solution whereby the density of the wall network will end up dominating the energy density of the universe, which is most undesirable.

In order to understand how to avert such catastrophic behavior, we delve into the consequences of evolving networks from biased initial conditions. We evolve our domain walls using numerical simulations relying on the PRS algorithm [3] and then observe their velocity and their density, as they vary with conformal time.

We proceed to test an analytic fitting formula proposed by [4] and find it is not in agreement with the simulation data (much unlike the biased potential case), as we have demonstrated in [5]. We also report on our attempts to explain the failure of this decay law for this specific case.

This work was done in the context of the project PTDC/FIS/111725/2009 from FCT (Portugal). C.J.M.

is also supported by an FCT Research Professorship, contract reference IF/00064/2012, funded by FCT/MCTES (Portugal) and POPH/FSE (EC).

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# Flexible Planar Thermoelectric Microgenerators Fabricated by Electrodeposition

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The growing energy consumption in our modern society calls for novel methods of energy storage and production [1]. Many energy sources have been discovered over the years, but more recently with the advance of nanoscience and nanotechnology. These developments led to higher efficiencies for many energy sources, such as thermoelectricity. Heat waste is a direct consequence of human activity, and being able to re-use this lost energy brings many advantages. Thermoelectric materials and devices allow us to harness heat and convert it back to usable energy. Their working principle is the Seebeck effect, in which a temperature gradient produces a voltage across the material [2]. This has led to the fabrication of several devices, through several techniques, some of considerable expense. Thus, we propose the fabrication of flexible planar thermoelectric micro-generators with a low-cost and highly scalable method as electrodeposition.

In this work, several steps and considerations for the fabrication of this device will be presented and discussed. Regarding the substrate, to create a flexible device, materials as indium tin oxide and polymers such as PET and polyamides, like kapton, can be employed. Afterwards, optimization of the overall electrodeposition process regarding deposition morphology and temperature dependence will be discussed, in order to maximize device performance. The electrodeposited materials will be characterized by X-ray diffraction and electrical resistivity measurements. Finally, several designs with different structures will be presented both in laboratory and in real life applications.

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# Further consistency tests of the stability of fundamental couplings

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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.

In a recent publication [1] we tested the consistency of current astrophysical tests of the stability of two fundamental couplings: the fine-structure constant –  $\alpha$  – and the proton-to-electron mass ratio –  $\mu = m_p/m_e$  – (mostly obtained in the optical/ultraviolet) with combined measurements of  $\alpha$ ,  $\mu$  and the proton gyromagnetic ratio  $g_p$  (mostly in the radio band). Given the significant observational progress made in the past year, we now revisit and update this analysis. We find that apparent inconsistencies, at about the two-sigma level, persist and are in some cases enhanced, especially for matter era measurements (corresponding to redshifts  $z > 1$ ). Although hidden systematics may be the more plausible explanation, we briefly highlight the importance of clarifying this issue, which is within the reach of state-of-the-art observational facilities such as ALMA and ESPRESSO.

This work was done in the context of the project PTDC/FIS/111725/2009 from FCT (Portugal). C.J.M. is also supported by an FCT Research Professorship, contract reference IF/00064/2012, funded by FCT/MCTES (Portugal) and POPH/FSE (EC).

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# Geometrical Optimization of a Thermoelectric Device: Numerical Simulations

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Energy is one of the main requests of our society nowadays since our modern world depends largely on energy to live. The request for the improvement of energy generation boosts the search of alternative energy generation with special attention on its harvesting. Thermoelectrics appear as one of the best candidates for this request since around 60% of the wasted energy is in the form of heat. In fact, the phenomenon was first discovered in the 19th century and since that it has been applied to a variety of situations, for example space research and investigation that use thermoelectric generators.

Thermoelectric generators are portable, small, have an easy maintenance and no moving parts. These qualities make them an improved way of generating energy. However, their efficiency is still low for them to have a wide usage, being the improvement of their efficiency a priority.

In order to improve the efficiency, simulations of thermoelectric devices and of its constituents, the thermocouples, were made, using the COMSOL Multiphysics software. Different geometries of these thermocouples were studied in order to optimize such a device. The thermoelectric material used was bismuth telluride Bi<sub>2</sub>Te<sub>3</sub> and copper Cu for the various connections of the devices. Two geometries of the thermocouples, cubic and cylindrical, were studied and the optimal number of thermocouples in a thermoelectric device was analyzed.

It was verified that the two geometries had identical performances under the same conditions. Furthermore, an optimal ratio between the height and the width of the thermocouples' legs was found to be  $\sim 1 \times 10^{-3}$  and that increasing the cross section area of the legs also enhanced the power produce. It was seen that in order to achieve the highest power possible, the number of TCs in a TE device has to increase. Further results that optimize a TE device were found in terms of other geometrical parameters and of the temperature of operation of the device.

In this presentation, the TE generators and devices are then explained. The studies of TE devices made are presented and its results are discussed, emphasising how they can optimize a device and improve the thermoelectric efficiency, an improvement extremely necessary if a broad use of these generators is to be made.

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# MgO and Chalcogenides Materials as Memristive based Devices for Applications in Neuromorphic Computing

**T.R. Phillip<sup>1</sup>, C. Dias<sup>1</sup>, L. Guerra<sup>1</sup>, P. Aguiar<sup>2</sup>, S. Cardoso<sup>3</sup>, P. P. Freitas<sup>3</sup> and J. Ventura**

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Progression in technology using current electronic devices are challenged by power efficiency and scaling size. These factors limit future prospects in the advancement of computational capabilities. To counter this problem an analogy to the human brain computational abilities and operating principles was proposed [1]. The discovery of a fundamental circuit device could make this analogy a reality. The Memristor is a two-terminal passive electronic device that typically exists as metal-insulator-metal structures. Memristive devices exhibit non-volatile memory that make it a strong candidate for memory applications [2]. It also has resemblance to neuronal synaptic function that can be used for adaptation in neuromorphic computing [3]. In this work we fabricated Memristive devices and studied their electrical properties.

A Memristive device of metal-insulator-metal structure was here fabricated to exhibit resistive switching properties. The device had an insulating oxide of MgO, which was used for its reversible switching between different resistive states. Fabrication methods for the Memristive device involved using lithography and ion beam deposition. An optimum Memristor device fabrication was achieved for Pt/MgO/Ta/Ru. Besides resistive switching, our device showed a variability in the electrical response. A statistical method using a random circuit breaker network (RCB) model was then used to help with understanding the variability of electrical switching characteristics [4].

For the future application of synaptic function we also used chalcogenides Memristive devices with Ag<sub>2</sub>Se and Ag<sub>2</sub>Te nanoparticles. Ag<sub>2</sub>Se and Ag<sub>2</sub>Te nanoparticles were synthesized by hot injection method and device fabrication methods involved spin coating and ion beam deposition of various electrodes. The novel memory device was fabricated in Si/Ag<sub>2</sub>Se/W,Ta,Ag,Cu and Si/Ag<sub>2</sub>Te/W,Ta,Ag,Cu structures. The electrical properties were studied using a modification scheme of electrical polarities.

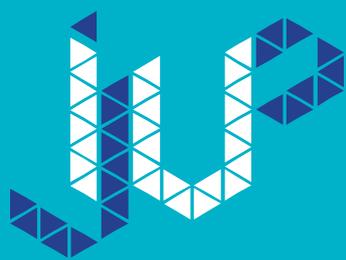
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**A3** **ENVIRONMENT II**  
PARALLEL ORAL SESSIONS VII

## Potential of constructed wetlands for the removal of veterinary antibiotics– response of microbial community

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Pharmaceuticals have been detected in wastewater treatment plant (WWTP) effluents, surface and groundwater. This presence results of their release into the environment which can occur in two ways: directly, by discharge or inadequate treatment of water; or indirectly, when manure is used as agriculture fertilizer. Antibiotics, due to the risk they represents to the spread of antibiotic resistance in the environment, are the most concerning group of veterinary pharmaceuticals. Understanding their environmental fate is of great importance, a subject that has being receiving increasing attention in an attempt to preserve or minimize the risks they may pose. Constructed wetlands (CWs) are artificial complexes with high sustainability, requiring low energy input and manpower, which can be used as alternative or additive low-cost wastewater treatment, for the removal of several contaminants. Considering the key role of microorganisms in CWs and the possible alterations in microbial communities resulting from the presence of a diversity of contaminants, the present study aimed to evaluate the response of microorganisms from CWs microcosms to the presence of veterinary antibiotics, both in terms of community structure and antibiotics removal performance. Three sets of microcosms planted with *Phragmites australis* (3 replicates each) were run in parallel: a set was feed only with livestock wastewater and other two sets were feed with the same wastewater but doped with antibiotics (enrofloxacin or ceftiofur at 100 µg/L). Wastewater was treated during one-week cycles. At the end of these one-week cycles wastewater was removed and replaced by new one (doped or not). The process was repeated for 18 weeks. At the end of week 1, 2, 4, 8, 14 and 18 treated water and sediment samples were collected. Antibiotics removal was evaluated by HPLC while microbial community characterization was performed by ARISA and by 454-pyrosequencing. Results show that microbial communities were dominated by the phylum *Proteobacteria* (38 to 48%), *Firmicutes* (20 to 27%) and *Bacteroidetes* (12 to 15%), independently of the presence of the antibiotics. The study also shows that the systems were able to remove more than 90% of the added antibiotics, pointing to the applicability of CWs for the removal of veterinary antibiotics from livestock wastewaters.

### Acknowledgements:

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# ***Solanum nigrum* L.: a strong candidate for the phytoremediation of paracetamol**

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*Solanum nigrum* L. grows worldwide in several polluted sites and is characterized by a fast growth rate, large shoot biomass, and an enormous phytoremediation potential [1]. Paracetamol (acetaminophen) is one of the most used drugs worldwide, used as an analgesic, antipyretic, and anti-inflammatory agent in medicine and one of the possible problematic compounds in water bodies [2]. This compound assumes an important role among pharmaceuticals reported in aquatic matrices due to its intrinsic toxicity [3]. In order to study the effect of paracetamol on *S. nigrum*, plants were grown hydroponically for 28 days and 3 treatments were carried out: watered only with Murashige & Skoog (MS) solution, control (C); MS solution supplied with 0.25mg/L (P1) and 0.5 mg/L (P2) of paracetamol. After this period, shoots and roots were used for biometry analysis (root length, shoot height, fresh and dry weight measurements) and for the determination of two enzyme's activities: Glutathione S-transferase (GST) and Gamma-glutamyl cysteine synthetase ( $\gamma$ -ECS). There was a significant increase in size with both paracetamol treatments for both organs, having shoots increased by 1.6x with P1 and 1.3x with P2, while roots increase by 1.3x with both treatments. There were no significant differences on the root's fresh and dry weights, neither on shoot's dry weight with P2. Shoot's fresh weight increased by 2.9x and 1.9x, with P1 and P2 treatments, respectively. Dry weight of shoots increased 2.3x with P1. GST activity suffered a significant increase in shoots of both treatments: P1 (2.0x) and P2 (2.6x), showing higher activity on the highest paracetamol concentration (P2). In roots, significant increases of GST activity were also observed in the treated plants, but the highest increase was obtained with P1 (4,2x) (P2 increased 3.6x). There were no significant differences of  $\gamma$ -ECS activity on both shoots and roots of P2 and on P1's shoots. Yet, there was a 5.6x significant increase of  $\gamma$ -ECS activity on roots of the P1 treatment. In conclusion, as shoot and root growth and GST activity seem to be stimulated upon paracetamol supply, these results strongly support the use of *S. nigrum* for the phytoremediation of paracetamol polluted sites.

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# Effect of O<sub>3</sub> and NO<sub>2</sub> atmospheric pollutants in the allergenicity of *Platanus x acerifolia* pollen

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In the last years, there has been a rising tendency towards respiratory diseases caused by pollen grains, especially in urban and industrialized areas. Previous studies suggest that air pollutants may increase the allergenicity of airborne pollen [1,2].

In an ongoing project, the effects of two of the most oxidizing atmospheric pollutants (NO<sub>2</sub> and O<sub>3</sub>) on the protein content, allergenic properties and biochemical composition of *Platanus x acerifolia* is being studied. Furthermore, the current work innovates previous studies by exposing the pollen to the two gases simultaneously. Pollen samples from 2010, 2011 and 2012 were *in vitro* exposed to atmospheric concentration of O<sub>3</sub> and/or NO<sub>2</sub> within the limit value for the human health protection in Europe, for 6h in an environmental chamber. In this chamber the sun light was simulated and the temperature and relative humidity were continuously measured, as well as the pollutant gas concentration [1,2].

In this communication the main results of this research will be presented, with focus on the observable differences between the non-exposed pollen and those pollen samples exposed to only one and both air pollutants. The discussion will be based on the soluble protein content, the allergenic properties using patient sera sensitized to pollen, infrared and X-ray photoelectron spectroscopies of the differently exposed pollen samples.

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# Individual and biochemical responses of the amphipod *Gammarus locusta* after chronic exposure to sertraline

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Due to its extensive and continuous use, pharmaceuticals have been found in aquatic environments. Very little is known about the chronic effects of pharmaceuticals in non-target organisms. It is necessary to be aware that aquatic organisms are exposed throughout their entire life to low concentrations of these substances and therefore sub-lethal effects may occur[1]. Considering the antidepressant pharmaceuticals, sertraline (SER) is among the most widely used worldwide and is therefore of emerging environmental concern. SER, belongs to selective serotonin reuptake inhibitors (SSRI)[2] and it is reported to be the most sub-lethal toxic SSRI in some aquatic organisms[2].

The goal of this study is the assessment of the ecotoxicological risk of sertraline, in ecological relevant concentrations, using chronic life-cycle studies with the amphipod *Gammarus locusta* (Crustacea, Amphipoda, Gammaridae). After an exposure to low levels (8, 40, 200, 1000 ng/L) of SER, chronic multi-level effects were analysed using several key endpoints at individual (survival, growth, reproduction) and biochemical (glutathione S-transferase, catalase, lipid peroxidation and acetylcholinesterase) levels. A behaviour analysis was also made considering the locomotion of the organism.

At an individual level, SER affected mainly the amphipods growth and at biochemical level the activity of several biomarkers were altered by SER exposure. The most predominant effect of SER was recorded in the behavioural analysis that revealed a significant impact on females' velocity exposed to 1000 ng/L SER.

The data here presented will improve the understanding of the low level effects of SER in aquatic ecosystems; contributing for the advancement of the field of pharmaceutical risk assessment. Further studies should however be undertaken to provide additional insight into the multigenerational effects of SER, as well as, on the toxicological mode of action of SER.

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# Scanning electron microscopy applied to minerals associated to gold mineralization in the Banjas mining field.

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The studied samples were collected in the area of the gold Banjas mine. These mineralisations are a part of Dúrico-Beirã mining district related with a major geological structure, with NW-SE orientation, the Valongo Anticline, associated with the first phase of deformation of the Variscan Orogeny (Ribeiro et al. 1987). The country rocks are of Palaeozoic age (Cambrian to Carboniferous). Mineralogical and paragenetic studies allowed distinguish four paragenetic associations: Sn-W, Sb-Au, Au-As and Pb-Zn-(Ag). Minerals associated to the Au-As type have been the aim of this study. This type of mineralisation is controlled by the Lower Ordovician layers (Couto et al. 1990, Couto 1993). The methodology focuses mainly on the study in scanning electron microscope coupled with energy dispersive spectrometer (SEM/EDS) analysis of some minerals of core samples, of small size not easily identified by optical microscopy. The studied minerals belong to black ferruginous layers and an argillaceous rose layer interlayered in the Lower Ordovician succession. In the ferruginous layers, it was possible to confirm the association of Pb and Sb sulphosalts, boulangerite - identified by Couto (1993) – and barite. In the argillaceous rose layer, kaolinite is dominant. The spectrum of a zoned crystal, belonging to spinels series, magnetite series division, corresponding to jacobsite that with the increasing degree of metamorphic, with the help of a fluid, turns into magnesioferrite becoming enriched in Mg and decreased in Mn (from inside to outside the crystal). Furthermore, in the same sample, it was possible to check inclusions of gold in arsenopyrite as checked before (Couto 1993). Barite and jacobsite were for the first time analysed. The mineralogy manifests a late hydrothermal deposition, related to the circulation of hydrothermal fluids, at low to moderate temperatures, with the filling of fractures and associated with sulphides.

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PARALLEL ORAL SESSIONS VII

# Self-Control, Victimization and Fear of Crime: A Quantitative Study

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The main goal of this study is to understand the relationship between self-control, victimization and fear of crime through a survey administered to a sample of 182 undergraduate students. Self-control has been largely discussed since the publication of the General Theory of Crime (Hirschi & Gottfredson, 1990), most fundamentally, its methods of operationalization, conceptualization, and relations with criminal offenses and victimization. According to the authors, self-control manifests in six components: impulsivity, risk-seeking, preference for physical and simple tasks, self-centrism and temperament. Recent studies reported that low self-control is associated with greater victimization (Pratt et al., 2014). The authors suggest that individuals with low self-control worry less with protection or avoidance behaviors, such as, protecting their homes or avoid situations in which is likely they would be victimized. Research also demonstrates that males are more victimized when compared with females (Reid & Konrad, 2004), as well as that self-control is lower in male subjects (Lagrange & Silverman, 1999). However, paradoxically females report more fear of crime, suggesting a complex relation between these variables. The relation of fear of crime and victimization is not consensual (see Hale, 1996).

Taking into account these results we first test the relationship between self-control and victimization. Secondly, we explore that this relation could be mediated by the cognitive dimension of fear of crime, as suggested by Pratt et al. (2014). Results confirm previous research, showing that individuals with low self-control experience more victimization. However, we do not find any results between self-control and the cognitive (risk perception of being victim of crime) and emotional (how safe the people felt in their area) components of fear of crime as hypothesized.

These findings will be discussed in the light of recent scientific literature on this subject, and future empirical directions for further exploration will be highlighted.

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# Trust and Satisfaction in Police: an analysis of *European Social Survey* (2010)

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Nowadays, the trust break towards the justice system constitutes a central issue on the public, political and academic debate. This break bring together several questions concerning to the trust, confidence, legitimacy and satisfaction with various institutions, namely courts and police forces, and to the people commitment towards the State [1]. The way through which the procedural justice influences the citizens' trust in justice entities, namely in the police, have been widely studied at an international level [2]. According to Taylor, the construction of the citizen's perceived legitimacy towards the police is related to the acceptance of its authority and to defer of its decisions. This acknowledgement relies on one hand, on the perception of police's effectiveness, and on the other hand, on the perception of its procedural fairness.

The present study tried to analyze the factors that might influence the trust and satisfaction towards the police, using as background the procedural justice theories [2]. It was used data from the *European Social Survey* (2010), namely from the module concerning to the *Trust in Justice*. This survey is used in different European countries in order to measure people's behaviors, beliefs and attitudes across several aspects of societal life. The used sample is representative for the Portuguese population aging 15 years or more. In order to verify if there is some kind of variation in trust and satisfaction towards the police, 2 linear regression models (OLS) were made.

Concerning to the demographic and political variables, it was verified that marital status, income proportion, interest in politics, social participation and trust in the others, predicted trust in police. Additionally, the results showed that the cooperation with the police, the perception of its effectiveness and procedural fairness, the consent towards its authority and moral alignment, also predicted trust in police. Regarding the satisfaction with the police work, several insecurity related variables, such as fear and trust in others, influenced the mentioned dependent variable. Also, the perceived police effectiveness, the trust in its procedural fairness, the consent towards its authority and the moral alignment, predicted the satisfaction with the police work.

The results will be discussed in the light of strong empirical evidence, being highlighted the importance of people's perceptions of the procedural justice. These perceptions must be used as analyzers of trust and satisfaction towards the justice system [3]. In fact, public policies and strategies must consider these findings in order to amplify the compliance and cooperation with the police [1].

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# **SOCIAL NETWORK ANALYSIS IN THE INVESTIGATION OF ORGANIZED CRIME IN PORTUGAL AND IN ORGANIZED CYBERCRIME**

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The Social Network Analysis (SNA) is now widespread in the academic community in the study of organized crime networks. It allows to analyze the network as a complex structure composed of actors or entities connected by links or relationships and in which a variety of resources is changed. This method, despite having valences and innovations at various levels, also has some limitations that need further attention. Nevertheless, the ARS is still taking its first steps in the fight against organized cybercrime and in Portugal. With this in mind, this article describes and analyzes critically the usefulness of SNA for the academic community, intelligence and criminal investigation services by focusing on: 1) the SNA in the study of organized crime networks, 2) the SNA in organized cybercrime research, and 3) the SNA in Portugal.

# Dissecting the role of apology in Restorative Justice: a review of the state of the art

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Restorative Justice (RJ) is full of results which demonstrate that victims in RJ processes present a significant decrease of their level of anger after the RJ process, as well as a decrease of their vengeance desire after the RJ process. Considering these results, a question arises: How does the RJ process work in order to produce these outcomes? The answer to this question does not appear to be straightforward because there is a lack of studies oriented to the process of RJ. Considering this gap, the present review tries to cast some light on the mechanisms that can be working in the RJ process and that can help to explain its success. With this purpose, the present review explores in detail the role of the apology in the RJ process.

Effectively, it appears that apology can be considered in the RJ investigation not only by the measurement of its frequency in the RJ processes, but also analyzed as a part of the process that, as an independent variable, affects other dependent variables. This hypothesis has been theoretically presented by authors such as Harris, Walgrave and Braithwaite [1] that defended that apology and remorse are very important mechanisms for the restorative processes. However, regarding apology, the definition of this concept by RJ authors is many times less clear. As a result, we often find in the RJ literature references to apology and remorse as different concepts when, as we will demonstrate, in Social Psychology remorse is conceptualized as a dimension of apology. Effectively, in this field of research, apology is presented as possessing several elements such as the offender's responsibility recognition for the wrongful act and the produced harm, a remorse expression, the offender's promise that he shall not produce more damage in the future and a harm reparation offer. Alongside the theoretical discussion, the exact ingredients for a good enough apology have been tested in laboratorial experimental studies, more precisely studies that employed scenario methodology. As a result, for example, Robbennolt [2] found that the participants expressed significantly less anger for the offender who presented a complete apology and expressed greater disposition to forgive him. Moreover, the participants who received a complete apology presented a higher chance of accepting the settlement [2].

Finally, considering the research produced about apology, mostly by Social Psychology, it is possible to conclude that the apology mechanism at work in RJ is much more than the verbal expression of "I'm sorry". On the contrary, the effective apology seems to be constituted by many other components.

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## OFFENDER'S CULPABILITY vs. FREEDOM OF THE PRESS

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The personality rights (including the offender's) and the press freedom are fundamental rights, since the first one is linked to the principle of personality and the second one is linked to the freedom of expression. Because Brazil is a democratic State is inevitable the collision between the fundamental rights, *in casu*, the so-called rights of personality (right to honor, to privacy, to intimacy) and the freedom of expression (freedom to inform and to be informed). The fundamentals rights are the entrenchment clauses --- in addition to having the same juridical status, they are in the same axiological level ---, and under the principle of unity of the Constitution, there isn't any legal hierarchy among constitutional rules, because in case of conflict between fundamental rights, the collision is not resolved suppressing one over the another. Both rights protect human dignity and deserve to be preserved as much as possible in the solution of the collision. This study aims to examine whether "the publication of an accused of a criminal acts' information, regardless of his authorization, generates damage to his personality?", as well as to investigate the legally appropriate criteria for its solution. For the development of research is being used the structuralist method, opting for the literature review procedure.

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# Administrative Acts: Sanctions Enacted by a National Competition Authority

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The current world situation, mainly influenced by the phenomenon of globalization, represents a change in political, social and cultural fields, determined by a set of procedures and institutions that are the result of multiple forces and multiple factors operating at different scales<sup>3</sup>, a problematic international context which make it impossible isolated solutions<sup>4</sup>.

Increasingly intense cross-border flow of goods, services and production processes begins to pierce the veil of sovereignty of the traditional nation-state and require more specific rules and standards beyond the territorial logic. With this development, which also gave rise to the "legalization" of access and participation, transnational procedures generation and introduction of knowledge in decision-making (including non-governmental organizations, NGOs, private companies and corporate actors), reflecting a higher level the complexity of the economic, political and legal decision-making process<sup>5</sup>.

Shipping to a multitude of interactions and volume of administrative actions at different levels of the global management system, special emphasis can be given to the administrative act of penalty in competition law, against transnational Global law, especially in European Law.

The sanctions imposed by the authorities under the competition law within the European Union are set out in Articles 81 and 82 of the EU Treaty and Articles 101 and 102 of the Treaty of Functioning of the Union and Council Regulation (EC) No 1 / 2003 of December 16, 2002 and give rise to at least two (2) issues: the effects of the administrative act of sanction, which repeatedly pierce the state borders and the prohibition of bis in idem, when such action is taken, applied even incomplete data.

The establishment of a free and dynamic internal market, with free movement of goods presupposes the existence of different rules on coordination between member states and the Union itself, in order to reconcile the interests of public protection, ensuring compliance with the rules on right competitive.

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# THE INFLUENCES OF PUBLIC LAW ON PRIVATE LAW AND THE CORRELATIONS OF THE CORPORATE SOCIAL RESPONSIBILITY VS. THE HUMAN RIGHTS

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Historically, the debate about the existing confrontation between Public and Private Law dates back to the middle twentieth century, primarily to the decision of the Federal **Constitutional Court of Germany (FCCG) on the *Lüth* case of 1958**. Since then, the discussion has been gaining some space, even being considered the “full Constitutionalization of Law” (*Vergrundrechtlichung des gesamten Rechts*), on one hand, and celebrated as the full accomplishment of the fundamental rights (Alexy, 2009)[1], on the other.

With the conflict between these two legal spheres (Public and Private Law), the legal protection of the fundamental rights was extended beyond its subjectivist affirmation, where individual rights were only confronted with the State. However, from that moment on, fundamental rights are seen as a consequence of the formation of objective principles, both determiners of legislative measures and inspirers of the legal order. They meant, therefore, the application of Law to individuals and, furthermore, allowed for the interpretation of the precepts of the German Civil Code (BGB) in direct harmony with fundamental rights. The *Drittwirkung* (irradiation of precepts) was acknowledged. Fundamental rights got free from the unilateral orientation towards the governmental intervention of the time and became universally valid (Grimm, 2006)[2].

Therefore, one might raise the following question: “how would the entailment of private law be applied to fundamental rights?”. German author Canaris (2003) [3] provides us with some orientation: the fundamental rights stand immediately given the norms of private law (dominant opinion), and this perspective is adopted by the FCCG, which has been trialling with a strong control of proportionality and of prohibition of the excess in light of the norms of fundamental rights in confrontation with the norms of private law.

In fact, how can we think the confrontation between the norms of Public Law and the ones of Private Law within Corporate Social Responsibility (CSR) – which, eminently, are not controlled by the Judiciary? This question is currently debated from the perspective of the purpose of the CSR: the business ethics. On the other hand, human rights are consecrated on the international order and its application is unquestionable within companies. All companies must respect human rights of stakeholders. Hence, is there any entailment between the norms of Public Law (here, human rights) and the ones of CSR? This is the debate we intend to present at this conference.

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# **Interpretation and application of the Fundamental Right to Health: a pragmatic approach of the decisions of the Courts of Alagoas (Brazil) and its consequences to the public administration**

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This study aims to find out more clearly the position adopted by the Courts of Alagoas (Brazil) concerning a variety of issues involving the Fundamental Right to Health. It is necessary to keep in mind that the mere generic description of fundamental rights should be avoided. For this purpose, this paper uses the legal pragmatism method, based on works of Oliver Wendell Holmes Jr. and Benjamin Cardozo. Their theories converge on the idea that Law must be apprehended in the reality of the Courts, that is, Law is a result of the Legal Courts decisions.

The methodology employed in this research includes theoretical and empirical approach, analyzing some concrete cases submitted to the Courts by the perspective of legal pragmatic method. In this aim, we empirically analyze the interpretation made by the local Courts of the Fundamental Right to Health guaranteed by the Brazilian Constitution and the consequences of this interpretation caused in the public administration, which has to deal with the growing demand to seek jurisdictional provision in healthcare.

The results are a product of a qualitative study, with no statistical approach. They demonstrate that the interpretation of the Fundamental Right to Health is generally based upon vague principles arguments and general propositions. The sentences usually bring the same grounding applied to different cases and situations, without a more accurate consideration about the specifics circumstances of the concrete case.

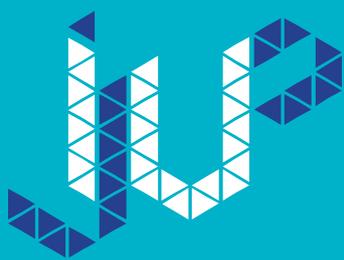
In conclusion, we have verified that the absence of an accurate analyze, that would demonstrate that, in some cases, the Right to Health should not be automatically applied, needing to have others factors counted, is causing serious damages in the public administration, that has to dealing with the problem of limited resources and, by the other side, has to guarantee the Fundamental Right to Health by promotion of public politics to benefit all the community. To sum up, the attempt to guarantee the Fundamental Right to Health jurisdictionally, case by case, without a consideration of certain particularities may turn unavailable the constitutional principle to guarantee a fundamental right to health to all the collectivity.



ENCONTRO INVESTIGAÇÃO JOVEM  
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**VIII** PARALLEL  
ORAL  
SESSIONS VIII



ENCONTRO INVESTIGAÇÃO JOVEM  
DA UNIVERSIDADE DO PORTO

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**A1** **ARTS & ARCHITECTURE**  
PARALLEL ORAL SESSIONS VIII

## Architecture in Blocks – toys as educators

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Toys hold a vital role in children's development and education. Many toys of the most varied forms help to teach children while they play. *"Blocks continue to be favored by many as a way of facilitating the developmental domains in children. In fact, blocks are considered the most useful and most used equipment in preschool and kindergarten programs."* [1]

This research relates a theoretical component that studies a specific toy – blocks – with a practical component of creating a prototype for a toy. This research took place in academic year 2013/2014, in the area of Architectural Toys of the Integrated Master's Degree in Architecture, with the collaboration of Gabriel Correia, under the supervision of Marco Ginoulhiac.

*"The importance of play as recognized mode of learning for young children is clearly reflected in the history of blocks as construction toys. As educators, we should appreciate the central historical and contemporary role of these toys in early childhood education."* [2] Countless games or toys take on the role of educator. Toys are often used as stimulators or teachers in the development and education of the child. Alphabet blocks provide a specific example of this.

The prototype "Architecture in Blocks" is inspired by toys such as the Eames Alphabet Blocks. Among others, Alphabet Blocks attempt to transmit forms of representation in architecture – plans, sections, elevations – and through the combination of these three elements, allow creation of a three-dimensional building. In addition, this toy not only familiarizes the child with the means of representation of architecture, but also allows creation of his or her own architecture. It is possible for a child to construct a building that may, eventually, be identified in reality. This prototype also has the versatility that the user may create a completely different building from that "intended." With the aid of various "extra openings" – reversing the plans – it is possible to create a building to the child's desire.

*"Playing with forms in space is an activity that has always been valued by artists, architects, and mathematicians as well as young children."* [3] In the same way, the prototype "Architecture in Blocks" is a toy that has a variety of characteristics and functions that help to develop the aptitudes of the child in the area of architecture. In addition, this toy may interest anyone of any age, since it is a well-known building by a renowned architect. Like Legos for architecture, this toy may also awaken great interest in the general public.

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# The right of the building to the euthanasia: of Riegl's prophecy to contemporary practices

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Dissertation directed by Dr. G. Furtado

The present project aims to discourse the thematic parallelism between monument and built heritage. It will unfold about the subject of the value loss, based on philosophies expressed by the authors: Alois Riegl, Françoise Choay and Kurt Forster, respectively, in: *The Modern Cult of Monuments, The Allegory Heritage, Monument \ Memory and the Mortality of Architecture*. Reaching parallels in theories of others authors, including: Viollet le Duc, John Ruskin, Robert Venturi, Aldo Rossi.

After an etymological observation necessary to understand the main concepts of the discourse - monument, heritage, false - about the reading of Choay's texts, the project collect, in the first part of the development, the prophetic position of Riegl about the relevance of the value to the validity of the work. Followed up the postmodern re-reading by Forster of Riegl's text and adding notions as: the isolated fragment, iconographic irony and pastiche, employed as false temporal values of the building. The theoretical basis of the dissertation ends with a neomodern vision found in the dichotomy between two neologisms *monumentalism* and *patrimonialism* that prove inside the logic of the negativist current, the perfidious architectural practices that blur, as effect, the reading of the time-value of the building.

In the second part will find as cases of thematic analysis three works: Piazza d'Italia of Charles Moore and Perez Architects (New Orleans) - It will become not only relevant in the temporal antagonism of the false ruin but also will promote the neological confrontation between the postmodern *monumentalism* and patrimony promoted by practices of the facadism -, Carandá Cultural Market of Eduardo Souto Moura (Braga) - offering an exceptional vision by treating of a re-valuing intervention through a partial demolition of the existing building of the same author - Temple of the Holy Family of Antoni Gaudí (Barcelona) - which will be developed on the historical-temporal paradox, face to the search for the end of the work. These cases promote a correlation between the *prophetic*<sup>1</sup> theories and the contemporary architectural practices, aiming to present the right of the building to the euthanasia.

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## **"Reliving Physical Landscape"**

**Maria da Cruz**

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The mountains, the valleys and the simple flow of water in a small stream playing a distinctive sound blend with memories. A combination of sweetness and freedom that express itself in a controlled and spontaneity gesture. The moments of controlled aggression and softness are registered in space and time. An exorcisement of the self as intermediary of the gesture is intended. The body is a mere mediator of tumultuous unconscious emotions, which are released through the sublime art of creating cosmos. Through expanded painting, the landscape turns out to be the revised body. My body becomes the link between my inner and outer. The organic nature, that are presented in my works are devoid of any objective representation. My motivations are influence in memories; revivify in a legible deconstruction of painting that expresses itself using glass as a fine art material, and create transparent landscapes.

## Painting and ceramics as narrative: Intrinsic link between sources, supports and work processes

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Based on the decoding of personal experiences, the "Land of life" acts on the perceptible and imperceptible features of each person, that come from his/her life path.

This research is currently underway within the Master in Painting and plastically is divided in two exploration fields that, by themselves, establish relations between the creation and provocations of controllable and uncontrollable, natural and synthetic features: experimentation / search that happens inside the universe of ceramic materials with different primitive techniques (pit firing [under the earth], paper kilns, wood kilns, trench kilns, Raku, etc.); and the use of trees' fragments that serve as support for the groove and painting.

While the ceramic forms are first created and subsequently undergo changes generated by fire, in the case of wood, the forms/shafts created by nature already exist and the painting then seeks to continue the story. In both, it is important to underline the significance of agreement between the cause and the consequence, between controllable and organic features, establishing a dialectic between what each human being defines for his/her life, between their choices and what naturally goes beyond them, sometimes leaving marks unconsciously.

With the inclusion in the works of familiar elements or trademarks of trees and roots of spaces known by the author, the parts seek to become a kind of reflective individual archive [1]. The final object is filled with a colossal coding, where in addition to the message and the reason of the work, also the technical procedures adopted reveal much of the meanings impregnated. The bases used are territories of work, both in terms of sculpture and painting, but more than that are living genesis of territories with rates from nature [2]. Each piece has its own story, that somehow developed in parallel with that of the author. This way, the narration of a path blends in with an element that was part of it.

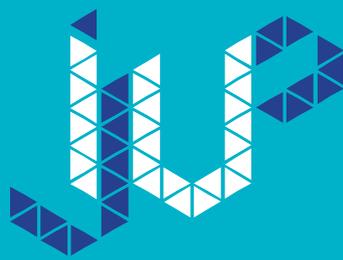
The support thus participates in the painting and the object itself is planned and executed for that spouse, to complement and to complete. "To think the support is to determine the nature of the painting's attitude." [3]. Each object is a territory of learning, an overlap of knowledge from previous experiences and the construction of new discoveries. The concerns of this research will continue to focus on that more or less present way that collective identities and spaces influence each individual, leaving marks that stay and follow them throughout life.

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**A2** **ASTRONOMY & PHYSICS II**  
PARALLEL ORAL SESSIONS VIII

# The effects of a biased potential on domain wall evolution

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The breaking of spontaneous symmetries at phase transitions that are thought to have happened in the early Universe led to the formation of topological defects [1]. A domain wall is a type of topological defect that occurs whenever a discrete symmetry is spontaneously broken. The study of domain walls has been fairly neglected since it was first remarked by Zel'dovich, Kobzarev and Okun [2] that the restoration of spontaneously broken discrete symmetries at high temperatures in the early universe causes serious problems for its subsequent evolution.

It is well known that standard domain walls evolve into an attractor linear scaling solution [3] implying that the density of the wall network will end up dominating the energy density of the Universe. So if there is no mechanism that leads to the disappearance of domain walls at a sufficiently early stage of the evolution of the Universe, this would lead to conclusions which are in contradiction with observations.

By studying the evolution of several types of biased domain wall networks, namely introducing an asymmetry between the two minima of the potential, we try to quantify whether or not the standard linear scaling solution persists and, when it does not, how the networks decay. Using the PRS algorithm [4] we carry out large numerical simulations and have confirmed that for a biased potential, scaling eventually breaks down and the networks decay. Taking advantage of the larger dynamic range of our simulations, we test an analytic model deduced by Hindmarsh [5], confirming it provides good fits for the results of our simulations [6].

This work was done in the context of the project PTDC/FIS/111725/2009 from FCT, Portugal. C.J.M. is supported by an FCT Research Professorship, contract reference IF/00064/2012, funded by FCT/MCTES (Portugal) and POPH/FSE (EC).

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# Nanofabrication of Palladium Nanowires and Dendrites through Pulsed Electrodeposition in Nanoporous Alumina Templates for Hydrogen Sensing

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Palladium is a metal that has the ability of dissociating the H<sub>2</sub> molecule at its surface and incorporate the resulting hydrogen atoms into its crystalline structure. Due to a change of the electronic structure during the hydrogenation, a modification of its refractive index occurs.<sup>1</sup> Unlike the standard resistive H<sub>2</sub> sensors, such phenomena can be used to design optical sensors that allow to measure, without sparking risk, H<sub>2</sub>(g) concentrations around the explosive threshold (4%v/v H<sub>2</sub> in atmosphere). Nanostructuring palladium, not only allows to accelerate the kinetics of thermodynamic reactions<sup>2</sup> involved, but also to explore the resulting Pd-based optical metamaterial's phenomena.

In this study we report the growth of palladium nanowires and dendrites through pulsed electrodeposition in nanoporous alumina templates. These templates are fabricated through a self-assembly process, where the controlled anodization of an aluminum sheet, results in honey-comb nanopore structure in which their diameters can be tuned.<sup>3</sup> After the pore/dendrites formation the filling is performed by electrodeposition in a Pd-based salt solution. In Fig.1 we show a micrograph of the final sample.

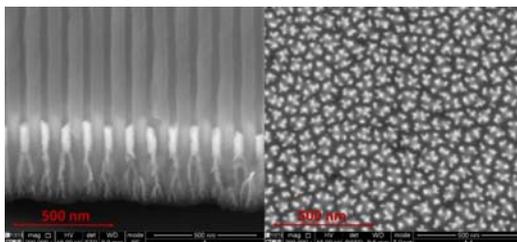


Fig. 1: Alumina template filled with palladium dendrites – cross-section and bottom view.

With this simple and industrially easy to implement fabrication technique we are able to detect in 50s a mixture of 4% v/v H<sub>2</sub> in atmosphere (explosive threshold), by simply measuring the relative variation of transmitted red light during H<sub>2</sub> exposure.

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# On the Number of Cosmic Strings in the Observable Universe

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Cosmic strings are line-like topological defects that can appear in cosmological phase transitions, whose existence was first theorized by Kibble [1]. These kinds of defects are particularly interesting, since the existence and evolution of a string network does not cause any major problems for standard cosmology. A large part of the work done in this area relies on numerical simulations, which are needed to account for the complex microphysics of these networks. However, one can use another approach and focus on treating the “thermodynamics” of the network, which can be done analytically.

In this project, we used a generalized version of Kibble's “one-scale” model [2], in which the strings are described by their velocity  $v$  and by a single correlation length  $L$ . This is the velocity-dependent one-scale model (VOS) [3,4] and it provides us with two coupled non-linear differential equations for the evolution of  $v$  and  $L$ , which although impossible to solve analytically (except in special cases), are fairly simple to integrate numerically. Using this model and the correct initial conditions we can predict the present values for these quantities, and thus forecast the possible effects that those strings would have in a range of cosmological models. A particular point of interest is the number of cosmic string in the present day observable universe [5], which is the relevant quantity for inferring many of the observable consequences of these networks.

We will report on ongoing work confirming the presence of an attractor solution for this model in the matter era, and also discuss how the number and average velocity of strings varies in different cosmological models and universes.

This work was done in the context of the project PTDC/FIS/111725/2009 from FCT (Portugal). C.J.M.

is also supported by an FCT Research Professorship, contract reference IF/00064/2012, funded by FCT/MCTES (Portugal) and POPH/FSE (EC).

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# Piezoelectric nanogenerators: harvesting energy from the environment

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With the consistent shrink of devices, micro-systems are now widely used in several fields, including biomedical research, electronics, automotive industries and in measurement devices. As devices shrunk, so did their power consumptions, making finally possible the use of nanogenerators (NGs) as power sources. These are based in scavenging energy present in the containing environment, including thermal gradients (thermoelectrics), physical deformations (piezoelectrics) and contact of static charges (triboelectrics).

The main focus of this work is to create a piezoelectric NG. These devices use a property of non-centrosymmetric crystallographic structured materials in which, upon an applied compressive or strain force, the atomic structure changes generating piezoelectric charges on opposite sides of the material [1]. This produces an electrical field that can be scavenged to power up a device. Such piezoelectric effect has been widely known for over a century, but only in the last decade have its properties been studied for nanoscaled power sources. The wide range of applications is due to its simple activation mechanism. In fact, in all dynamic environments there is a movement which can be transformed into energy using a device. Imagine, for example, our own shoes and clothes generating energy as we walk/move, feeding incorporated LEDs [2] which could light our path. To accumulate the piezoelectric charges so they can be used to feed a system like this, the piezoelectric effect in NGs must be combined with their semi-conductive properties in the presence of a Schottky barrier. Further improvements can be made using rectifying bridges, which makes both positive and negative peaks of voltage row in a single direction.

This work aims to develop zinc oxide (ZnO) nanowired NGs [3] in flexible substrates. The growth in flexible materials will allow a wider approach to future applications, putting us in the front line of the ultimate energy power source revolution where renewable and clean electricity production will be the most important factor.

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# Triboelectric nanogenerators: a new dynamic route for energy harvesting

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There is a large amount of energy sources in our living environment, such as dissipated heat, mechanical deformation and static charges, which can be harvested and converted into electricity. With this in mind, self-powered nanosystems, the so-called nanogenerators (NGs), have been developed for several different applications in a wide range of areas, such as personal electronics, environmental monitoring and medical science. In particular, triboelectric nanogenerators (TENGs) convert external mechanical energy into electricity by the conjunction of triboelectric and electrostatic effects, which are a universally known phenomena. They have the potential to harvest energy from our own body movements (as in our muscle stretching and contracting), rotating tires, acoustic/ultrasonic waves, ocean waves and tides, mechanical vibration, wind and blood flow [1].

Triboelectric materials possess the property of becoming electrically charged upon friction with other triboelectric materials, creating positive or negative charges depending on the materials' tendency to gain or lose electrons. A TENG is basically composed by two triboelectric materials with different polarity, a spacer between them and two metal electrodes. When these two materials are periodically contacted and separated, the potential difference between the metal electrodes of the two triboelectric surfaces periodically varies, which drives the electrons to flow between the two metal electrodes and generate a continuous output [2]. To optimize the performance of TENGs, one requires more practical designs and careful optimization of the tribo-pair materials, including the modification of its surface morphology.

Our project work allowed a greater insight on the extensive development on the field of triboelectric nanogenerators, ranging from the intrinsic Physics behind the technology to the optimization of triboelectric materials, namely surface alteration through nanofabrication, which enlarges the surface area, and therefore, substantially increases the TENG efficiency. Energy harvesting nanodevices promise to be a new highlight in nanotechnology since they enable the capture of green energy from the environment.

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## Wearable energy storage in textiles

**José Valente<sup>1,2</sup>, Ana Silva<sup>1</sup>, Andreia Oliveira<sup>1</sup>, André M. Pereira<sup>1</sup> and Clara Pereira<sup>2</sup>**

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The assembly of energy in wearable devices is one of the great challenges of our Society, since our resources are becoming more limited to produce energy [1]. The application of supercapacitors (SC) as smart and functional clothing is an important goal for the Portuguese economy since the Textile Industry is one of the pillars of the Portuguese Industry, being recognized worldwide due to its quality and competitiveness. In particular, the Textile Industry represents 8% of the total exports and corresponds to 20% of the manufacturing industry [2].

The SC technology can be integrated in textiles to provide energy to technologies such as mobile phones, wearable devices and flexible displays, improving our day life routines [1].

Allying science and textile, the main purpose of this work is to assemble flexible energy storage devices in textile fabrics. This can be achieved through dip-coating, screen-printing and build-up yarn / fiber processes. The work starts with a brief introduction to energy storage principles, giving examples such as Lithium-batteries and supercapacitors (SCs) [3]. Afterwards the working principles between the two main types of SCs are explained – electric double-layer capacitor (EDLC) and pseudocapacitor – and the components that are relevant for the construction of flexible solid-state SC textiles are highlighted.

Finally, conductive textile electrodes were fabricated by dip-coating cotton fabrics with carbon nanomaterial inks. This coating process was selected since it is low-cost and of easy implementation in the textile industry. The influence of the number of dip-coating steps on the DC electrical conductivity and impedance was studied. The results showed that after more than two dip coatings the impedance almost stabilized. In parallel, cotton fabrics were coated with Fe<sub>3</sub>O<sub>4</sub> magnetic nanoparticles. Vibrating sample magnetometry revealed the superparamagnetic nature of the functional textiles.

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**A3** **PUBLIC HEALTH &  
EPIDEMIOLOGY**  
PARALLEL ORAL SESSIONS VIII

# Burden of ophthalmic procedures in public hospitals of Portugal

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**Introduction** The eye is one of the most exposed structures of the human body, however, to our best knowledge, no study was performed in order to understand trends on Ophthalmic Procedures (OP) and to access their evolution throughout time in an in-patient based population. The study of trends on OP is a valuable asset to understand their role in hospitalizations registered in the different databases and to direct medical policies and education towards the most performed procedures.

**Methods** We performed a retrospective observational study using administrative and clinical data, provided by the Central Administration of Health System of the Portuguese Ministry of Health (ACSS), from a national database. we used the Agency for Healthcare Research and Quality's Clinical Classification Software (CCS) to summarize ICD-9-CM codes into a small number of clinically meaningful groups. For each hospitalization episode identified, the following variables were analysed: age, sex, length of stay(LOS), procedures performed (coded according to ICD-9-CM and grouped in CCS categories), discharge date and status, mean costs and type of admission.

**Results** Hospitalizations associated to OP rose 377%, from 28.554 episodes in 2000 to 107.756 in 2010. Lens and cataract procedures were the group of OP with the highest frequency throughout the entire period, reaching 59% of all the hospitalizations analysed, of those episodes, 38% were performed in male patients. The overall median LOS associated to each group of OP decreased in all groups of OP, except for "other intraocular therapeutic procedures" where it remained equal from 2000 to 2010 (1 day). The group of OP with the highest mean charges associated were the "diagnostic procedures on eye" (6.207€). That same group of OP also presented the highest in-hospital mortality (2.69%) and median LOS (7 days). The outpatient surgeries increased in all groups of OP, e.g. lens and cataract procedures increased from 8.1% in 2000 to 92.4% in 2010.

**Discussion** From 2000 to 2010 hospitalizations associated to OP increased substantially. In the majority hospitalisations associated to OP the median LOS has decreased and the frequency of outpatient surgery has increased.

## Perception of Caregivers about School Meals

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The requirements of the modern world leave parents with less and less time for their children, which determines that an increasing number of children attend to kindergartens where they consume much of their daily diet. Since it is during the first years of life that we acquire eating habits that persist into adulthood, parents should have the perception about the food service which their children attend in order to be able to participate on the improvement of its quality. Objective: To evaluate the caregivers perceptions about their children school meals. Methods: An autofill survey was applied to caregivers. Statistical analysis of the data collected was carried out using the IBM SPSS program version 22. Results: The results seem to indicate that caregivers give little importance to the menus and are not concerned about their children food intake at the kindergarten. There was also insufficient knowledge about the rules of good practice and hygiene in establishments by caregivers. Conclusion: It's clearly insufficient the knowledge that caregivers have about kindergarten's food service.

## Clonal dissemination of clinically relevant multidrug-resistant *Salmonella* Typhimurium, S. 4,[5],12:i:- and S. Rissen in Portugal

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Non-typhoidal *Salmonella* infections represents a global public health burden, being crucial to identify and follow clones of clinically relevant serotypes to contain its spread. Our goal was to assess clonal distribution trends in three clinically relevant *Salmonella* serotypes and their association with antimicrobial resistance in Portuguese isolates. Results were also compared with previous data from the last decade (2002-2009).

Isolates of *S.*Typhimurium/n=253, *S.*4,[5],12:i:-/n=158 and *S.*Rissen/n=26 from different sources (clinical/food/animal) and regions (2009-2014) were screened by PCR for sulfamethoxazole resistance genes (*sul1/sul2/sul3*) and genes linked with class 1 integrons (*intI1*/antibiotic resistance gene cassettes). Detection of other antibiotic resistance genes by PCR, resistance to 10 antibiotics [ampicillin-A/chloramphenicol-C/gentamicin-G/kanamycin/nalidixic-acid/ciprofloxacin/streptomycin-S/sulfametoxazol-Su/tetracycline-T/trimethoprim-Tr] and DDST by disk diffusion (CLSI/EUCAST) and clonal relatedness by PFGE were performed in representative isolates.

In *S.*Typhimurium we detected 3 predominant clones: i) "*S.*Typhimurium DT104" (43%; *intI1*; 5'CS-*aadA2*±5'CS-*bla*<sub>PSE-1</sub>; *sul1*±*sul2*; *qacEΔ1*), with identical MDR profiles (mostly ACSSuT-*bla*<sub>PSE-1</sub>-*floR*-*aadA*-*sul1*-*tetG*) and PFGE-types to those described since 2002; ii) "*S.*Typhimurium European clone" (23%; *sul2* and absence of *intI1/sul1/qacEΔ1*) with the same or very closely related PFGE-types and MDR profiles (mostly ASSuT-*bla*<sub>TEM</sub>-*strA*-*strB*-*sul2*-*tetB*) as the "European clone" of *S.*4,[5],12:i:-; iii) "*S.*Typhimurium OXA-30-producing" (17%; *intI1*; 5'CS-*bla*<sub>OXA-30</sub>; *sul1*±*sul2*; *qacEΔ1*), with the same MDR profile (mostly ACSSuT-*bla*<sub>OXA-30</sub>-*catA*-*aadA*-*sul1*-*tetB*) and PFGE-type circulating since 2002. In *S.*4,[5],12:i:- it was found the presence of the 3 clones currently circulating in Europe: i) "European clone" (75%; *sul2* and absence of *intI1/sul1/qacEΔ1*; mostly ASSuT-*bla*<sub>TEM</sub>-*strA*-*strB*-*sul2*-*tetB*), which has expanded throughout this study period; ii) "Spanish clone" [6%; *intI1*; *qacEΔ1*+*qacH*; mostly AC(G)SSuTTr-*bla*<sub>TEM</sub>-*cmlA*-*floR*-(*aac*(3)-IV)-*aadA*-*sul1*-*sul2*-*sul3*-*tetA*-*dfrA12*] and iii) "Southern-European clone" (1%; *intI1*; *qacH*; CSSuTTr-*cmlA*-*aadA*-*strA*-*strB*-*sul3*-*tetB*-*dfrA12*) mostly with similar MDR and/or PFGE-types described since 2002. In *S.*Rissen stands out the maintenance of the clone frequently associated with class 1 integrons [38%; *intI1*; 5'CS-*drfA12*-*aadA*/5'CS-*drfA1*-*aadA*; *sul1/sul3*; *qacEΔ1/qacH*] and MDR profiles [mostly A(C)SSuTTr-*bla*<sub>TEM</sub>-(*cmlA*)-*aadA*-*sul1*-*tetA*-*dfrA12*].

In the three *Salmonella* serotypes we observed the persistence of the MDR clones and corresponding PFGE-types more frequently identified in the last decade. It is of note the decreased frequency of *S.*4,[5],12:i:- "Spanish" and "Southern-European" clones and the expansion of a new clonal group with ASSuT profile among isolates of *S.*Typhimurium and *S.*4,[5],12:i:-. The follow-up of population dynamics and the understanding of factors promoting survival/persistence of *Salmonella* clones is crucial to improve effective strategies and interventions in food safety at global level.

# Extended Spectrum $\beta$ -Lactamase producing *Enterobacteriaceae* in recreational river waters of the North of Portugal

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River water is one of the most important reservoir and vehicle of antibiotic resistant bacteria dissemination, in nature <sup>(1)</sup>. The overexposure of aquatic environments to human and animal intestinal commensals and pathogens, and the selective pressure exerted by factors as antibiotic residues, are important sources of resistance <sup>(2)</sup>. Our previous work showed multi-resistant ESBL producing *E.coli*, in river water in Portugal, leading us to improve our research about this public health threat.

Our aim was the detection of ESBL and carbapenemase producing *Enterobacteriaceae* in recreational river waters in different cities of the North of Portugal.

Water samples of 5 different rivers with recreational activities, were analyzed by membrane filtration on MacConkey agar and MacConkey Agar with cefotaxime, ciprofloxacin and meropenem (2mg/L). Representatives of different colony morphologies were tested for antimicrobial susceptibility and carbapenemase screening, according to CLSI and EUCAST. ESBL producers were confirmed by the double-disk-synergy-test and PCR was performed for detection of relevant  $\beta$ -lactamases: *bla*<sub>TEM</sub>, *bla*<sub>OXA</sub>, *bla*<sub>SHV</sub>, *bla*<sub>CTX-M-group-1</sub> and *aac(6')-Ib-cr* genes. Isolates were identified by ID-32-GN.

Our study showed the dissemination of *bla*<sub>CTX-M-group-1</sub> producing *Enterobacteriaceae* and the presence of gram-negative-non-fermentative bacteria with reduced susceptibility to carbapenems in aquatic environments in Portugal. From 56 isolates analyzed, 17 were ESBL producers and 33 showed multi-resistance. Isolates of *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter spp.* showed positive for *bla*<sub>CTX-M-group-1</sub> (13), *bla*<sub>OXA</sub> (12), *bla*<sub>TEM</sub> (1), *bla*<sub>SHV</sub> (6) and *aac(6')-Ib-cr* (1) genes. Results indicate that recreational river waters of the North of Portugal are reservoirs of ESBL producing *Enterobacteriaceae* that were also resistant to non- $\beta$ -lactam antibiotics. This reality combined with the dissemination of non-fermentative-gram-negatives with reduced susceptibility to carbapenems suggests that recreational waters can be an indubitable source of resistance genes persistence and dissemination and a probable colonization source of multi drug resistant bacteria in humans and animals.

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# A lifetime of antibiotherapy: Intestinal colonization of long-term care facilities patients with Extended Spectrum $\beta$ -Lactamase producing *Enterobacteriaceae*

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Population aging in developed countries creates new challenges to public health and microbiology. The increasing need for long-term care facilities (LTCF) brings a whole new paradigm for bacterial resistance dissemination, since these institutions act as complex reservoirs of multi-resistant bacteria[1]. Patients in this facilities experience several hospital admissions and are more likely to have had contact with antibiotic therapy during their life, therefore they are predisposed to have antibiotic resistant bacteria in their fecal flora.

The aim of our work was the detection and characterization of extended-spectrum beta-lactamases (ESBL) producing *Enterobacteriaceae* in the intestinal flora of patients institutionalized in a Portuguese LTCF in the North of the country.

Fecal samples of 30 LTCF patients, with an average age of 73 years old, were collected and stored for further analysis. Isolates of interest were randomly selected in MacConkey agar with cefotaxime (2mg/L), using MacConkey agar as control. Susceptibility to antibiotics was determined by disk diffusion methods, according to the CLSI guidelines and ESBL detection was confirmed with addition of clavulanic acid to oximiino antibiotic disks. Isolates were subjected to PCR amplification for detection of *bla*TEM, *bla*OXA, *bla*SHV, *bla*CTX-M-group-1. The chromogenic medium, Chromagar Orientation, was used for preliminary identification of selected strains.

Eighty percent of the analyzed fecal samples contained 29 ESBL producing *Enterobacteriaceae* isolates: 12 *Escherichia coli* and 19 of KESC group (*Klebsiella* spp., *Enterobacter* spp., *Serratia marscescens*, and *Citrobacter* spp.) according to Chromagar Orientation medium. Isolates were positive for *bla*CTX-M-group-1 (16), *bla*TEM (21), *bla*OXA (19), *bla*SHV (18) genes. ESBL producers showed resistance to other antibiotics namely fluoroquinolones, aminoglycosides, tetracyclines, folate pathway inhibitors, phenicols and fosfomycin.

Our results confirmed the dissemination of ESBL producing *Enterobacteriaceae* in intestinal colonization of LTCF patients who may act as vehicles of resistant bacteria within the health care facilities and community. This work emphasizes the importance of rigorous infection control measures to prevent the dissemination of multidrug resistant bacteria as well as the importance of screening for colonization before hospital admission of risk patients from LTCF.

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## Filling the map for antimicrobial resistance in sub-Saharan Africa: ampicillin resistant enterococci from non-clinical sources of Angola

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In sub-Saharan Africa (sSA) the antibiotic consumption is rising but surveillance capacity is still minimal. Insufficient antimicrobial resistance (AMR) data impairs a snapshot of true extension of the problem and the application of adequate control measures. *Enterococcus* causes a diversity of human infections in nosocomial and community settings, especially in patients with risk factors (e.g., immunosuppression), abundant in sSA. In a geographical area where other antibiotics, like glycopeptides, are not easily available, beta-lactams are critical for the treatment of enterococcal infections. Ampicillin resistance (AmpR) among *Enterococcus faecium* (*Efm*) is associated with worldwide nosocomial epidemic clones, being the genetic background of AmpR-*Efm* in sub-Saharan Africa (sSA) still barely known. Among the countries with scarce AMR data from clinical and community sources is Angola, an emergent economy, still with a precarious health system. As part of a surveillance study on the occurrence of Gram-positive and Gram-negative resistant bacteria to key antibiotics used to treat human infections, we searched AmpR-*Efm* in human, animal and environment from Benguela province (June 2013). Samples from healthy humans (faeces n=20), a wild park (faeces from grey monkeys-n=2 and goats of range-n=3, animal drinking water-n=2), farm production animals [faeces from poultry-n=5, veal-n=3, pigs-n=2; facilities walls/floor-n=2; drinking water-n=3], aquatic environment [river-n=3; treated-n=7 and untreated-n=5 human drinking water; residual waters-n=5] were included. They were enriched (1:10) in peptone water supplemented+8mg/l of ampicillin (37°C/24h) and 0,1ml was plated in Slanetz-Bartley+8mg/l of ampicillin. Resistance to 12 antibiotics was evaluated by disk diffusion/Etest (EUCAST/CLSI). Species identification, search of antibiotic resistance genes [*tet*(MLOS), *erm*(B), *aadE*, *pbp5*] and virulence factors (*IS16*, *esp*, *hyl*, *acm*) were searched by PCR/sequencing and clonality was determined by MLST. AmpR was detected in 8 *Efm* from 7 samples (hospital+community residual water, poultry farm facility, poultry and pigs faeces). They were identified within diverse Bayesian Analysis of the Population Structure (BAPS) groups [BAPS 2.3a-ST610; BAPS 2.1b-ST245, ST650, ST971], some described in human infections in sSA. The PBP5 amino acid analysis of 4 AmpR-*Efm* showed 3 different sequences, corresponding mostly to the AmpR consensus region previously described. Two sequences were new but a poultry-*Efm* PBP5 was previously identified in 19 *Efm* (Europe/USA/Israel-bloodstream infections-n=10, unknown origin-n=9) belonging to hospital associated clonal lineages. All, but one AmpR-*Efm*, were multidrug resistant. The *acm* gene was identified in 4 poultry isolates. In conclusion, AmpR-*Efm* carrying diverse antibiotic resistance genes are dispersed in animal and environmental settings of Angola, suggesting a beta-lactams selective pressure. The great number of immunocompromised individuals, the poor hygiene favoring AMR transmission and the non-controlled antibiotics use, justify the monitoring of the emergence and evolution of the clonal lineages of AmpR-*Efm* found, to clarify their impact on public health of Angola and other regions of sSA.

## “Operação Mãos Limpas” – a Community Intervention Project

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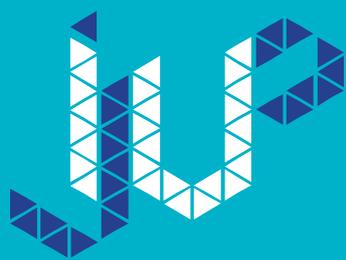
“Operação Mãos Limpas” was a community intervention project, developed by Didáxis V.S. Cosme in cooperation with FCNAUP (one food microbiology teacher and three students), between 2013 and 2015, funding by Ciência Viva - Programa Pais com a Ciência. The main goals were to promote hand hygiene habits and to improve the scientific knowledge in microbiology and food safety among students and school community. This project was developed due to perception of poor personal hygiene, including lack of students hand-washing at mealtime in the local canteen. In order to promote hand hygiene among school community, theoretical (for teachers, parents/caregivers and students) and practical sessions (for students) were designed and performed. To achieve success in this educational program, sessions design took into account several key points such as target audience (e.g. previous knowledge/skills), supporting material (e.g. appropriate to capture attention and to facilitate learning; containing clear, concise and simple messages) and presentation/discussion of recent examples about food safety failures and its health impact. To facilitate learning throughout sessions, simple concepts were previously defined, being the most relevant: “hands have microorganisms and are a major vehicle for spreading pathogens”; “cleaning hands appropriately is an important health protection measure”; “hands can contaminate food”; “foodborne diseases can be serious”, and “wash your hands properly and frequently”. In the theoretical sessions, important key concepts related to foodborne diseases were firstly introduced (e.g. definition, symptoms, associated costs, risk factors and transmission routes), including examples about large and severe outbreaks related to poor hand hygiene and appropriated educational videos and figures to highlight the importance of prevention practices (e.g. showing effective procedures to hand-washing). In the practical session, we included the same introductory contents, but the main focus was the experimental approach, where students learned how to collect hand samples and to apply several microbiological techniques. The students were organized in groups (2 students) and they had the opportunity to collect at least one hand sample and to perform poor-plate method using two different culture medium (*Plate Count Agar* and *COLI-ID*). A lack of knowledge related to health risks associated with poor hand hygiene and food-borne diseases in this school community was observed, but from our experience food safety key messages were well accepted and learned by all the targeted audience. In summary, community intervention projects about food safety and hygiene issues should largely be promoted among school communities (students, teachers and parents/caregivers) in order to improve knowledge and personal hygiene/safe-food handling behaviours.



ENCONTRO INVESTIGAÇÃO JOVEM  
DA UNIVERSIDADE DO PORTO

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**P** POSTER  
SESSIONS



ENCONTRO INVESTIGAÇÃO JOVEM  
DA UNIVERSIDADE DO PORTO

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13<sup>th</sup> **WEDNESDAY**  
POSTER SESSIONS

# Abacus for Pre-Sizing Walls base Ventilation Systems

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Rising damp is a degradation factor specially in old buildings and monuments. It is a result of slow upward movement of water in the lower sections of walls and other ground-supported structures by capillary action. There are various methods to treat rising damp among which is the wall base hygro-ventilation system best suited for high thick walls with considerable heterogeneity [1,2].

A number of studies have been undertaken around this method which have been implemented and validated in several heritage buildings. Such success has led to the adoption of the patent of the HUMIVENT system which aims to increase evaporation through peripheral channels on the base of ventilated walls. A pre-sizing programme has also been created through Excel called HUMIVENT PREDIM. [3,4]. A scheme about the wall base ventilation System is illustrated in figure 1a [4].

This study aims to develop a set of auxiliary abacuses for pre-sizing wall base ventilation systems. This approach aims to provide fast and expeditious systems sizing in the design and rehabilitation offices. It allows for easy perception of changes in the various parameters that influences the wet front and preliminary design of the ventilation system itself. It is a very useful tool for designers because it allows them to obtain pre-sizing without the need of extensive knowledge of the programme and determining the associated parameters. An example of abacus is represented in figure 1b.

Figure 1a - Scheme about the wall base ventilation system.

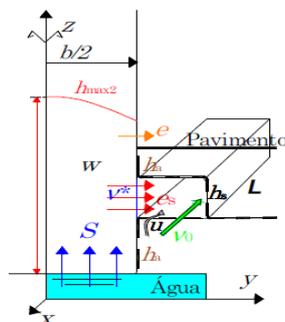
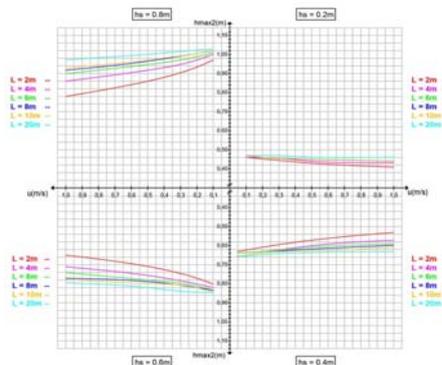


Figure 1b - Example of abacus.



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# Ambulatory knee monitoring system to prevent injuries in volleyball athletes

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Ligament injuries such as patellar tendinitis are the most common injuries affecting professional volleyball players [1]. To prevent the occurrence of such injuries a system capable of collecting data related to the knee acceleration and the foot pressure level was designed in the present study. For this purpose, two tri-axial accelerometers and a pressure sensor were used [2][3].

Then the collected data were processed and analyzed in order to construct an effort pattern considered as ideal. The establishment of this pattern allowed comparison between different patterns of different players, giving us significant deviations from the ideal pattern as can be seen in Fig. 1.

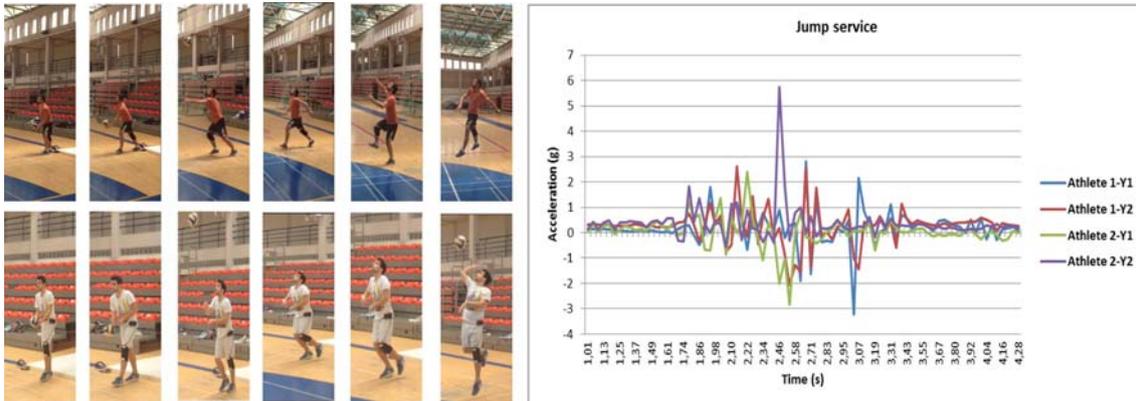


Figure 1 - Comparing two athlete's patterns of effort in jump service. Athlete-1 was considered as the ideal pattern. As can be seen, the accelerations from both athletes have a similar patterns.

The Y component is the most important variable analysis since it represents the lateral flexing of the knee. In fact, according to the study of acceleration values collected in the Y component, conclusions can be drawn in order to study ligament effort level. These conclusions can be drawn by analyzing the behavior of the acceleration in Y1 and Y2 on their movement graphs.

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# Biodegradable films obtained from chitosan and whey protein isolate

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The use of non-biodegradable components in plastics formulation has caused serious ecological problems and much debate in recent times. In this sense, several studies have been developed in order to find viable alternatives to address this environmental scourge [1]. One recurring alternative is the formulation of packages using biopolymers, which are biodegradable, and allow to create alternatives to the 245 million tons of non-biodegradable synthetic polymers produced annually [2].

Polysaccharides, lipids and proteins are important renewable and natural raw materials for packaging film's formulation. The functionalities of these biopolymer films are known to depend on several factors including the biopolymer structure and properties, films composition, plasticizer type and concentration, fabrication process, and environmental condition [3].

Whey protein isolate (WPI) is a byproduct of the dairy industry, obtained during the industrial production of cheese or casein. Edible films from whey protein isolates usually show good oxygen and aroma barriers but poor mechanical properties. Chitosan (Q) is a cationic polysaccharide with antimicrobial features produced by deacetylation of chitin, a polysaccharide found in the exoskeleton of crustaceans.

The aim of this work was to study the potential application of synergistic effects between whey proteins and chitosan to improve whey protein-based films' properties. For this purpose, aqueous chitosan and whey protein isolate solutions buffered at pH 6.0 were prepared. At this pH, chitosan is positively charged while whey proteins are negatively charged. Chitosan/whey protein, chitosan and whey protein-based films containing glycerol as plasticizer were prepared by the casting method to evaluate their physical properties. The mechanical properties (tensile strength and elongation at break) were assessed with stress tests. Sensitivity to moisture was evaluated through water vapor permeability measurements. Color and opacity were characterized using a portable spectrophotometer and morphology was studied by scanning electron microscopy (SEM). The obtained results evidenced transparent and optically clear films, with an enhanced moisture resistance and improved mechanical properties when compared to the biofilms prepared only from whey protein.

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# Evaluation of lead chromate pigments alternatives in paint industry

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Lead is a toxic metal that can have its greatest adverse health effects on children, but also causes harm in adults, including exposed workers. There is no known level of lead exposure that is considered to be safe [1]. Lead sulfochromate yellow, also known as C.I. Pigment Yellow 34 (PY34) is a key product for the paint, plastic and road marking industries. PY34 is classified as a carcinogenic and toxic to the reproduction [2]. In this study, technical and economic aspects of non-toxic alternatives to the use of PY34 were evaluated. We tested 7 commercially available yellow pigments in an oil alkyd resin based paint at 14% (w/w) concentration. The selected pigments were: the organic O1–5,5'-(1H-isoindole-1,3(2H)-diylidene)dipyrimidine-2,4,6(1H,3H,5H)-trione, 2 inorganic I1 and I2 – both based in bismuth vanadium tetraoxide, and 4 blends of organic and inorganic compounds with undisclosed composition (B1-B4). Additionally, we formulated a blend of organic and inorganic pigments (B5) based in pigments O1 and I2. The contrast ratio and color differences ( $\Delta E$ ) of the paints, in full shade and mixed with 1:4 TiO<sub>2</sub>, were determined by colorimetry using the CIE units (L\*, a\* b\*).

The best results were obtained with the blend that we formulated (B5), with a contrast ratio of 94,3%, in comparison with PY34 that as a contrast ratio of 87,3%, and the best overall results in color differences,  $\Delta E=2.31$  in full shade and  $\Delta E=2.77$  when mixed with 1:4 TiO<sub>2</sub> (Fig. 1). In an economical perspective, B5 has a total formulation cost *ca.* 200% higher than the yellow sulfochromate. All the other alternatives presented costs 50-200% higher than the PY34 formulated paint. It can be concluded that there are several viable alternatives to the application of yellow lead pigments. However, costs of these new pigments represent an economic issue for the companies, but the health and environmental gains with the substitution of lead in paints are tremendously higher.

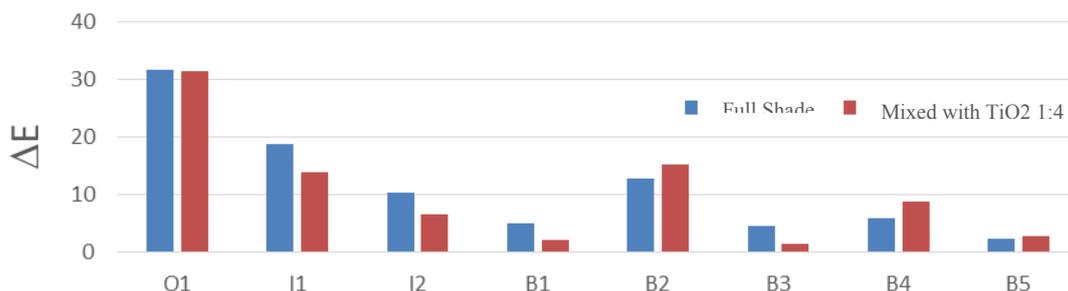


Fig. 1 – Color differences of the various tested pigments when compared to PY34.

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# Flood Risk Analysis – Development of a model applied to Historical Buildings

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Architectural and cultural heritage is of greatest importance, which justifies the need to safeguard it from extreme climatic phenomena such as floods. The most prestigious buildings were built, for historical reasons, near natural waterways and are frequently threatened by the flood phenomenon.

The climate change models suggest this phenomenon will increase throughout the 21st century as an impact of human activity, either caused by global warming or by inappropriate management of hydric resources and construction in flood plains. This situation, combined with the geomorphological and meteorological conditions of the territory, creates a favourable scenario for the occurrence of floods. Consequently, it is necessary to adopt preventive measures to minimize, mitigate and control these adverse effects.

This work proposes an analytical model for flood risk calculation that considers three different groups of parameters: the monument location in relation to a waterway, the characteristics of the waterway and the behaviour of the monument construction material when in contact with water.

The model is represented by a final equation (Eq. (1)) composed by the factors (Fig. 1), which are defined as the most influent in flood risk determination. The results obtained allow for the creation of risk maps that permit an easy identification of the places in which it is necessary to adopt preventive measures.

$$FR = 0.2 D_{WL} + 0.6 \Delta h \times UF \times Ob + 0.1 WL \times Q + 0.1 A \times \Delta L_H \quad (1)$$

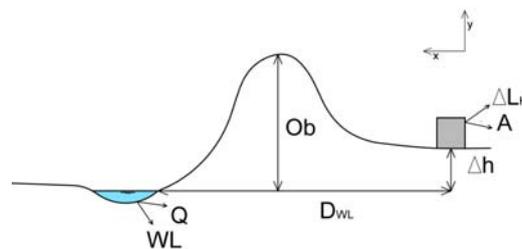


Figure 2 - Schematic representation of the Risk Factors

where, FR is the flood risk, DWL is the horizontal distance to the waterway, Δh is the height difference between the building and the waterway, UF is the existence of underground floors, Ob is the existence of obstacles, WL is the waterway type, Q is the discharge, A is the water absorption coefficient and ΔLH is the hydric expansion.

The model was applied to the Portuguese National Monuments. A risk map was created on the basis of the classification developed. Overall, the flood risk model showed good and promising results and it is possible to observe that a significant amount of Portuguese monuments are classified as medium to high risk of flooding.

# Membrane technologies for water desalination and purification

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The exponential population growth and global warming have increased the demand for clean water worldwide, in particular in arid and semi-arid regions. The current water sources are decreasing and, hence, novel strategies have to be adopted for obtaining fresh water from seawater or brackish groundwater through desalination technologies [1]. Nowadays, forward osmosis (FO) is gaining attention in wastewater treatment and seawater desalination because of its many advantages, including reduced energy requirements. On the other hand, carbon materials such as carbon nanotubes and graphene are demonstrating a huge potential in the modification of membranes for water desalination [2-3].

In this work, polysulfone (PS) membranes blended with graphene derivatives were prepared by the phase inversion method. Different synthesis parameters such as polymer content, composition of the coagulation bath, carbon loading and surface chemistry of graphene were studied. The membranes were characterized by scanning electronic microscopy, porosity, contact angle determination and water flux measurement in filtration experiments at different trans-membrane pressures (TMP). Then, PS membranes were used as support in the preparation of thin film composite (TFC) membranes, which were tested in FO of salty water. In general, PS membranes blended with graphene derivatives showed higher water fluxes than the bare PS membrane, that prepared with 0.1 wt.% of GO presenting the largest water flux at any TMP (0.5 and 2 bar). PS membranes prepared with isopropanol/water (30:70) as coagulation bath presented better performance than those prepared with water only. These conclusions were also applicable when TFC membranes were tested in the FO process, higher water fluxes being obtained with the active layer facing the draw solution.

Acknowledgments:

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# Optimization Study of the p-nitrophenol Degradation by an Advanced Oxidation Process

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Optimize treatment processes can be a major problem since many variables and their interactions are directly related to the final results of the process [1]. The application of experimental optimization shows the need for planning, one of the most important phases of scientific research [2]. The application of Central Composite Design (CCD) planning is characterized as an alternative since it predicts the use of a smaller number of tests than a parametric study and gives optimal answers in the process [3].

The objective of this study was to identify the level of influence of the variables, and their interactions, in order to optimize the conditions that provide the maximum mineralization and degradation, with minimal toxicity, of a solution containing p-nitrophenol (PNF), a compound of industrial importance. PNF is present in liquid effluents of various chemical processes and exhibits high toxicity [4-5], inhibiting biological degradation. The treatment process considered is by homogeneous Fenton reaction, an Advanced Oxidation Process (AOP).

The selection of variables and the ranges of the study were based on preliminary studies. All experiments were carried out in a batch reactor with a reaction time of 60 min and pH 3.5 (optimal values obtained in this study). The variables studied were as follows: Temperature ( $T = 19.8-79.8$  °C), concentration of Hydrogen Peroxide ( $[H_2O_2] = 25.4-2945.6$  mg.L<sup>-1</sup>) and concentration of iron ion ( $Fe^{2+} = 5.9-174.1$  mg.L<sup>-1</sup>).

It was concluded that for the operating conditions adopted, the quadratic models of mineralization, degradation and toxicity reduction have a good fit to the experimental data. Moreover, the variables T and  $[H_2O_2]$  have a direct influence on the answers considered. The experimental design technique allowed to determine the optimum conditions for the variables that influence the responses.

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## Application of a CdTe-MPA quantum dots packed reactor in clean reactive oxygen species generation

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Reactive oxygen species (ROS) are peroxides and ions including oxygen involved in cell signaling and homeostasis of living organisms. However, different circumstances lead to increased ROS production, oxidative stress and ultimately to cell and tissues damage. Thus, development of analytical methods for the quantification, in vitro evaluation of their effects and study of new biocompatible antioxidants often require simple and clean ways to produce ROS in laboratory. In this work, an irradiated column packed with mesoporous silica embedding CdTe quantum dots capped with mercaptopropionic acid (MPA) is described for this purpose. Semiconducting II-VI quantum dot nanostructures (QDs) are recognized by the high photostability, luminescence quantum efficiency, and wide emission tenability and accordingly explored in (bio)imaging and sensing approaches<sup>1</sup>. The additional ability to generate reactive oxygen species (ROS)<sup>2</sup> was also evidenced despite the hazardous nanoparticles chemical composition and use in bulk solution. Alternatively, the CdTe-MPA QDs were added to pre-hydrolyzed tetramethyl orthosilicate sol in an acidic medium containing Triton X100 as surfactant. The assessment of the obtained xerogel was performed by SEM-EDS, confirming uniform distribution of QDs agglomerates in vicinity of the surfactant vesicles. The confinement effect in immobilized QDs remained practically unchanged, as well as particles quantum yield (QY), but excitons lifetime increased almost 185 %. A small column packed with 100 mg of xerogel was inserted in a continuous flow-system, and afterwards irradiated with commercial LED illumination lamp, when ROS production is required. Deionized water was made to flow through the column and down stream merged with luminol alkaline solution to yield reproducible luminescence. In conclusion, immobilized CdTe-MPA QDs avoided the use of expensive reagents for reactive oxygen species oxygen generation and provided a start point for future applications in ROS studies.

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# Automated evaluation of ionic liquids toxicity through the inhibition of cytochrome c oxidase

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Ionic liquids (ILs) have raised interest in the academic and industrial fields for their particular and notable characteristics. Several studies revealed their potential application in areas such as synthesis, biocatalysis, electrochemistry, nanotechnology [1] and pharmaceutical industry [2]. However, this raises a new concern in terms of security, e.g., what is the real impact of these compounds on the environment and human life. This issue led the scientific community to focus on the assessment of ILs toxicity in the last years. Enzymatic assays have been preferably used for the evaluation of ILs' human toxicity [3]. Cytochrome c oxidase is commonly used as a marker for the study of mitochondrial membrane integrity and therefore toxicological study of several xenobiotics [4]. Nonetheless, it was never applied in ILs toxicity studies.

In this context, we developed a versatile, economical and robust automated methodology for the study of ILs toxicity through the inhibition of cytochrome c oxidase. The assay was implemented in a sequential injection analysis system, and was based on the decrease in absorbance at 550 nm of ferricytochrome c by cytochrome c oxidase. In each analytical cycle 40  $\mu\text{L}$  of enzyme, 12.5  $\mu\text{L}$  of assay buffer and 25  $\mu\text{L}$  of substrate were aspirated sequentially to the holding coil and after sent to the reaction coil, by flow reversal. The flow was then stopped for 180 seconds and after that period the reaction zone was propelled to the detector.

The selected compounds exhibited variable chemical structure and incorporated different structural elements, namely phosphonium, imidazolium and pyrrolidinium in what regards to cation core; and methanesulfonate, tetrafluoroborate and chloride, which represent some of the generaliest used anions. We expect that the obtained results can provide important information about the safety of the studied ILs. It is also our purpose that the automated methodology developed can be applied in the study of other classes of compounds and used as alternative to batch assays described in the literature.

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# Automation of peroxidase activity assay using a lab-on-valve system

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In the present work, an automatic methodology, allowing the evaluation of peroxidase activity, was developed. Automation was carried out using a miniaturized sequential injection analysis system, usually called lab-on-valve (LOV) [1]. This kind of system allows downscaling of current flow injection and sequential injection systems and facilitates automation of different techniques including the spectrophotometry [2].

For the automatic evaluation of horseradish peroxidase activity it was used as substrate hydrogen peroxide and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate (ABTS) as electron donor. In the presence of hydrogen peroxide, peroxidase catalyzes the dehydrogenation of ABTS, resulting in the formation of a resonance-stabilized and colored radical cation of ABTS that was measured spectrophotometrically at 405 nm. The green-blue radical is taken as a measure of the peroxidase activity.

Within the flow system, the effect of different parameters as reagent ratios, volumes and concentrations, pH, temperature and reaction time, were tested and evaluated in the peroxidase activity.

The use of the automatic and miniaturized system for the implementation of the described reaction, enables the precise and exact time control of fluidic manipulations leading to a repeatable and reproducible measurements. In addition, it allowed a decrease of reagent consumption and in wastes generation. Thus, the developed methodology constitutes a robust, versatile, rapid and simple alternative for the evaluation of peroxidase activity, adapted to the actual demands of reagents economy and reduced residue production, being in good agreement with the actual recommendations of green chemistry.

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# Canned Tuna: Nutritional Characteristics and Quality Evolution After Opening

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Fish consumption, due to its high nutritional characteristics, is receiving increased attention from consumers. Canning is a preservation method widely used by industry, and also highly recognized. More recently, canned tuna with vegetables emerged as a convenience product, which combines the benefit of both fish and vegetables. In addition, a question that arises frequently by consumers is the shelf life after opening. Therefore, this work intended to compare the quality of classical canned tuna with two products of tuna with vegetables, focusing on the shelf life under refrigeration after opening, and using several chemical, sensorial and microbiological parameters.

Tuna samples in vegetable oil, with corn and red beans, and with vegetable salad and mayonnaise, were evaluated at the time of opening, and after 1, 2, 3, 4 and 7 days of storage under refrigeration. Microbiological analysis consisted in total counts of aerobic mesophilic, psychrophilic and thermophilic as well as sulphite-reducing anaerobes mesophilic and thermophilic microorganisms, following respective ISO guidelines. Chemical analyses were based on lipid deterioration (reactive substances to 2-thiobarbituric acid, polyunsaturated fatty acid content and vitamin E), protein deterioration (total volatile nitrogen, biogenic amines content, protein oxidation), complemented by pH, moisture, and total phenolic compounds. Sensory analyses were performed using a descriptive test with a structured scale for different attributes, while color was assessed instrumentally.

The microbiological parameters studied were considered of satisfactory quality for the 3 types of canned tuna evaluated under the total refrigeration period [1,2]. The chemical parameters evaluated revealed a similar trend, with insufficient variations to consider the product unfit for consumption, but with statistically significant differences for some parameters/samples ( $p < 0.05$ ). Only the sensorial analyses allowed defining true quality limits. The changes observed for the evaluation of attributes related to color, smell, taste, and texture determined that the tuna samples with vegetable salad and mayonnaise should be eaten in the same day of opening, while tuna in vegetable oil, and tuna with corn and red beans can be consumed within 1 to 3 days, respectively.

Acknowledgments: The authors thank the collaboration of Ramirez & C<sup>a</sup> (Filhos) S.A.

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# Chiral HPLC enantioresolution of synthetic cathinones “Legal Highs”

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In recent decades, "Legal Highs" sold in old "smart shops" and on Internet for recreational use have been fabricated exponentially. Within these new drugs, derivatives of cathinones have gained a huge popularity among consumers. These compounds are analogues of the natural cathinone, the active ingredient of Khat, and are chemically modified generating huge number of different derivative molecules. All the described cathinone derivatives are chiral and enantioselectivity must be considered for their toxicological effects [1]. Because these is a recent concern, there is little information on racemic mixtures and almost nothing is known about the biological activity of each individual enantiomer [2]. Thus, the aim of this study was to study the enantiomers of cathinones in these “Legal Highs” and evaluate the respective toxicological effects.

In this work we describe the enantioresolution of 9 synthetic cathinones (MDPV, Flephedrone, Pentedrone, 4-MEC, 3,4-DMMC, Metedrone, Buphedrone, Methylone and Etcatinone) present on 13 “Legal Highs” commercially available in drugs sold on old "smartshops" by chiral HPLC method. The separations were performed with the Chiralpak® AS-H column under normal-phase elution conditions. The best results were achieved with n-hexane:2-propanol: TEA (97: 3: 0.1) as mobile phase at a flow rate of 0.5 ml/min. The analyses were performed at room temperature in isocratic mode and UV detection at a wavelength of 254 nm.

We successful separate both the samples with only one cathinone as in samples with more than one cathinone. It was found that, with the exception of MDPV all cathinones were enantioseparated with very high enantioselectivity and resolution with  $\alpha$  and  $R_s$  values always greater than 1.25 and 4.00, respectively. The overall results of this study indicate that it is possible to scale-up the enantioresolution in order to obtain the single enantiomers of these derivatives of cathinones.

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## **Chondroitin sulfate or fucoidan crosslinked with a sol – gel network as sorbents for metal cations.**

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Chondroitin sulfate (CS) and Fucoidan (Fd) are compounds of marine origin extracted from the cartilage and tissues of animals and seaweed respectively [1, 2]. Both are linear acidic polysaccharide, composed of repeating disaccharide units and modified with sulfate residues at different positions.

The aim of this study took into account the valorization of marine resources by aiming at innovative applications. In this context, the reticulation of CS/Fd via a sol-gel process was explored with the purpose of preparing sorptive materials for metal cations such as  $\text{Pb}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$ . The same process was also attempted for the molecular imprinting of target cations ( $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$ ) envisaging an increase in the selectivity of the adsorbents. Simultaneously controls, corresponding to adsorbents without CS/Fd, were performed. All sorbents were structurally characterized and its efficiency in sorption of the cations under study was briefly assessed by solid phase extraction (SPE) and monitored by atomic absorption spectrophotometry (AAS).

The developed sorbents presented low surface areas (range of 4 - 6  $\text{m}^2/\text{g}$ ) and low pore volume (range of 0.003 - 0.004  $\text{cm}^3/\text{g}$ ) but the adsorbents with CS/Fd showed significant SPE retention of  $\text{Cu}^{2+}/\text{Zn}^{2+}$  (aprox. 90% and 84 % respectively) and  $\text{Pb}^{2+}$  (aprox. 70%), which demonstrated that the compounds of marine origin greatly benefit the adsorption of cations in study. The  $\text{Ag}^+$  was the cation with lowest SPE retention with all the adsorbents (< 15%).

Sorbents composed of CS showed better retention capacity of metals as compared with sorbents composed of Fd. However the later appears to retain more strongly the cations. The molecular imprinting with CS did not increase the retention of the imprinted cation, however it increased selectivity for the metals used as templates.

The data concerning the kinetics and isotherms adsorption is currently being collected and will also be presented during the meeting.

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# Determination of diacetyl and other volatile compounds in solid and liquid samples by gas-diffusion microextraction

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Membrane-based extraction can be used to develop simple, high throughput, low cost and low solvent consumption sample preparation procedures. Gas-diffusion microextraction (GDME) is an extraction technique, for volatile and semi-volatile compounds, that was recently developed and patented by our research group [1, 2]. GDME consists in a small polytetrafluoroethylene (PTFE) module with a microporous hydrophobic PTFE membrane at its bottom; the extraction process is based on the compounds transfer from the sample (donor phase) through the membrane into an acceptor phase, usually liquid. The mass transfer occurs essentially by diffusion of the analytes in the gas form across the gas layer separating the two phases. One of the main advantages of GDME is the possible combination with a derivatization reaction, which improves separation and/or detection of the analytes and enhances extraction selectivity. So far, GDME has been successfully applied in the analysis of important food quality markers like diketones, aldehydes and biogenic amines in several liquid and solid samples [3, 4].

In this work, the GDME methodology was applied to the determination of diacetyl (butane-2,3-dione) and other  $\alpha$ -dicarbonyl compounds in solid (popcorns, bread) and liquid (wine) samples. Analytes were extracted from the sample and derivatized with *o*-phenylenediamine before analysis by high-performance liquid chromatography with UV detection. Several extraction conditions were studied and optimized, such as the influence of membrane nature, acceptor solution composition, temperature of extraction, mass of sample used for extraction as well as the calibration procedure.

Acknowledgements: This work is funded by FEDER funds through the Operational Programme for Competitiveness Factors - COMPETE and by National Funds through FCT - Foundation for Science and Technology under the Pest-C/EQB/LA0006/2013 and NORTE-07-0124-FEDER-000069. I.M.V. receives a postdoctoral grant through the project NORTE-07-0124-FEDER-000069 – Ciência do Alimento. RMR (SFRH/BD/88166/2012) wishes to acknowledge FCT for his PhD studentship.

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# Determination of polyphenols in blueberries using salting-out assisted liquid-liquid extraction

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Salt-assisted liquid-liquid extraction (SALLE) is a homogeneous liquid-liquid extraction technique that has been receiving considerable attention for sample preparation of various types of samples. The procedure consists in adding a water-miscible organic solvent to an aqueous sample followed by the addition of a salt (or a mixture of salts) to induce the phase separation between water and the organic solvent, due to the salting-out effect. This process is very interesting for sample preparation since extraction of compounds to the organic solvent can occur, being the analysis performed over the organic phase [1].

Studies over the formation of two liquid phases in acetone-water-inorganic salts mixtures go back to the early 20th century. Since then, the interest in this type of liquid-liquid extraction procedure has been increasing, particularly for the analysis of biological samples [2]. Main advantages of SALLE are its low cost, simplicity and low solvent consumption. Besides, the extracts are readily compatible with various instrumental techniques such as gas and liquid chromatography [1, 3-4].

In this work the application of SALLE for the determination of polyphenols in blueberries is presented. Samples were extracted with acetonitrile-water-ammonium sulphate by decoction at room temperature, followed by centrifugation to improve liquid phases' separation. Finally, the organic extract was collected and analysed by high performance liquid chromatography with UV detection. The extracts' composition was characterized by liquid chromatography with mass spectrometry detection for compounds identification and confirmation.

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# Development of a sequential injection analysis system for the evaluation of protein binding affinity of pharmaceutical ionic liquids

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In recent years, ILs with biologically active ions were synthesized and showed to retain the activity of both the original cation and anion, proving to be potentially valid active pharmaceutical ingredients (IL-APIs) [1]. The research on this field has been focused mainly on the synthesis of novel compounds through the association of active ions and their physico-chemical characterization with only a few studies regarding their *in vivo* behavior [2].

In this work, the protein binding affinity of IL-APIs based on the association of non-steroidal anti-inflammatory drugs with choline was evaluated resorting to an automated methodology specifically designed for this purpose. For this study, human serum albumin (HSA) was selected as model plasmatic protein due to its significance and intrinsic fluorescence that can be quenched through the interaction of drugs with one of its binding sites. The fluorescence quenching assay was implemented in a sequential injection analysis system and the main physical and chemical parameters were optimized. The analytical cycle comprised the sequential aspiration of 100  $\mu\text{L}$  HSA 6  $\mu\text{M}$  and 100  $\mu\text{L}$  of IL-API (variable concentration) at a flow rate of 1 mL/min. Then the flow was reversed and the aspirated zones were sent to a reaction coil where the flow was stopped during 60sec. The fluorescence intensity was then measured at the detector.

In the optimized conditions the effect of increasing concentrations of choline ketoprofenate and choline naproxenate (and respective starting materials: ketoprofen and naproxen) on the intrinsic fluorescence of HSA was studied and the dissociation constant ( $K_d$ ) was calculated by means of models of drug-protein binding in the equilibrium. The results evidenced that the tested IL-APIs and respective starting materials quench the intrinsic fluorescence of HSA in a concentration dependent mode. The calculated  $K_d$  showed that all the compounds bind strongly to HSA ( $K_d < 100 \mu\text{M}$ ) and that the use of the drugs in the IL format does not affect or can even improve their HSA binding.

#### Acknowledgements:

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# Dispersion of Carbon Nanotubes in Water by Gemini Surfactants: a Systematic Study

Jessica Rocha<sup>1</sup>, Ricardo Fernandes<sup>1,2</sup>, Oren Regev<sup>3</sup>, István Furó<sup>2</sup> and Eduardo F. Marques<sup>1</sup>

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Carbon nanotubes (CNTs) are a quasi-one-dimensional nanomaterial with unique properties and hence with a high potential for a variety of applications [1]. However, due to the strong van der Waals interactions established between them, CNTs are prone to agglomeration into big bundles, which makes their handling and usage rather difficult. The dispersion and isolation of individual CNTs is important. One of the most common procedures to suspend and separate this material in water is using non-covalent dispersion methods, which involve the adsorption of amphiphilic molecules (such as surfactants, polymers and proteins) on the surface of CNTs through hydrophobic interactions, and colloidal stabilization by electrostatic and/or steric repulsions provided by the surfactant headgroups [1–3]. In this work, we have carried out a systematic study of the dispersion ability of a set of di-cationic gemini surfactants of the bis-quat type, with variation of main chain length ( $n = 12$  and  $16$ ), and spacer length ( $s = 2, 6$  and  $12$ ), cf. Fig. 1a). The exfoliation and de-bundling of the nanomaterial was achieved by a dual ultrasonication-centrifugation procedure, followed by accurate quantification of the concentration of dispersed by CNTs by thermogravimetric analysis and UV-Vis absorption spectroscopy. Multi-walled carbon nanotubes (MWNT, Fig. 1b) were employed, covering a wide range of surfactant concentration, below and well above the critical micelle concentration ( $cmc$ ). The dispersion curves obtained allowed us to extract parameters such as critical surfactant concentration for dispersion, maximum dispersed CNT concentration, respective surfactant concentration at that point, dispersion efficiency and performance index for each surfactant. The effects of the gemini surfactant structure (e.g. spacer and chain length) and  $cmc$  in the dispersion process are presented and critically interpreted.

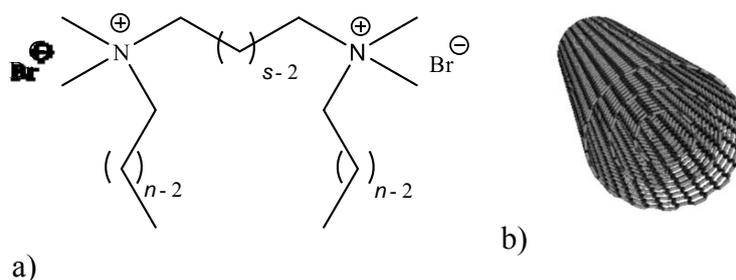


Fig.1: a) Molecular structure of the used gemini surfactants ( $n = 12, 16$ ;  $s = 2, 6, 12$ ); b) MWNTs.

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# Eco-sustainable modification of wood fibre by oxidation through metalloporphyrin catalysis

**A. Patrícia Pereira, Susana L. H. Rebelo and Baltazar de Castro**

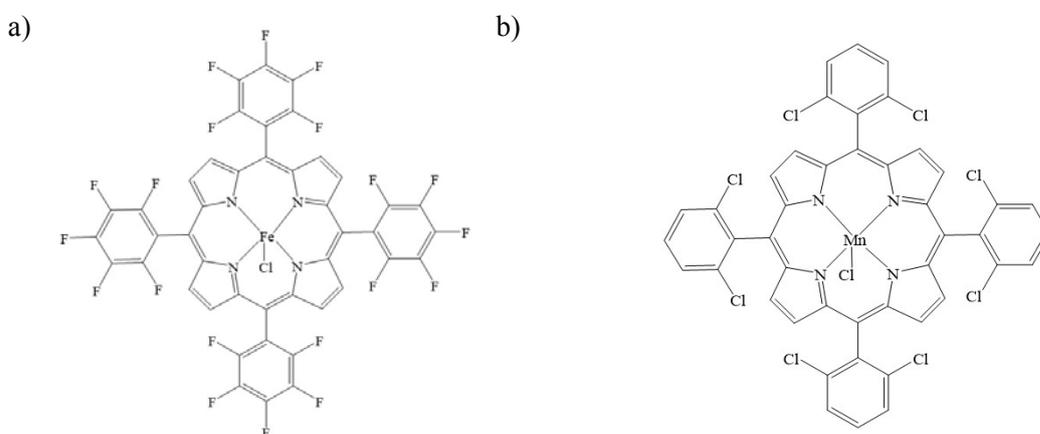
REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, 4169-007 Porto.

In recent years, specific iron (III) and manganese (III) porphyrins have shown prominent activity as biomimetic models of heme enzymes, namely cytochrome P450 monooxygenases and lignin peroxidases [1,2]. These methodologies also allowed mild or eco-sustainable conditions and fast processes.

In the present work, diverse catalytic systems based on metalloporphyrins (figure 1) were tested in the oxidation of wood fibre, a natural lignocellulosic material. Our aim was to obtain more functionalized materials for composites production or the oxidative lignin depolymerization, in order to produce platform chemicals or a wood fiber bleaching process, in eco-compatible conditions.

Hydrogen peroxide was chosen as a green oxidant that produces water as the only by-product and its cost has been decreasing in the recent years [1].

The work considered the optimization of reaction conditions and the characterization of materials before and after the oxidation process by techniques such as SEM, FTIR, XPS and solid state  $^{13}\text{C}$  NMR CPMAS.



**Figure 3 Metalloporphyrins used as oxidation catalysts : a) [Fe(TPFPP)Cl] and b) [Mn(TDCPP)Cl]**

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# Evaluation of the effect of ionic liquids on the sensitivity of the copper-catalyzed chemiluminescence reaction

**A.R.F. Costa, A.R.A. Sousa, M.L.M.F.S. Saraiva, P.C.A.G. Pinto**

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Ionic liquids (ILs) have been used in numerous processes like synthesis, catalysis and chromatography due to characteristics as low vapor pressure, which made them an interesting alternative to the use of organic solvents [1]. In the field of analytical chemistry the utilization of ILs has been mainly associated to separation/extraction procedures and electrochemical measurements. It is also important to highlight the well-known benefits of using ILs in chemiluminescence determinations [2]. Amongst these reactions, the catalyzed CL reaction of luminol is well known by its high sensitivity and low background signal. However, these reactions require strong oxidants and high pH which creates difficulties regarding its applicability in bioassays. Metal catalyzed CL reactions emerged as good alternatives in these cases due to their softened conditions. In particular, sensitivity enhancements of copper-catalyzed CL determinations were already reported in the presence of the IL 1-ethyl-3-methylimidazolium ethylsulfate through the interaction of the copper catalyst with the imidazolium ring.

The main goal of this work was to evaluate the influence of several ILs on the copper-catalyzed chemiluminescence (CL). Considering the previous results reported for this reaction, in this work six ILs of the imidazolium group were tested. Three ILs with pyrrolidinium and pyridinium cations were also evaluated with comparative purposes.

The evaluation of the effect of the selected ILs on the CL reaction was performed in an automated sequential injection analysis (SIA) system [3]. Initially, the copper catalyzed reaction was implemented in the flow system in aqueous media. The optimized analytical cycle began with the sequential aspiration of 50  $\mu\text{L}$  of luminol 300  $\mu\text{M}$ , 50  $\mu\text{L}$  of  $\text{H}_2\text{O}_2$  1 mM and 50  $\mu\text{L}$  of  $\text{CuSO}_4$  1.67 mM followed by the propulsion of the reaction zone to the CL detector at a flow rate of 5 mL/min. In the optimized conditions, the effect of copper/IL catalysts on the CL signal was studied. The concentration of IL was varied between 0.25 and 1.5 M. The results evidenced that all the ILs of the imidazolium group enhanced the sensitivity of the CL reaction and the ILs with pyridinium and pyrrolidinium cations decreased it markedly, both in a concentration dependent mode. The most promising ILs showed to be those based on the 1-ethyl-3-methylimidazolium cation combined with methanesulfonate or trifluoromethanesulfonate anions, with sensitivity enhancements of about 3 times.

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## Extraction and solubilisation of eggshell membrane

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Over the years, hen's eggs consumption has been increasing around the world, including in Portugal. This increase comprises both industrial and household, leading to an eggshell accumulation that can be responsible for environmental and public health problems. Eggshell treatment has become essential to minimize these effects, so as to find rentable applications. In last decades, based on eggshell constituents biological properties some studies have been performed in order to promote its valorisation. However, some applications are expensive or without added value. Concerning the eggshell membranes, which consists essentially in protein, their applications are limited due to their insolubility that results from the presence of multiple disulphide bonds. Eggshell membranes are already applied in cosmetic field; however their use on food industry is an unexplored area <sup>[1-4]</sup>.

Thus, this work leads to extraction and solubilisation processes optimization for eggshell membranes in a fast and economic way with a view to their application in the food industry. Experiments were performed with different conditions in order to optimize time and yield. It was concluded that the best extraction method was the immersion of eggshell in acetic acid 5 %, during 1h at room temperature. As regards to solubilisation process, the best results were obtained when it was made with a low concentration of 3-mercaptopropionic acid in eggshell membranes previously separated from eggshell. In order to eliminate this solvent, the obtained extract was washed with ethanol or methanol which were completely evaporated from the final protein powder. Regardless the wash solvent used, the solubilisation yield was of 63 %.

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## Heterogeneous acid catalysts for ring opening of epoxides

A. Silva, S. M. Silva, D. Oliveira, A. T. Silva, A. F. Peixoto, C. Freire

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Epoxides are useful intermediates for organic synthesis, due to their high reactivity as a consequence of their ring strain. The reaction of epoxides with amines is particularly interesting, because it leads to the formation of  $\beta$ -amino alcohols which are the key intermediates to many organic compounds, including biologically active natural and synthetic products, and chiral auxiliaries for asymmetric synthesis [1]. Usually, the epoxide ring opening reactions involves a well-known variety of Lewis or Brønsted acid catalysts, already reported in literature. [1,2] The majorities of these catalysts are homogeneous and are always associated with high reaction temperatures, prolonged reaction times, non-catalytic nature of the reagents, low conversions and poor selectivity, difficulty of separation and the impossibility to recover from the reaction media. [3] The use of heterogeneous catalysts provides a perfect solution to solve these problems.

In this work, sulfonic acid heterogeneous catalysts were used in ring opening of styrene and cyclohexene oxide with aniline under solvent-free conditions and room temperature (Fig.1). All catalysts tested showed good catalytic activity. Good results were also obtained in the reutilization of the catalysts with no significant loss of catalytic activity until the third reaction cycle.

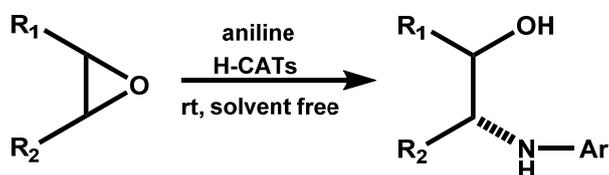


Fig. 1: Schematic representation of ring opening epoxides with aniline.

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# Implementation of a laccase air-breathing cathode for self-powered sensors

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The proposal of sensor based technologies to provide chemical information is one of the most active research areas in several applied branches of analytical chemistry, particularly in agro-food, health and environment fields. Such interest relies on the capability of such devices to gather real-time information without harvesting representative numbers of samples or take measurements overcoming complex treatments. Their typical small size enables portability as well and paves the way for remote sensing and screening tasks. Nevertheless, when this last usage is equated the need of an additional power source for sensor operation can be a cumbersome problem particularly if one considers sensors use as implantable device. A possible solution was firstly advanced by Katz and col [1] and comprised a 1 $\mu$ W bio-fuel cell generating different potentials according the fuel/analyte concentration. The cell was implemented with an anode with immobilized glucose oxidase or lactate dehydrogenase over its surface, and a cathode based on immobilized cytochrome *c*/cytochrome oxidase responsible for dioxygen reduction. Either glucose or lactate was used as a fuel/analyte and the cell generated a voltage signal which increased logarithmically with the increase of the fuel concentration. To extent such scheme to other analytes or simply get further miniaturization, more efficient electrodes must be searched in order to maintain or even provide more power. In this work, a nanostructured air-breathing biocathode based on laccase from *T. versicolor sp.* is described to provide high current density. The electrode was implemented with a 2 mm pencil mine over which 20  $\mu$ L of graphene oxide (1 mg/ml) were dropped and further electro-reduced for 33 hours. The follow-up of characteristics and performance of the electrode, namely regarding response modeling and evaluation of heterogeneous electron transfer reaction was performed by means of cyclic voltammetry and electrochemical impedance spectroscopy. An increase current density of more than 5 times range was noticed relatively to the graphene oxide surface electrodes (without electro-reduction). Such response resulted from both double layer charge capacitance and lower heterogeneous electron transfer resistance which reduced the order of magnitude from k $\Omega$  to  $\Omega$ . The electrode was finally coated with the enzyme immobilized in a silica xerogel film. Comparative direct electron transfer from the electrode to the T1 catalytic laccase center through single walled carbon nanotubes or chemically carbon black nanoparticles is under way.

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## Innovative strategy for the determination of ammonia in maize silages

**C. D. Vaz<sup>1</sup>, M. J. Cerqueira<sup>1</sup>, R. M. Ramos<sup>1</sup>, I. M. Valente<sup>1</sup>, H. M. Oliveira<sup>2</sup>, A. J. M. Fonseca<sup>2</sup>, A. R. J. B. Cabrita<sup>2</sup> and J. A. Rodrigues<sup>1</sup>**

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Sample preparation is frequently considered the bottleneck in analytical procedures, as it can be time and reagent consuming; it may involve several steps and has an important influence in the precision, accuracy and limits of detection. Membrane-based extraction techniques have been successfully applied for sample preparation, since simple, high-throughput, low cost and low solvent consumption procedures can be developed. The major advantage of this type of processes is the extraction of compounds from the sample (donor phase) to an acceptor phase without direct contact between the phases. In this work, a membrane-based sample preparation technique, gas-diffusion microextraction (GDME) [1], was used to develop a methodology for the determination of ammonia (NH<sub>3</sub>) in maize silages. The extraction is based on the NH<sub>3</sub> transport from the sample (donor phase) through a gas-permeable membrane into a liquid acceptor phase. The membrane (impermeable to both phases) embodies a thin air space inside its pores, and the mass transfer occurs by gas-diffusion of the analytes across the gas layer separating the two phases [2]. The experimental procedure consists on the extraction of NH<sub>3</sub> from the samples by volatilization. To achieve this, a sodium hydroxide (NaOH) solution is added to the sample to increase pH and promote the analyte volatilization [3]. NH<sub>3</sub> passes across the membrane pores and is collected in a liquid acceptor solution containing a labeling reagent (*o*-phtalaldehyde, OPA). The extract is analysed by fluorimetric detection. Several experimental parameters were studied and optimized: OPA concentration, pH of the acceptor solution, NaOH concentration in the sample and sample mass. Other studies are currently being performed, namely the effect of temperature and time of extraction.

Acknowledgements: This work is funded by FEDER funds through the Operational Programme for Competitiveness Factors - COMPETE and by National Funds through FCT - Foundation for Science and Technology under the Pest-C/EQB/LA0006/2013 and NORTE-07-0124-FEDER-000069. I.M.V. receives a postdoctoral grant through the project NORTE-07-0124-FEDER-000069 (Ciência do Alimento). RMR (SFRH/BD/88166/2012) and HMO (SFRH/BPD/75065/2010) wish to acknowledge FCT for their PhD studentship and post-doctoral grant, respectively.

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# Lamb's lettuce as a good source of chlorogenic acid

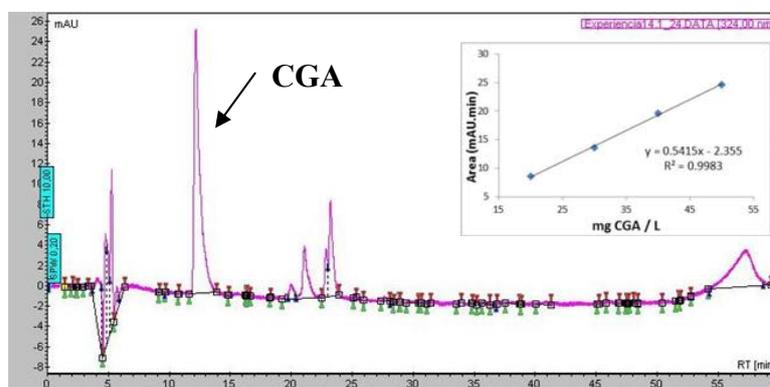
**I. Oliveira, L. F. Guido**

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Lamb's lettuce (*Valerianella locusta*) is a green leafy vegetable, which is well appreciated in France, Britain, Germany and Italy [1]. Lamb's lettuce is achieving importance as a major source of phenolic compounds, especially chlorogenic acid (CGA). The multiple pharmacological effects of CGA, due to its antioxidant properties, are widely recognized. Classically, CGA are a family of esters formed between certain trans-cinnamic acids, like caffeic acid, p-coumaric acid or ferulic acid, and a quinic acid. The commonest individual CGA is 5-O-caffeoylquinic acid (5-CQA) [2].

The content of CGA was determined, based on an ultrasonic methodology recently developed for extracting and quantifying this compound from lamb's lettuce's fresh leaves. CGA was extracted by using an ultrasound assisted extraction (UAE) in the following conditions: solvent/sample ratio of 1:30, 100% methanol as solvent, 3 successive extractions during 40 minutes each on UAE. The detection and quantification of CGA was carried out by HPLC-DAD (high-performance liquid chromatography/diode array detector) that enables continuous monitoring and recording of the ultraviolet and visible absorption spectrum. The calibration curve and the method of least squares were applied as calibration method.

The calibration curve of CGA showed a good linear response over the concentration range measured ( $r^2$  of 0.9983). A chromatogram obtained for a methanolic extract of lamb's lettuce (detection 324 nm) is shown below.



Considering the moisture content of approximately 90%, the total CGA content of lamb's lettuce found was 883 mg/100g of dry weight. The relative standard deviation (coefficient of variation) was 5.6% (six replicates), demonstrating the repeatability of the applied method. Moreover, the results show that this matrix contains significant amounts of CGA, suggesting that lamb's lettuce is an excellent natural source of CGA. Work is in progress to assess the method in terms of intermediate precision and robustness.

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# Methodological study of the gel to liquid crystalline phase transition of phospholipids by DSC

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Liposomes are widely used both as model membranes (for eukaryotic and pathogen membranes) [1] as well as nano-carriers for drugs and food components (nano-delivery systems) [2]. In the last decade large resources were used in this research and it is nowadays one of the leading research fronts in Horizon 2020.

In order to use liposomes quantitatively and in a solid knowledge-basis, comprehensible methodological work is needed to fully characterize their characteristics – thermal behavior, shelf life, morphology, response to medium variables.

The objective of this Project (3<sup>rd</sup> year of Chemistry) is the establishment of an optimized liposome preparation protocol for use in DSC (differential scanning calorimetry). DSC is the method of choice and the most widely used to characterize the thermal behavior and thermodynamic properties of liposome temperature induced transitions [3].

Both multilamellar vesicles (MLV) and unilamellar vesicles (LUV) obtained from an extrusion technique were used. DMPC (1,2 dimyristoyl -sn- glycerophosphocholine -3) was chosen as probe lipid, and the suspensions were prepared in HEPES buffer 10 mM, 150 mM NaCl. Two liposome preparation methods were used – direct hydration of the powder and hydration of the lipid film (Bangen method).

The samples were run in a VP-DSC (Microcal/Malvern) to characterize the observed transitions – the pre-transition (“ripple” to gel phase) and the main transition (gel to liquid crystalline phase), and the temperature ( $T_m$ ) and enthalpy change ( $\Delta H$ ) were calculated from the obtained thermograms.

In order to establish the referred protocol, several variables were systematically changed: i) the scanning rate; ii) the width of the data filter; iii) gain; iv) pre-period; v) number of scans; vi) time between preparation and scan; and vii) sample concentration.

The influence of these parameters in the obtained results will be presented and discussed.

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# Novel POM@CNTs nanocomposites: from synthesis to electrode modification

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Carbon nanotubes (CNTs) have become a subject of increasing interest in different fields of Materials Chemistry, mostly due to their unique combination of properties<sup>[1]</sup>. CNTs consist of cylinders made of graphene layers that maybe closed or open at both ends. Both single (SWCNTs) and multi-walled nanotubes (MWCNTs) have diameters of a few nanometers and lengths of the order of 1  $\mu\text{m}$ . Among the several molecular species that can be immobilized on carbon nanotubes are the polyoxometalates (POMs). These anions represent an important class of bulky clusters with applications in a variety of scientific fields<sup>[2]</sup>. Immobilization of POMs into CNTs is expected to increase the number of active centres that would improve electrochemical properties, but also their electrocatalytic properties<sup>[3]</sup>.

In the present work, single-walled and multi-walled carbon nanotubes were functionalized with cobalt ( $\text{K}_6[\text{SiW}_{11}\text{Co}(\text{H}_2\text{O})\text{O}_{39}] \cdot 12\text{H}_2\text{O}$ , (K-SiW<sub>11</sub>Co)) and manganese-substituted ( $\text{K}_6[\text{SiW}_{11}\text{Mn}(\text{H}_2\text{O})\text{O}_{39}] \cdot 13\text{H}_2\text{O}$ , (K-SiW<sub>11</sub>Mn)) Keggin-type polyoxometalates, respectively. Polyethylenimine (PEI), for SWCNT, and polydiallyldimethylammonium chloride (PDDA), for MWCNT, were used functionalization agents to improve the polyoxometalates attachment to the carbon nanotubes.

Investigations were focused on the structural characterization and electrochemical properties of the POM@CNTs nanocomposites. FT-IR spectroscopy confirmed the synthesis of the desired compounds and nanocomposites. Cyclic voltammetry experiments with electrochemical probe  $\text{K}_3[\text{Fe}(\text{CN})_6]$  at glassy carbon electrodes modified with pristine CNTs and POM@CNTs nanocomposites showed an improvement in the electroactive surface area which is advantageous for future electrochemical applications.

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# ORAC-pyrogallol red methodology under microplate format for assessment of antioxidant reactivity of phenolic compounds

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Peroxyl radicals (ROO<sup>•</sup>) are chain-carrying species of lipid peroxidation that react with a wide spectrum of biomolecules with consequent impairment of their functions. Phenolic compounds are known for their scavenging ability against this type of radicals, reducing the extent of their oxidative damage. When present in trace amounts, phenolics should afford protection to susceptible molecules from oxidation in order to meet the definition of antioxidant. When characterizing a given molecule, both antioxidant reactivity (reaction rate between a given free radical and specific antioxidants) and antioxidant capacity (amount of free radicals scavenged) should be considered in order to establish more realistic antioxidant outputs.

Oxygen radical absorbance capacity (ORAC) methodologies are currently used for the assessment of peroxyl scavenging ability. In general, the protection afforded by an antioxidant compound to a target molecule against oxidation by peroxyl radicals is measured [1]. Depending on the kinetic profile of target molecule oxidation, it is possible to determine antioxidant capacity and reactivity. For instance, the coloured reagent pyrogallol red (PGR) is a suitable target molecule to estimate antioxidant reactivity as it presents a higher reactivity for radicals, when compared to other targets [1]. Simultaneously, PGR works as spectrophotometric probe for reaction development since its oxidation by ROO<sup>•</sup> generated from AAPH causes progressive absorbance decrease at 540 nm (pH 7.4).

The present study was conducted towards establishing a structure-antioxidant reactivity relationship for several phenolic compounds employing a microplate-based ORAC-PGR protocol [2]. Differences in reactivity were interpreted considering structural differences such as the number and position of OH groups and the influence of conjugated double bonds in the side-chains. For example, pyrogallol has shown 100 times higher reactivity than catechol, which can be explained by an additional hydroxyl group in its structure. Also, hydroquinone has proven to be 100 times more reactive with peroxyl radicals when compared to catechol, which is an isomer, proving that hydroxyl groups' position within the aromatic ring influences antioxidant reactivity.

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# Organic vs. Conventional agriculture: hydrophilic antioxidant compounds in olive oils

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The increasing interest in Organic agriculture among consumers and within the scientific community is driven by concerns on human health, food safety and environment. The possible increased nutritional quality of organically produced food represents one of the consumers' reasons for choosing these products. Olive oil is the essential component of the Mediterranean diet, a nutritional regimen gaining renown due to its beneficial effects in the prevention of cardiovascular and neurodegenerative diseases and oxidative stress. Apart from its lipid composition, olive oil is considered an important source of polyphenols, with a recent nutritional allegation for cardiovascular health published by the European Commission, for olive oils with at least 5 mg of these polyphenols per 20 g of olive oil. It is crucial to understand the impact of production systems (Organic vs. Conventional) in the amounts and composition of these hydrophilic antioxidants.

An extractive and chromatographic methodology was optimized for a fast analysis of total hydrophilic antioxidants in virgin olive oil by HPLC-DAD as is [1], or after direct hydrolysis with HCl 2M, 25°C/6h [2]. The Folin-Ciocalteu method was also applied [3]. All methods were applied to commercial samples from Organic production system (n=19) and using Conventional commercial olive oils as control (n=21).

No statistical differences were observed between the total hydrophilic antioxidant compounds and the production systems obtained by HPLC-DAD, with 255±64 mg/kg in the Organic production system, and 232±48 mg/kg in the Conventional ones. The same applies for total hydroxytyrosol and tyrosol, obtained after hydrolysis, ranging from 273±93 mg/kg in Organic production system and 294±70 mg/kg in Conventional production system. A total of 26% and 10% of Organic and Conventional samples, respectively, did not fulfill the nutritional allegation.

The practice of Organic agriculture is considered a promising type of production to meet the challenges of modern agriculture but, in the particular case of olive oil, not necessarily granting increased bioactivity. This study should be complemented by other recognized important parameters more sustained conclusions.

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# Phenolic profile of grapes from Dão region: evolution of non-colored compounds

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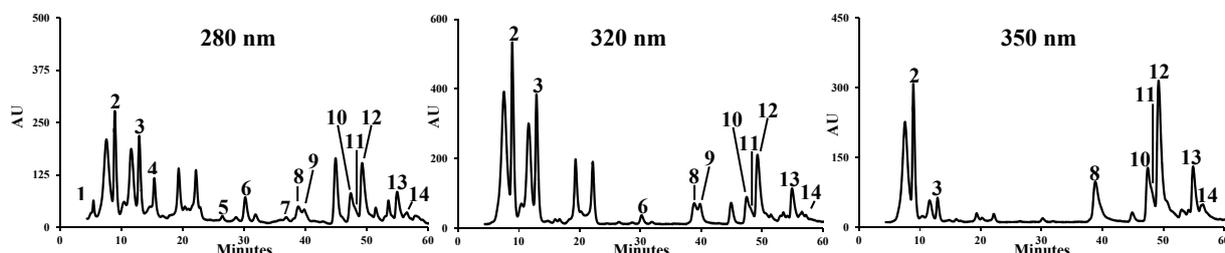
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Grapes are a rich source of phenolic compounds, which are mainly present in the seeds and skin. As so, the knowledge of these compounds is important for their recovery in winemaking. Phenolic compounds play an important role in the growth and reproduction of the plant, but also in oenology, due to their influence on some important sensory properties of grapes and wine, such as color, flavor, astringency, bitterness and structure, contributing to wine quality [1]. The phenolic compounds generally found in grapes are distributed among phenolic acids, stilbenes and flavonoids, the last constituting the main group. Among flavonoids, flavanols, flavonols and anthocyanins were reported as the most important constituents of grapes [1].

In this work, non-colored phenolic compounds from 14 different varieties of Portuguese grapes harvested in Dão region, at two different maturity stages, with a week difference, were studied. The qualitative profile of the different varieties revealed to be similar, but some quantitative differences between varieties and maturation degree were found. The most abundant compounds were coumaric acid, caftaric acid and epicatechin.



**Fig. 1** HPLC-DAD chromatogram of *Vitis vinifera* cv Alvarelhão grapes hydromethanol extract. (1) Gallic acid; (2) Caftaric acid; (3) Coumaric acid; (4) Catechin; (5) Epicatechin; (6) *p*-Coumaric acid; (7) Epicatechin gallate; (8) Myricetin-3-*O*-glucoside; (9) Resveratrol-3-*O*-glucoside; (10) Quercetin-3-*O*-galactoside; (11) Quercetin-3-*O*-rutinoside; (12) Quercetin-3-*O*-glucoside; (13) Kaempferol-3-*O*-glucoside; (14) Isorhamnetin-3-*O*-glucoside.

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## Rapid method for the detection of chloroanisoles in cork stoppers

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The presence of chloroanisoles in cork stoppers is considered the most frequent cause of organoleptic defects in wines bottled with natural corks. These compounds, namely 2,4,6-trichloroanisole (TCA), are generally associated with musty off-flavors and odors in food products. Having this in mind, the major aim of the present work was the development of a rapid and low cost screening method for TCA detection in cork stoppers prior to wine bottling.

The proposed methodology comprises the preconcentration of TCA using solid-phase extraction (SPE) in disk format and analyte detection through measurement of fluorescence quenching of a specific probe previously immobilized in the disk, after irradiation with UV light. SPE was performed using C18 disks as sorbent and methanol as eluent. The selected probe was 8-anilino-1-naphthalenesulfonic acid (ANS). Different parameters were tested and evaluated, namely probe concentration and volume, analyte concentration and eluent volume. TCA selective retention in C18 disks was assessed through eluate analysis by HPLC-DAD at 282 nm.

Envisaging the future application in the wine industry, the developed detection device was used to determine TCA in cork stoppers. The device was adapted to a plastic bag containing cork stoppers spiked with TCA. The air of the bag was filtered by vacuum through the SPE C18 disk with immobilized ANS until there was no air left inside the bag. Subsequently, the device was disassembled, the disk was exposed to UV light and a digital image was captured. The visual comparison with a blank assay (cork stoppers not contaminated) permitted to conclude if there was fluorescence quenching of the ANS probe due to the presence of TCA.

The proposed strategy constitutes a valuable, simple and rapid alternative for TCA detection in cork stoppers. The whole procedure was completed after approximately 20 minutes. Values as low as 2.5 µg of TCA could be determined.

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## Sulfated coumarin glucosides: synthesis and anticoagulant activity

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The growing importance of natural sulfated molecules, as modulators of a number of physiological and pathological processes, has inspired the synthesis of non-natural sulfated scaffolds [1]. Until the 1990s, the synthesis of sulfated small molecules was almost restricted to derivatives of flavonoids and aimed mainly at structure elucidation and plant biosynthesis studies. Currently, the synthesis of sulfated compounds concerns structurally diverse scaffolds and is aimed at the development of potential drugs and/or exploitation of the biological effects of sulfated metabolites [1]. Particularly in the field of anticoagulant agents, some important sulfated flavonoids and other polyphenols are emerging [2,3].

Being coumarin a relevant scaffold for anticoagulant therapy (e.g. warfarin), the introduction of sulfated groups could be a promising strategy to obtain new coumarin derivatives with different mechanisms of action.

In this work, three new sulfated coumarins were obtained. Sulfation was accomplished with triethylamine-sulfur trioxide adduct, under microwave radiation. The structure elucidation of the synthesized compounds was established by IR, <sup>1</sup>H NMR and <sup>13</sup>C NMR. The anticoagulant activity was measured by the classical clotting times - activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) - in five different concentrations. The derivatives were found to prolong the APTT and PT in a dose-dependent manner, and no effect was observed on the thrombin time (TT).

Future work will consist in the investigation of the mechanism of action of these compounds on factor Xa.

Acknowledgements: This research was developed under the Project PEst-OE/SAU/UI4040/2014, and partially supported by the ERDF through COMPETE and FCT (PEst-C/MAR/LA0015/2013).

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# Synthesis of a sulfated flavonoid - deoxycholic acid conjugate as a potential orally-active antithrombotic agent

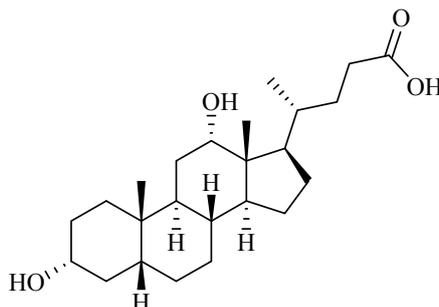
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# Master student of Pharmaceutical Chemistry, FFUP.

The search for new alternative anticoagulant agents with a well-defined composition, feasible synthesis, fewer secondary effects, and orally-active is one of the major challenges to medicinal chemists. In our research group, a new class of heparin-like antithrombotic agents, polysulfated glycosidic flavonoids, was identified [1]. Although they showed interesting anticoagulant activity, these sulfated small molecules were not active after oral administration. Deoxycholic acid (DOCA) (**Figure 1**), a bile acid recognized by specific receptors in the small intestine [2], was successfully used to improve oral bioavailability of heparin [3]. Conjugation of DOCA with the antithrombotic polysulfated flavonoids is expected to improve their oral bioavailability.



**Figure 4** – Deoxycholic acid (DOCA).

Conjugation of a natural flavonoid with DOCA was achieved through an esterification reaction using TBTU as coupling reagent. Following, sulfation was accomplished with triethylamine-sulfur trioxide adduct, under microwave radiation. The structure elucidation of the synthesized compounds was established by IR, <sup>1</sup>H NMR and <sup>13</sup>C NMR. Biological activity/permeability tests will be performed to compare antithrombotic activity and oral bioavailability of the conjugated with the nonconjugated sulfated flavonoid.

**Acknowledgements:** This research was developed under the Project PEst-OE/SAU/UI4040/2014, and partially supported by the ERDF through COMPETE and FCT (PEst-C/MAR/LA0015/2013).

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# Synthetic and natural dyes as models for bioactive compounds

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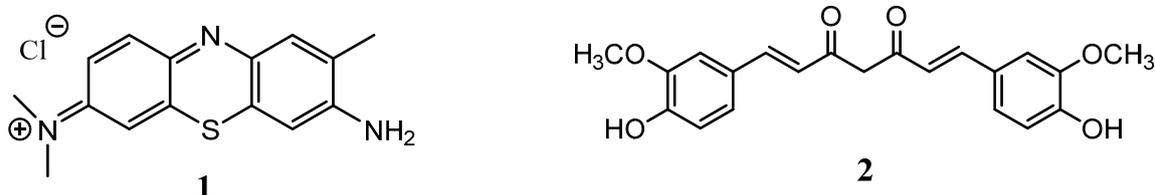
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Natural and synthetic dyes are used for many purposes, namely in fabrics industry and as analytic reagents, but one important field related with “dye molecules” is related with Medicinal Chemistry.

Some examples of the involvement of dyes in therapeutics is the methylene blue derivatives that originated compounds with antihistaminic, antipsychotic, antidepressant activities and *prontosil rubrum* as antibacterial activity.<sup>1</sup> Toluidine blue is a synthetic dye with application in a variety of fields: staining of cells and tissues, oral cancer screening and photodynamic therapy.<sup>2</sup> Curcumin is a natural dye that is undergoing through a lot of studies due to its many biological activities such as antioxidant and anti-inflammatory, but most importantly it is known to act as a multi-drug resistance modulator.<sup>3</sup>

In this communication, the syntheses of new derivatives of the dyes, toluidine blue (**1**) and curcumin (**2**) are presented (Fig. 1). The strategy of molecular modification concerning toluidine blue followed the formation of dimers using tartaric acid as a linker. As for curcumin the strategy followed the synthesis of more chemically stable analogues, particularly, with a piperidone moiety.



Preliminary results concerning the synthesis and structure elucidation by spectroscopic techniques of these new derivatives will be presented herein. Future work will consist in the biological investigation of these derivatives.

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# The safety of lipsticks: Results from a study on metals content in low cost products from de Portuguese market

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For safety reasons, a long list of metals and their compounds are prohibited by current EU Cosmetics Directive (76/768/EEC). However, due to their ubiquitous and persistent nature, the presence of metals at trace amounts in cosmetic products is considered virtually unavoidable, even under good manufacturing practices.

European regulation doesn't define quantitative limits for metal impurities in cosmetics (it simply requires that products are subjected to a careful safety assessment before being placed on the market). However, for the most toxicologically relevant metals (Pb, As, Cd, Hg, Sb), some national health authorities (e.g., Germany, Canada) have set limits above which levels in cosmetic products are seen as technically avoidable [1].

Lipsticks are widely used by most women and present special concern because of its higher potential for being ingested. At the end of the 2000s, several reports on the presence of Pb in lipsticks at levels that would pose safety concerns were published in the media and on the internet [2], and several surveys were performed, namely in de USA [3] and Europe [4].

In 2014, at our Laboratory, using an analytical procedure combining a microwave-assisted acid digestion for samples mineralization with inductively coupled plasma-mass spectrometry (ICP-MS) for elemental determination, we performed a work aiming to assess the metal contamination of lipsticks from the Portuguese market (n = 43; 7 different brands). Since the most known international brands have already been studied in the abovementioned European survey [4], the study focused on products manufactured in Portugal and low cost products available in the Portuguese market.

Overall, the results showed no reasons for safety concerns regarding the studied elements. In particular, results (median / max) for Pb, As, Cd, Hg and Sb were well below the Canadian limits (the most restrictive ones): Pb 0.77 ppm / 2.46 ppm [limit: 10 ppm]; As 0.10 ppm / 3.11 ppm [3 ppm], Cd 0.02 ppm / 0.97 ppm [3 ppm], Hg 0.01 ppm / 1.37 ppm [3 ppm] and Sb 0.08 ppm / 1.01 ppm [5 ppm].

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# Toxic metals in cigarettes from the Portuguese market

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Tobacco use is the leading cause of preventable death and disease worldwide. It is a major cause of cardiovascular disease, chronic obstructive lung disease and lung cancer. Globally, tobacco use is considered responsible for about 1/10 of adult death worldwide [1].

For 2015, the World Health Organization (WHO) estimates as about 20% the percentage of the Portuguese population aged 15 years and over (approximately 1,800,000 persons) with smoking habits [2].

Smoking-related diseases can be attributed to the inhalation of a long list of potentially harmful substances, including heavy metals, which have a host of well-known detrimental health effects. However, few studies were conducted on the role of heavy metals as causes of smoking-related diseases [3].

The metals most commonly associated with health effects are arsenic (As), cadmium (Cd), chromium (Cr), nickel (Ni) and lead (Pb). Arsenic and inorganic As compounds, Cd and its compounds, Cr (VI) compounds and Ni compounds are classified by the International Agency for Research on Cancer as “carcinogenic to humans” [4]. Those metals may also display relevant non-cancer toxicities affecting, namely, the cardiovascular, nervous and renal systems.

In this context, we conducted a study aimed to assess the metal content in the cigarettes of the most sold brands of the Portuguese market (n=19 different brands). Cigarette samples (n=38) were digested through a microwave-assisted acid digestion procedure in closed vessels. Sample solutions were analyzed for their content of As, Cd, Cr, Ni and Pb by inductively coupled plasma-mass spectrometry (ICP-MS).

The obtained results (mean±SD; maximum) were: As = 0.11±0.06; 0.36 µg/g; Cd = 0.92±0.72; 5.61 µg/g; Cr = 1.50±0.51; 2.72 µg/g; Ni = 1.93±0.45; 3.61 µg/g and Pb = 0.55±0.11; 0.97 µg/g. These results are in good agreement with the values found in cigarettes from the US market [3]. For a definite assessment of the effective contribution of heavy metals to the toxic and, particularly, to the carcinogenic potential of tobacco smoke, an accurate determination of the fraction of the metal present in cigarettes that actually passes to the smoke is necessary. This will be the next step of this study.

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# Zirconium based metal organic frameworks for heterogeneous desulfurization catalysis

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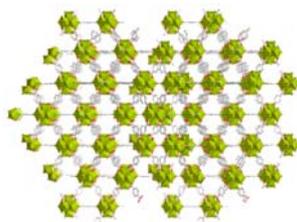
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Porous metal-organic frameworks (PMOFs) are crystalline materials build-up with metal centres interconnected by rigid organic molecules (ligands) to form extended structures that can be uni- (1D), bi- (2D) or tri-dimensional (3D).[1] The structural diversity of PMOFs has led to their application in a wide range of fields including gas storage, sensors and catalysis. [2] The coordination of Ln<sup>3+</sup> ions to lacunary units of polyoxometalates results in stable complexes with unique properties that have been extensively studied in a wide range of applications, especially in catalysis and luminescence.[3]

The work herein described involves the synthesis and posterior application in heterogeneous desulfurization catalysis of potential catalyst materials. The UiO-66 (Figure 1) and the NH<sub>2</sub>-UiO-66 MOFs were chosen due to their high surface area and remarkable chemical stability, being prepared by previously reported procedures. [4]

The incorporation of the [EuW<sub>10</sub>O<sub>36</sub>]<sup>-9</sup> anion within the framework of UiO-66 was also performed. There is an increasing interest in the design of heterogeneous systems using MOFs as solid supports for the immobilization of catalytically active species. The POM was chosen due to its peculiar luminescent features combined with catalytic activity. The characterization data obtained by several techniques (FTIR, XRD and SEM/EDS) point out to the successful preparation of the materials. The UiO-66 type materials were evaluated as heterogeneous desulfurization catalysts in the dibenzothiophene (DBT) oxidation using a green oxidant, H<sub>2</sub>O<sub>2</sub>.



**Fig 5.** Schematic representation of the UiO-66 framework.

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# AMAZON FOLKLORE: A PSYCHOANALYTIC READING THE LEGEND JURUPARI

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Among the many characteristics that make up the identity and the imagination of a people, are their folk tales. Thus, addressing the legends and other cultural events is to preserve the rich and diverse cultural and historical code of a population. Many of the mysteries that make up the Amazon and its people are in their legends. Psychoanalysis, since the time of Freud, always dialogue with the cultural events, showing what can be unconsciously such content, going beyond the obvious phenomena.

The objectives of this research were to highlight, from a psychoanalytic reading, unconscious contents of Jurupari legend and, in particular, try to describe the legend of Jurupari; analyze the selected legend light of Freudian theory; and finally redeem this legend in discussions about this culture.

In the methodology, Bardin's content analysis (1977), with the north the following: 1) The pre-analysis; 2) The material exploration and 3) treatment of results, inference and interpretation [1]. Because it is a qualitative research, produce results of subjective order and we realized to answer some questions about the psychoanalytic meaning of Jurupari legend.

We conclude that an Amazon cosmogony is updated when the approach to psychoanalysis. The redemption of our legends, stories, myths and fantasies allow us to symbolize the mysteries that surround our desires and impulses, to beyond the reason or science.

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# An organizational intervention's process: Implementation of a Card Wall

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This work focuses on a process of organizational development, in a division of a Portuguese local authority, and which resulted in the implementation of a *Card Wall*, in order to improve the workflow and enhance other tools and processes.

After identifying the need to enhance the process of conceptualization and implementation of training activities in the division; since its creation to of its execution's evaluation, the implementation of a *Card Wall* was planned. This tool which will be used by the team enables communication, sharing, decision-making and the reduction of constraints [1].

After dealing with the team's resistance to change during the tool's implementation, the use of accidental observation and the interaction with the team allowed to observe people willing to change. Specifically, the *Card Wall* enabled the emergence of a micromanagement [2] within the team due to the visibility that the work of all individuals gained [3]. However, the evaluation of the change's impact needs more time, as the project is still going on.

This implementation's process allows us to realize the importance of not neglecting the management and leadership planning activities during the change's implementation phase. Even when evaluating, at earlier stages, if people are open-minded regarding change. It also gives the chance to realize the simultaneous changes connected to the implementation of a given tool.

The main contribution of this work is the fact that it reflects the introduction of a change within a team in a bureaucratic organization, observing how it reacts and takes part in the development process and the strategies that can be embraced to manage resistance to change when it is not managed proactively.

**Keywords:** *Card Wall*, organizational development, resistance to change, communication, decision making.

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# Building the relationship between subordinates and supervisors: an exploratory study

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Little is known about the process of building a relation between subordinate and supervisor. The purpose of the given study is to identify critical incidents experienced by the subordinate while interacting with his direct supervisor, and that influence the process of building their relation. Its aim is also to explore in what way the attachment style can relate to the subordinate reactions in those critical incidents. Hence, we intend to answer the following research questions: (1) which events characterize the process of building a relation between supervisor and subordinate? (2) What is the subordinate's reaction to the supervisor's behaviour? And (3) what is the impact of those events in the relation between subordinate and supervisor? We adopted a qualitative approach with an exploratory and descriptive focus, and did interviews, applying the Critical Incidents Technique to 12 participants in a supervision relation. We also applied the Categorical Model of Assessing the Style of Adult Attachment (Hazan & Shaver, 1987). Results indicate three types of the supervisor's behaviour that characterize the relation, namely, caregiving behaviour, support in performing tasks and solving problems and authority abuse. The supervisor's behaviour sets different reactions in the subordinate, namely activating their care-seeking and self-defence systems, where the dominant behaviours are fight and freeze. Regarding the impact of those critical situations in the relation, it was shown that it has positive and negative consequences. The positive consequences lead to a higher closeness and trust towards the supervisor; the negative manifests in disappointment. The theoretical and practical implications associated with the obtained results are addressed, as well as the limitations of the study.

**Key words:** subordinate-supervisor relationship; attachment style; critical incidents

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# Changes on Mental Representations in patients of Psychodynamic therapy

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The present study aimed at verifying the physical development through a survey with teenagers between the ages of 12 and 18 who frequented the psychology service at a University in southern Brazil. The Inventory of Object Relations (ORI) was used to investigate the changes that occurred in the psychotherapeutic process [1]. This inventory evaluates the characteristics of the patient's self and object representations through a semi-structured interview, in which it was requested for the subject to describe each of the following individuals: his/her mother, his/her father, a friend or boyfriend/girlfriend, him/herself, the therapist and a pet. The ORI was applied at the beginning of the treatment and after four months of psychotherapy. The ORI is composed by the Differentiation-relationship scale (evaluates the capacity to represent the self and the object), the Conceptual Level (evaluates the description's structure of the self and the object) and the Thematic Analysis of verbalizations (evaluates the adjectives used to describe him/herself and the others) [1]. The interviews were carried out with two subjects. The results obtained in the first interviews regarding Differentiation-relationship scale show that, in general, patients were at more regressive levels of object differentiation. In the second description an evolution on the differentiation between self and object became clear [2]. In addition, regarding the conceptual level, it was noticed that the first interview patients's self and object descriptions were narrower, however, the second interview descriptions contained a wide range of physical features, activities and internal psychological dimensions[3]. The mental representation in adolescence is critical to monitor the therapeutic process. The patient's ability to represent him/herself and the others in a stable and continuous way can become a source of investigation related to the changes that occur in the autonomy and identity construction process. Therefore, the intervention should build a more cohesive identity [4]. Furthermore, it is possible to observe significant changes in the self and object representations when subjects undergo brief psychodynamic therapy.

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# FORENSIC PSYCHOLOGICAL ASSESSMENT IN BRAZIL: A SYSTEMATIC REVIEW OF THE LITERATURE

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Within Psychology, in Brazil, the Forensic Psychology is one of the most promising areas<sup>1</sup>. In this context, the psychologist exercise the role of expert and is notable the increased frequency with which have been called to perform that function, in order to make up for the inadequacy of Jurists with regard to technical knowledge<sup>2</sup>. Even understanding the psychological assessment does not involve strictly the use of psychological tests, the proponent study aimed to conduct a bibliographic survey about that most instruments used in forensic psychological evaluation in Brazil and are in favor of the CFP through the SATEPSI.

A systematic review was made through electronic search of scientific productions over the last 8 years in national database of Virtual Health Library – VHL such as: PEPSICO, SCIELO and LILACS. Found a total of 133 articles according to the search criteria, but have chosen for this study a total of 13.

The results show that there is a pattern of psychological assessment instruments for use in forensic context, that there are few instruments designed for this practice in Brazil and that there is little offer of good qualifications in this area. Also, the methods of evaluation depend on the demand, and the psychologist use observation and interview in conjunction with projective psychometric scales and instruments. Among the instruments that have been mentioned, the following are recognized as favorable for CFP: Factorial Range of Socialization; Factorial of Extraversion scale; Hare Psychopathy inventory and inventory of social skills. Worth pointing out that the Rorschach test that is unfavorable for the CFP was quoted considerably.

The misuse of instruments can result in consequences in the life of the subject that is being evaluated and in society. Therefore, it is of paramount importance to the field of Forensic Psychology developing instruments that support the specific demands of this context. This is a gap that can be filled by the highest bidder qualification, to the advances of studies in the area and the development of specific tests for this scenario.

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# **Metalinguistic awareness and Literacy: The Importance of Linguistic and Cognitive Activities Inclusion in Early Childhood Education and Elementary Education Home.**

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The linguistic and cognitive skills are highlighted in the Reference Matrix for Literacy Assessment (MEC, 2012), which subsidizes items of the Provinha Brazil. Contemporary research demonstrates that metalinguistic skills are fundamental to the acquisition and development of reading and writing, especially those involving phonological processing. Some studies support the view that specific activities in order to develop phonological awareness significantly increase the chance of success in learning the reading and writing and contribute to the advancement of children who are with difficulties. The aim of our study was to investigate the relationship between reading ability at the end of the first year of elementary school and phonological awareness, taking into account the knowledge of letters, from a preliminary study carried out in 2012.

The study included 49 children of the 1st grade of elementary school who underwent a reading test (adapted), a task of letter knowledge, and phonological awareness test.

Found great performance in task of letters knowledge, and correlations between the performances in reading and phonological awareness test, particularly between reading pseudo-words and words and performance in phonological awareness test, can be observed that the words-pseudo reading difficulties correlated to the difficulties in the more complex tasks of phonological awareness supports the idea that proficient reading depends on a strategy to process the composition of graphemes in the words, in order to convert the graphemes sequence in a sequence sound.

The reading level at the end of the school year was influenced by phonological awareness. The assessment of phonological awareness not only indicated the importance of the level of linguistic segmentation but also the cognitive level required by the nature of the task. In addition, there are some misunderstandings in the use and construction of activities for working with phonological awareness, thus requiring a look more careful about these activities.

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## **PENTECOSTAL CONVERSION IN BRAZILIANS PRISONS: THE DIVINE SUPEREGO**

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The Brazilian prison system is in crisis. According to the National Council of Justice and the National Penitentiary Department, Brazil has the third largest prison population in the world (about 500 thousand), only behind the United States (2,3 million) and China (1,7 million). Precarious, subhuman conditions, unsanitary, overcrowded, drugs, riots etc. are words that always appear when talking about the prisons in Brazil. Especially, the criminal recurrence is one of the most discussed and matters of concern that appears in the Brazilian prison system scenario. No consolidated data about recidivism in Brazil, it is estimated that their content is around 70%. In parallel, we see the growth of the Pentecostal movement in prisons.

This study is part of a research project conducted between 2012/2013, in which we seek to understand the representations of religiosity in the speeches of men serving time in prison in a closed system of the city of Manaus. In this survey, we made a psychoanalytic interpretation of speeches by ministers in services held in the prison in question.

The results show the possibility of Pentecostal discourse function as fierce and superego imperative, descendant of maternal and paternal complex of detainees. Finally, we question this Pentecostal discourse in prisons as a device that can promote the reintegration of the convict in the social bond.

# The innovation and involvement of employees: an organizational development project in a Portuguese company

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This work focuses an organizational intervention process intended to stimulate CORK.IN, the Amorim Revestimentos' idea generation program, due to the fact that its initial implementation in June 2012 did not present the expected results. The intervention took place mainly according to the integrated model of Cummings and Worley (2005) [1], which defines the process of organizational intervention in five stages from entering and hiring, to diagnosis, design and implementation of a plan of action, and evaluation. Since the beginning of the process it was stipulated the importance of involving various organizational stakeholders (e.g., management, direct supervisors, employees and the human resources department) throughout the all process, adopting a collaborative perspective in the pursuit of the success of the intervention.

The intervention was evaluated throughout time and at the end of the process (impact evaluation). Results show that most of the defined objectives were achieved, resulting in a general improvement in the program at various levels, namely the significant increase in the number of participations in the program, the improvement in the knowledge regarding the same, greater autonomy of the facilitators, improvement in the level of skills regarding the instruments used to submit ideas and a faster process of evaluation and implementation of submitted ideas.

It is possible to conclude the importance of doing organizational intervention according to a systematic process, managing inherent time constraints, highlighting the important role of the change agent and the effective management of some aspects, such as communication and workers' engagement, while assuring the autonomy of those involved. It is important to emphasize the benefits of initiatives as the CORK.IN program, since it allows the involvement of employees, who often do not have the opportunity to formalize their ideas for the development of the organization, that can bring significant gains to the company and have an impact on recognition of self-competence, on feeling of belonging with the company and motivation.

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# BRINQUE CEU-UNIFESP: Toy library, culture and the playful

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The project planning, implementation and management of university / community toy BRINQUE-UNIFESP / CEU, has as main objective the implementation and management of the first university toy geared toward children of the surrounding Peppers Neighborhood, Guarulhos in Sao Paulo. The specific objective is to provide a place for the development of the play culture, the right to play and socio-cultural relations between children, and insert Faculty of Education students in the management of a *Toy Library Academic*.

During the first deployment process (CEU - UNIFESP), was held cataloging 786 toys and 96 materials for various uses. The second stage of the project was to develop workshops for university students and teachers from the Guarulhos network.

It also indicated a greater participation and integration projects by other teachers of the campus Education area, in addition to students (the vast majority of Pedagogy) to develop curricular activities from the materials present in the Toy Library, namely: sound materials, sensory, docking, literature from various genres among others. With the availability of space for students and teachers there was a greater link between theory and practice, especially in developing didactic sequences for Early Childhood Education and Elementary Education, respecting the child's right to have their knowledge and experiences to their articulated their learning needs. Note also the reflections favored this Toy space about the teacher's role as a mediator of that knowledge, as an articulator of meanings to children. Thus, the continuity of design Toy library community/academic, have the effect of increased participation of students and teachers for both development projects involving theory / practice as research and extension projects in the area of early childhood education, the main pillars of the children cultures, child development and educational proposal linked to the child's vision for the mediation and investigation of the educator.

# Curricular expressiveness of the 1<sup>st</sup> cycle of basic education in Portugal

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The curriculum of the 1<sup>st</sup> cycle of basic education promotes the learning of socially relevant competences with a focus in four big disciplinary programming, Portuguese language, mathematics, the analysis of the means and the expressions. In school is defined the curricular priority, by the Public Education System convenience, exalting their interests. This attention, about the contents, should include the multiplicity of senses, emotions and experiences with the enrichment of young citizens, motivating them to the continuous search of feelings. The dignity that they've right, has as objective to ensure that they receive a general education common to everyone, since it concerns to their first four years of contact with the environment and different persons, with whom they' find their way to interact socially. We must question what are the essential, primary, general and common dimensions that may be explored and oriented by the teachers in the contact with the children, during their basic cycle.

This study intends to identify, in the teacher's perspective, which use is given to expressive dimensions in a 1<sup>st</sup> cycle child of State School's curriculum from urban and rural area, in curricular units with required attendance.

Understanding the condition of the artistic expressions with the curriculum and the processes of transformation that teachers passed are objects of study. The qualitative investigation method was used to involve the 1<sup>st</sup> cycle expressions and the problematics, having as goal their identification, promoting the improving of the practices. In this study, we had a document analysis and had the realistic confrontation of working teachers, through semi-structured interviews. The experience of teachers and their narratives are crossed references to curricular studies.

The results, until the moment, have not been completed because the research is under development.

## **Ethnic diversity in early childhood education: inequalities minimizing or spreading stereotypes?**

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Ethnic diversity is the basis for the work of early childhood education, as the socio-political function of this stage of basic education is based on respect, celebration and promotion of differences. In this sense, the official documents of the Ministry of Education, MEC, show is great value, and the reasons for directing practices of teachers.

The research objectives were to analyze the similarities and differences of a particular municipal kindergarten Municipal Center for Child Education - CMEI, with the National Curriculum Guidelines for Early Childhood Education – DCNEI's, to work with the theme of diversity ethnic front of the personal training process and social the child; and identify the views of teachers about the importance of ethnic diversity theme for the small child's socialization process in preschool space.

For data analysis, we used the phenomenological view, the concept of Marx Weber ethnicity; of ethnocentrism Everardo Rocha, historical analysis of the race term of Poutignat and Streiff Fenart and official documents of MEC, for didactic guidelines on ethnic diversity. The instruments used for data collection were observation and interview.

With the final results, we can consider that teachers of early childhood education are still working in the perspective of races, which leads to a theoretical mistake and thus practical. In official documents realize the guidance for the development of plurality, but at the same time working on race that valuation perspective and teachers still conceive the idea of humans rating. The view of teachers as the ethnic diversity in the studied institution, brings us to the myth of democracy established in Brazilian society, demonstrating that even with educational public policies to minimize inequalities, it is necessary to implement these policies determine what, so therefore we can work together for change actions and views about ethnic diversity.

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# Law of education and optional character of the sports practice in public schools of Maceió, Brazil

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This paper stems from the supervised training in teacher formation degree at the Federal University of Alagoas, Brazil, carried out among the students of Public Schools of the State of Alagoas. It was observed that the students of the schools did not apprehend the contents that were being demanded in the High School National Examination (HSNE), because apparently there was no Sport class for the students. The justification was found in the Law 9.394 / 96, which provides your practice. Thus, the study aimed to know what were the implications of the Law 9.394 / 96, in its optional character, art. 26. § 3 generated in State Public Schools in Maceio, regarding the realization of the High School National Examination (HSNE). It was qualitative and exploratory-descriptive research, where five state schools in the city of Maceio were investigated. To this end, five principals, 2 males and 3 females, formed the group of participants in this investigation. It was used as a tool for data collection semi-structured interview. So the data were interpreted through content analysis as suggested by Guerra (2006)[1]. The data interpretation suggests to us that with the insertion of Sports discipline in the HSNE, the law brought the possibility that students could take the exam without attending the course. The lack of consensus on the interpretation of the rule brought each school a different perspective of how it should be included or offered discipline in its curriculum, based on the culture, schedules and interests of students, generating an academic, professional and personnel loss to the whole school community.

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# Teaching Mathematics in the indigenous public schools in Manaus

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Despite the achievements in the area of specific and differentiated education for indigenous people in Brazil (Federal Constitution of 1988 and the Law of Guidelines and Bases for Education - LDB 9.394 / 96), recent studies focused on the Indigenous Education show that many people still have little schooling that converges with guaranteed by law, therefore the question arose: how the pedagogical proposals of the Municipal Department of Education Manaus - SEMED for indigenous education are being implemented in Indigenous Education Schools in respect the teaching of mathematics?

The research aimed to investigate the relationship between the pedagogical proposals SEMED-Manaus for indigenous education and its implementation for teaching mathematics.

Search owned qualitative, based on field research and case study. The instruments for data collection were systematic observation and structured with interviews and data analysis using the method of content analysis.

As final results, it is evident the lack of effective proposals by the SEMED regarding the teaching of mathematics; the sparse advice has led to the emptying of the principles of indigenous education (bilingualism, intercultural and communitarianism) which consequently brings the overvaluation of mainstream education in these communities.

Although teachers have knowledge of laws and official documents, the lack of a solid foundation for the development of discriminatory minimization related to traditional knowledge and others, advocates a practice arising from the wider society: the school as an area without real sense for the educating. So, we launched as a suggestion to redraft the pedagogical proposals SEMED in partnership with all communities served, from a focused research to meet existing concerns and problems in communities about school education.

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# Aerobiology of *Pinus* spp. and effects of different O<sub>3</sub> levels on pollen fertility, proteins and allergenicity

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Respiratory allergic diseases are increasing in both prevalence and severity in most industrialized countries, being linked, among other factors, to urban air pollution which increases airway responsiveness to aeroallergens [1].

This work aims to study the aerobiology and ozone's effect on *Pinus* spp. pollen viability, germination rate, protein content and allergenicity.

Airborne pollen monitoring was continuously performed during 2014, using a 7-day Hirst-type volumetric trap set on the roof of the Faculty of Sciences. Pollen grains were trapped on a Melinex tape coated with silicone oil, which was then cut into 7 daily segments and mounted on slides with glycerol jelly. The daily mean concentration of pollen was estimated using an optical microscope (x400) 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. *Pinus pinaster* L. pollen samples, collected directly from the tree, were exposed to O<sub>3</sub> in an environmental chamber, under artificial solar light, controlled temperature, and relative humidity [2]. Each pollen sample (150 mg) was exposed during 6h to two different concentrations: the hour-limit value acceptable for human health protection in Europe (0.06 ppm) (Directive 2008/50/EC) and around two times this value (0.11 ppm). Pollen fertility was assayed by viability (Trypan blue dye) and *in vitro* germination tests. Pollen total soluble proteins were quantified colorimetrically, the polypeptide profiles were determined by SDS-PAGE and the immunoreactivity was assessed by Western Blot using patient sera sensitized to pollen.

*Pinus* spp. highest airborne pollen concentrations were found from late March to mid April. There was a decrease in viability, germination rate and total soluble proteins in pollen exposed to both O<sub>3</sub> concentrations tested. The polypeptide profiles did not show differences however it was observed an increase in sera IgE reactivity for pollen exposed to 0.06 ppm but a decrease when exposed to the highest gas level.

Our results indicate that airborne pollen fertility and sera IgE reactivity can be influenced by atmospheric pollution.

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## ***Anemonia sulcata*: more than a sea anemone?**

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Plants and microorganisms have been targeted in intensive investigation on the search of new bioactive molecules. Nevertheless, in the recent years we have witnessed a growing demand on marine biomolecules. In fact, marine environment serves as reservoir for many species with unimaginable biological potential.

In this work, an anemone, *Anemonia sulcata* Pennant, was studied. This species is present in the northeastern Atlantic, Mediterranean and English Channel. The attention was focused on an ethanol extract prepared from this marine invertebrate, namely on its chemical composition and bioactivity.

The analysis of this extract by HPLC-DAD allowed the identification of several carotenoids (Fig.1). GC-FID revealed the presence of saturated and unsaturated fatty acids.

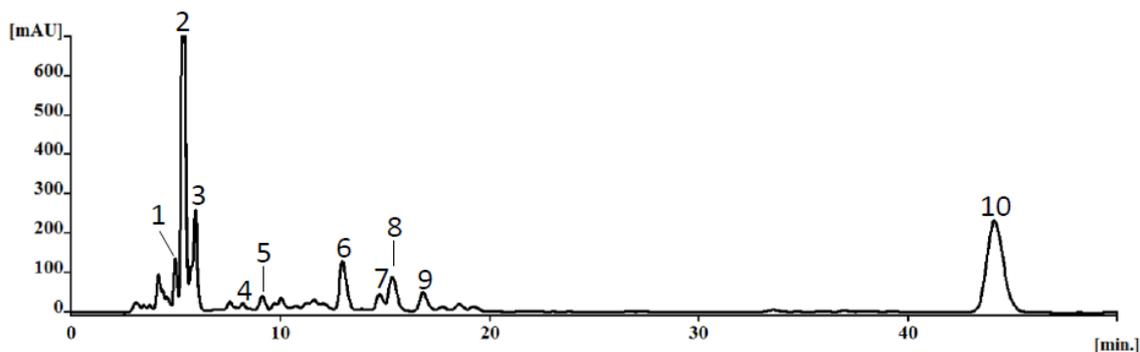


Fig. 1. HPLC-DAD chromatogram of the ethanol extract of *A. sulcata*. Detection at 450 nm. Peaks: fucoxanthin (1); unidentified xanthophyll (2 and 3); violaxanthin (4); unidentified carotenoid (5, 7 and 8); lutein (6); chlorophyll *a* isomer (9 and 10).

A reduced antiradical capacity was noticed against both DPPH• radical and nitric oxide. No effect was observed against selected Gram+ and Gram- species.

Further studies with other kinds of extracts and addressing other activities should be performed, to give a more complete idea of the potential of this anemone.

This work was developed within the optional curricular unit “**Bioactivity of Natural Matrices**” of the 5th 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 David Pereira.

# Antimicrobial potential of marine bacteria isolated from macroalgae

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In their evolution, macroalgae have been colonized by a variety of microorganisms, including bacteria. Therefore, it is not surprising that a complex array of interactions has evolved between macroalgae and bacteria which basically depends on chemical interactions of various kinds. Marine secondary metabolites are key bioactive compounds responsible for the mediation of ecological interactions between organisms and their immediate surroundings. As bioactive natural products have important biochemical properties they have been used in the treatment of several diseases like bacterial infections and cancer. This study aims to evaluate the capacity of bacteria isolated from *Ulva* sp., *Porphyra dioica* and *Sargassum muticum*. to produce secondary metabolites by molecular analyses of the polyketide I synthases (PKS-I) and nonribosomal peptide synthetases (NRPSs) genes. 227 bacteria were isolated from macroalgae sampled in autumn, in Porto, Portugal (41.153680 N, -8.679332 W 41° 18' North, 8° 44' West 41°09'N, 8°40'W). Potential PKS-I genes were detected in 15 *Phorphyra dioica* strains, 34 in *Ulva* sp. and 30 in *Sargassum muticum*. Presence of NRPSs gene was confirmed in 17 strains *Phorphyra dioica*, 26 in *Ulva* sp. and 7 in *Sargassum muticum*. These results show the potential of the epiphytic bacteria associated with these macroalgae as producers of bioactive compounds.

## Application of cross-species microsatellite markers in sexual selection studies with *Gammarus locusta* L.

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*Gammarus locusta* L. is a species with a relatively short life cycle (about 4 weeks) and a highly pronounced sexual dimorphism. Besides these features, it is a species that can be maintained and reproduced in laboratory, which makes it a great model for sexual selection studies. However, to do so, it is necessary to identify the individuals and establish the genetic relationship among them, which is achieved using molecular markers. In previous studies, several microsatellite markers were developed for *Gammarus fossarum*[1] and *Gammarus roeselii*[2], however there are no known microsatellite developed for *Gammarus locusta* L.

The current work primarily aimed to develop a set of markers that could be used in *Gammarus locusta*. In order to achieve that, we tested a wide range of primers that had been previously used in the species mentioned above, and applied them to *G. locusta*. After optimizing the DNA extraction protocol, we selected fourteen primers designed for *G. fossarum* and *G. roeselii* to be optimized through polymerase chain reactions, testing them at different conditions. Out of the fourteen, eleven worked and were improved through changes in MgCl<sub>2</sub> concentrations. Our preliminary results indicate that six primers showed great potential in *G. locusta* with enough variation for individual assignment and to establish genetic relationships among the individuals. We will further study the *G. locusta* mating system and sexual selection using the developed microsatellites.

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# Arginase 1 and iNOS expression at the fetomaternal interface during *Toxoplasma gondii* infection

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*Toxoplasma gondii* (*T. gondii*), is considered to be the world's most successful zoonotic parasite, causing congenital toxoplasmosis, the second most common fetal intrauterine infection, leading to toxoplasmosis, often causing neonatal mortality, spontaneous abortion and blindness [1]. In the last years, scientific breakthroughs have been made in understanding the immune response to toxoplasmosis, but there is still lack knowledge about which immune players are responsible for the pathology associated with this infection during pregnancy, specifically at the maternal-fetal interface. Macrophages have a variety of activation states, being able to adapt their functions to environmental changes of cytokines, described as innate (TLR ligation), classical/M1 (TLR ligation with IFN-gamma) or alternative/M2 (IL-4/Ralpha ligation) [2]. Both human and animal studies support that aspects of both classical and alternative activation occur in macrophages responding to *T. gondii*. Thus, while classical macrophage activation is able to control replication through induction of inducible nitric oxide synthase (iNOS), alternative activation can control parasite replication through induction of arginase 1 (Arg-1) and depletion of arginine [3].

Our study focused on the effects of *T. gondii* infection on Arg-1 and iNOS expression at the maternal-fetal interface using the mice model. Infection of BALB/c and C57Bl/6 mice, resistant and susceptible strains respectively to *T. gondii* infection and the use of a type II strain of *T. gondii* allowed the follow-up of pregnancy. *In vivo* infection was performed using viable tachyzoites obtained from *in vitro* infected-confluent human foreskin fibroblasts. Pregnancy outcome was evaluated both in infected and control animals by the quantification of normal and resorbed implantation sites. Morphometric analysis of decidua and placenta was performed using hematoxylin-eosin sections of the fetoplacental units. The evaluation of parasite loads in the organs was done by quantitative Real Time-PCR using Taqman probes (qRT-PCR). Arg-1 and iNOS protein expression was evaluated by immunohistochemistry.

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# Attempting to establish gastric cancer multidrug resistant cell lines

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The incidence of gastric cancer is high in the Portuguese population<sup>1</sup>. Treatment of gastric cancer usually consists in surgery, chemotherapy, radiotherapy, chemoradiation or targeted therapy<sup>2</sup>. Most patients with advanced stages of gastric cancer require chemotherapy with drugs such as cisplatin or fluorouracil, which have been used in the treatment of gastric cancer for decades<sup>2</sup>. One of the greatest obstacles to effective chemotherapy is the development of drug resistance, including multidrug resistance (MDR)<sup>3</sup>.

MDR consists in cellular cross-resistance to a range of structurally and functionally unrelated compounds and may be caused by different mechanism such as inadequate drug uptake into cancer cells, activation of detoxifying systems, escape from drug-induced apoptosis or increased drug efflux<sup>3</sup>. Even though the etiology of MDR is multifactorial, the classic resistance to the cytotoxic drugs mentioned above has most often been linked to the overexpression of P-glycoprotein (P-gp), causing increased drug efflux from the cancer cells. Inhibition P-gp as a method to reverse MDR in cancer patients has been extensively attempted, but the results have so far been disappointing<sup>4</sup>.

The aim of this study is to develop models of MDR gastric cancer in order to allow future identification of compounds to counteract this problem.

Cells from the AGS epithelial cell line (human gastric adenocarcinoma) are currently being cultured at 37°C in a 5% CO<sub>2</sub> humidified incubator and maintained in RPMI medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and with increasing concentrations of doxorubicin.

Further continuation of the selection process should provide multidrug resistant cells which will provide an ideal model to study multidrug resistance and identify novel compounds to counteract this problem. In addition, selection with different drugs such as cisplatin or fluorouracil will be conducted.

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# Bacterial resistance to antibiotics in faecal samples of cattle in North of Portugal

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The evaluation of the impact of livestock systems in earth microbial pollution reveals to be an important subject [1] due to the presence of antibiotic resistant microorganisms in bovine cattle feces. The concept of “One Health” must be highlighted, due to the worries about antibiotic bacterial resistance, its influence on the different ecosystems and, consequently, how it affects public health [2].

This is an exploratory study. Twelve faecal bovine samples were collected from one production for meat in the North Portugal. After collection, enrichment was performed with the medium Trypticase Soy Broth (TSB), in 2g of bovine cattle feces, and other enrichment was done in the same way but under pressure with addition of cefotaxime (CTX) 30µg disk. After overnight incubation at 37°C, isolate selection in MacConkey agar with antibiotics: cefotaxime meropenem, ciprofloxacin and tetracycline (TT). Susceptibility to different antibiotics was tested, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [3].

Twelve samples were submitted to this study and 8 of them presented results, selecting 23 gram negative isolates in total. All of them were evaluated according to the antibiotic resistance for lactose fermenting bacteria, corresponding to 10 isolates, and for lactose non-fermenting, corresponding to 13 isolates. In the first case, is possible to see that 100% of the isolates are TT resistant and this data can be corroborated by the detection of this trend in other studies [1]. Seventy percent are resistant to amoxicillin and 30% to amoxicillin plus clavulanic acid. In the second case, the lactose non-fermenters, was observed that 69,2% of this isolates are resistant to one or more beta-lactam antibiotics class and 61,5% are resistant to nitrofurantoin (F) [3].

The results showed 66% of samples resistant to one or more antibiotics used in selection. For lactose fermenting isolates, 2 isolates showed multi-drug resistance. This study should be continued in order to get consistent information about bacterial resistance in the fecal flora of bovine in Portugal.

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## Bioactivity assays of marine *Actinobacteria*

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The extensive Portuguese coast line possesses a rich marine biodiversity, which comprehends microbial communities including bacteria of phyla like *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Firmicutes* and *Planctomycetes*. Marine bacteria, especially *Actinobacteria* [1], are well known for their ability to produce bioactive compounds as first line defence against natural competitors. These natural molecules are secondary metabolites possessing many valuable bioactivities with pharmacological interest [2,3]. In this study, the effects of the bioactive compounds produced by *Actinobacteria* isolated from *Erylus* sponges [2,4] have been assessed using genetically modified Reporter *Bacillus subtilis* 186 [5-7].

Previous screening assays allowed the selection of strains with antimicrobial activity. To study the mechanisms of action of the antimicrobials produced, *Actinobacteria* extracts were assayed against four reporters' *B. subtilis* with the promoters *yorB*, *ypuA*, *yvgS* and *yheI*. These reporters signal the presence of inhibitors of DNA, cell wall, RNA and protein synthesis respectively. Signalling of inhibitors was obtained with 3 and 5-days aqueous and acetone extracts of *Dermacoccus* sp. (#91\_17), *Microbacterium phyllosphaerae* (#91\_35), *Microbacterium foliorum* (#91\_37), *Gordonia* sp. (B02\_22.2 and B02\_78), *Agrococcus* sp. (SM 115 and SM 116) and *Microbacterium* sp. (B02\_79, B02\_79a and B01\_119c). These encouraging results stimulate further studies to understand the mechanisms of action behind the bioactivities of these *Actinobacteria*.

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# “Browning” of White Adipocytes: Melanocortins as Essential Players

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White adipose tissue (WAT) is specialized in energy storage whereas brown adipose tissue (BAT) burns fat to produce heat via an uncoupled metabolism [1]. The discovery of beige or brown-like cells within WAT [2] set off the conversion of white into brown adipocytes as an attractive option in the management of obesity. Beige adipocytes arise by transdifferentiation of white adipocytes when subjected to certain conditions, such as chronic  $\beta$ -adrenergic stimulation and exposure to cold. It is known that the melanocortin neuropeptides have a lipolytic effect in white adipocytes [3] and promote thermogenesis in brown adipocytes [4], but it was not yet described their role in the transdifferentiation of white to beige/brown adipocytes, and that was the aim of this study.

For this, 3T3-L1 adipocytes were stimulated with the melanocortin alpha-MSH and the expression of the browning hallmark genes uncoupling protein 1 (UCP-1) and peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 $\alpha$ ) and the WAT characteristic gene resistin was measured through real time PCR. It was observed an upregulated expression of UCP-1 and PGC-1 $\alpha$  and a decreased expression of resistin mRNA. To evaluate mitochondria biogenesis, which is increased in brown adipocytes, the binding of the fluorochrome 10-N-nonyl acridine orange to mitochondria membrane was measured. Data revealed that 3T3-L1 adipocytes, stimulated with alpha-MSH for 24h, present a significantly higher content of mitochondria. In accordance, oxygen consumption, measured in a Clark-type electrode, indicated that melanocortin treatment increases the total oxygen consumption rates (OCR) and improves uncoupled OCR in 3T3-L1. These results are in accordance with a higher UCP1 expression. In conclusion, the melanocortin alpha-MSH induces beige/brown adipocyte characteristics in 3T3-L1 adipocytes.

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# Characterisation of Potentially Allergenic Proteins of *Acer negundo* pollen – molecular cloning, expression and purification

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Previous studies performed in Porto, Portugal, revealed a correlation between production peaks of potentially allergenic pollen and maximum hospital emergency admissions for asthma- or dyspnea-related respiratory diseases. *Acer negundo* is an ornamental tree predominant on the streets of the city and it has been verified a hypersensitization to proteins of *A. negundo* pollen [1]. Two potentially allergenic proteins were later identified - profilin and calreticulin. Profilins are highly conserved due to their role in cytoskeleton formation, have low molecular weight (12-15 kDa) and promote cross reactivity [2]. Calreticulin is a molecular chaperone of 46-60 kDa involved in protein folding and Ca<sup>2+</sup> homeostasis, predominant in the endoplasmic reticulum, highly conserved and ubiquitously expressed in all eukaryotic organisms. Due to profilin's and calreticulin's important roles in subcellular processes and the lack of information about their allergenic potential, this study aims at isolating their coding sequences, in order to produce and purify recombinant protein, and to verify and characterize their allergenic profile. Degenerate primers were designed using known partially conserved sequences for both proteins, followed by corresponding amplification of 5'- and 3'- cDNA ends by RACE techniques and the design of *A. negundo* specific primers to obtain complete sequences. Complete and partial cDNAs were obtained for profilin and calreticulin respectively and it was possible to produce and purify both recombinant proteins from a prokaryotic expression system, recognizable by both anti-profilin and anti-calreticulin specific polyclonal antibodies, and sensitized patient sera, hinting at an allergenic potential of both proteins. Further studies will ascertain if there are any changes in protein sorting or expression levels when the plant grows under stress conditions (such as air pollution, water stress, etc.) that might affect pollen allergenicity.

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# Antifungal susceptibility testing of clinical and environmental isolates of *Aspergillus fumigatus*

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During the last few decades the incidence of invasive fungal infections (IFI) has increased dramatically, especially in immunocompromised and critically ill hospitalized patients [1]. Invasive Aspergillosis (IA) has been increasing, particularly in patients who underwent bone marrow or solid-organ transplantation. Presently, IA is one of the most important causes of mortality in these cases, even after the implementation of antifungal therapeutic. The primary cause of IA is spores inhalation present in environment, being *Aspergillus fumigatus* the most common agent of IA. Considering the three classes of antifungal drugs in use against IA, actually the first line therapy recommendation is voriconazole, while amphotericin B, posaconazole, itraconazole and echinocandins are alternatives [2]. However, one of the current problems is the appearance of some drug resistance, particularly to the azoles, in clinical and environmental isolates.

The aim of the present work was to evaluate the susceptibility profile of *A. fumigatus* using Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution protocol M38-A2 [3]. Minimal inhibitory concentrations (MICs) and minimal effective concentrations (MECs) were measured for amphotericin B, the triazoles voriconazole, itraconazole and posaconazole, and the echinocandins caspofungin and anidulafungin. Forty isolates of *A. fumigatus*, obtained from respiratory and air samples, were tested.

Although categorization of filamentous fungi susceptibility is not established yet, according to the working breakpoints published in M38-A2 [3] the isolates were susceptible to the tested drugs, with one exception. One strain obtained from a biological sample showed resistance to azoles. Moreover, the environment isolates studied did not show resistance to any of the antifungal tested.

The results obtained for the first forty isolates of this study cannot prove correlation for the resistance to azoles between the environmental isolates and fungi infection with clinical occurrence. However, the antifungal susceptibility testing of *A. fumigatus* will continue and the resistance mechanisms will be study by molecular methods.

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## Antioxidant activity of polar extracts from different botanical parts of *Coleostephus myconis*

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Under stress, human body produce more reactive oxygen and nitrogen species (ROS/RNS) than enzymatic antioxidants and non-enzymatic antioxidants (*e.g.*, vitamin C, vitamin E, glutathione, carotenoids, and flavonoids), causing an imbalance that may lead to cell damage and health problems [1]. Antioxidants can block the complex networks of oxidative damage pathways at different levels, yielding an overall therapeutic effect. Consequently, antioxidants that are exogenously acquired from diet may have important functions in redox cell homeostasis and can be important for cellular function and disease prevention [2]. Antioxidant compounds might be isolated and characterized from different plant parts like roots, stem, bark, leaves, flowers, fruits and seeds [3].

Herein, different polar extracts (water, ethanol and water:ethanol 1:1) obtained from the green parts and three floral stages of *Coleostephus myconis* (*Asteraceae*) were evaluated for their antioxidant activity, using different *in vitro* chemical and biochemical assays. All the studied botanical parts showed high antioxidant potential, either considering their reducing power, radical scavenging activity and lipid peroxidation inhibition. Independently of the studied botanical part or antioxidant assay, the hydro-alcoholic extract gave the best results. Furthermore, significant differences were detected among the green parts and each of the flowering stages. In conclusion, the results highlighted the possibility of using *C. myconis* as a potential source of bioactive compounds with several possible application.

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## Carbapenem resistant *Pseudomonas* spp. in clinical isolates from a hospital in the Minho region

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*Pseudomonas* spp. are Gram negative bacilli considered important nosocomial opportunistic pathogens, causing infections in immunocompromised or hospitalized patients and rarely affecting healthy people<sup>1</sup>. Production of carbapenemases is the most important resistance mechanism against carbapenems in *Pseudomonas* spp.

The aim of this study is the characterization of carbapenem resistant *Pseudomonas* spp isolates, collected from June 2014 to March 2015 from different biological products and hospital wards, from a hospital in the northern region of Portugal. We analyzed 93 pre-selected carbapenem resistant *Pseudomonas* spp with resistance to at least one carbapenem (imipenem and/or meropenem), according to the hospital information. Antimicrobial susceptibility tests were performed by disk diffusion methods according to the CLSI guidelines in order to confirm resistance to carbapenems (imipenem, meropenem and doripenem). Carbapenemases were screened by the Blue-Carba test, EDTA disk test, Carbapenem Inactivation Method (CIM) and PCR for detection of *bla*<sub>VIM</sub>, *bla*<sub>NDM</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>KPC</sub> and *bla*<sub>IMP</sub>. Fifty seven percent of these pre-selected carbapenem resistant isolates showed resistance to imipenem, 88% are resistant to meropenem, and 66% are resistant to doripenem. Six of the 93 isolates revealed the presence of *bla*<sub>VIM</sub> metallocarbapenemase, all of which had positive results with the Blue-carba and CIM tests.

This study demonstrates VIM producing *Pseudomonas* spp in clinical isolates from a hospital in the Minho region. Our findings are in accordance with other studies performed in acute care hospitals, which demonstrate that VIM is the dominant  $\beta$ -lactamase implicated in carbapenem resistance in *Pseudomonas* spp. It represents an important threat, as it is the main carbapenemase associated with *Pseudomonas* nosocomial outbreaks<sup>2</sup>. Multi-drug resistant (MDR) bacteria are increasing worldwide, both in clinical setting and in the community, with immunocompromised patients functioning as true reservoirs of multiple resistances. This increases the probability of resistance mechanism dissemination across bacteria from different species<sup>1</sup>. The absence of new agents for the treatment of infections caused by these bacteria may lead to treatment failures with increased morbidity and mortality, making their presence in the hospital environment a worldwide concern.

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# Carboxymethylation of flavonoids: synthesis, purification and structure elucidation

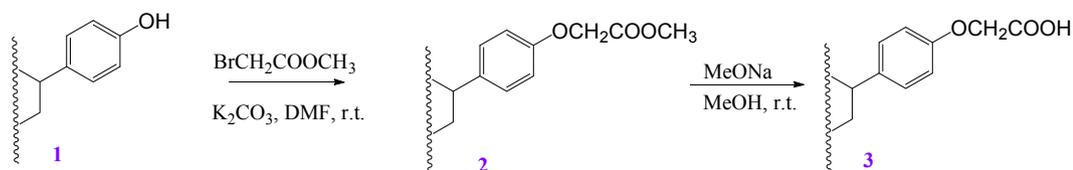
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Bifunctional linkers are generally used in medicinal chemistry for various conjugation applications [1]. In a project involving the synthesis of bioactive flavonoids -bile acid conjugates to improve flavonoids oral bioavailability, bromoacetic acid was selected as a bifunctional linker to accomplish the coupling of both bioactive compounds with the use of EDC/NHS chemistry (**1**, Figure 1).



**Figure 6** – Reaction conditions used to obtain compounds **2** and **3**.

To avoid undesirable lateral reactions, the protected carbomethoxymethylene group is firstly introduced in a phenol group of the desirable target molecule following a deprotection of the linker in basic conditions [2]. The representative flavonoid **1** reacted with methylbromoacetate at room temperature, for 48 hours. After this period, compound **2** was isolated by flash chromatography. The deprotected compound **3** was obtained after treatment of compound **2** with MeONa solution, at r.t. The product was neutralised using a cationic exchange solid phase extraction column. The structure elucidation of the synthesized compounds **2** and **3** was established by IR, <sup>1</sup>H NMR and <sup>13</sup>C NMR.

Future studies will involve the coupling of compound **3** with the bile acid deoxycholic acid by EDC/NHS chemistry to investigate the potential oral bioavailability of the conjugate.

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# Caregivers: a theoretical and reflective description

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The caregiver is the support in the attention to the person with some dependence. As consequences, often demonstrates ambivalent feelings: stress, fatigue and exhaustion. Other times they have positive relationships with gratification personal, social benefit and the quality care [1]. This paper to aims theoretical reflection about the caregivers' definition. The research was an analysis of documents [2], the theoretical-reflective type. We conducted a literature review, based on descriptor: "caregiver\*". We selected 13 full papers in language Portuguese and deleted the texts that were not in the area of nursing science. The results were grouped in a conceptual framework (Fig. 1) that had the function to know: How the concept has been referred to in scientific production?

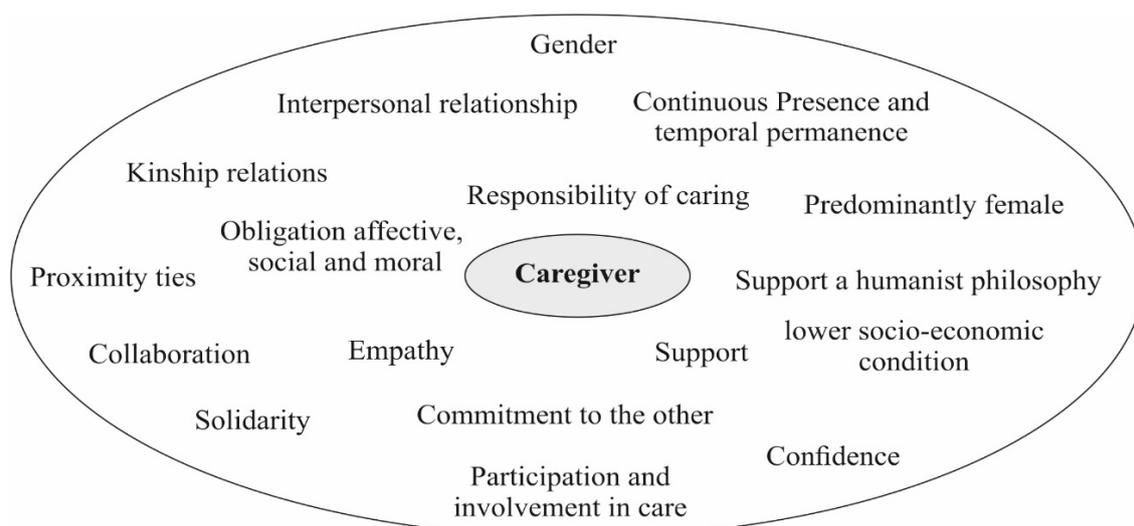


Fig. 1 Attribute of the caregiver

The caregiver was defined as the person or group of people that in face of human frailty, physical, emotional and/or social, through an interpersonal relationship of proximity or professional, with emotional bond or labor, they are available to the other, taking the responsibility of care, continuously or permanently. The concept was found coherence and consistency in the application in the context of nursing and international reference' taxonomy due to similar definition to the International Classification for Nursing Practice (ICNP).

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## Characterization of the semen microbiome in infertility cases and controls

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Infertility is defined as the inability of a couple to conceive after 1-2 years of regular sexual intercourse.<sup>1</sup> Abnormal semen parameters including low spermatozoa number, reduced motility, altered morphology and increased viscosity are different phenotypes associated to male infertility.<sup>2</sup> Sexually transmitted diseases (STDs) are recognized as one of the multiple causes for male infertility and often these are asymptomatic, undiagnosed and untreated. Up till now, only a limited number of studies have attempted to address the impact of STDs in the aetiology of male infertility.<sup>2,3</sup> Therefore, in this study, we propose to evaluate the semen microbiome of cases with different infertility phenotypes (N=127) and controls (N=54). In a first step, we extracted DNA from semen samples and assessed by PCR the presence of 7 common STDs agents (HPV, HSV1, HSV2, *Chlamydia*, *Ureaplasma*, *Mycoplasma* and *Neisseria*). The most prevalent STD agents in our cohort were HPV (31% in cases and 28% in controls), *Chlamydia* (28% in cases and 37% in controls), and *Ureaplasma* (14% in cases and 11% in controls). However, none of these results reached statistical significance. Regarding *Neisseria* agent this was only identified in 2% of cases and no positive results were obtained for *Mycoplasma* or HSV1/2. In the end 18% of cases and 15% of controls were confirmed to be infected by at least 2 STD agents. Currently, we are exploring the occurrence of additional bacterial strains in the semen by cloning and sequencing V3-V6 hypervariable region of the 16S ribosomal gene. So far, our results have shown that the most abundant genus in the control samples is *Enterococcus*, while in cases, a large variety of bacteria is observed, including *Prevotella*, *Gardnerella* and *Aerococcus* genus. In near future, we plan to use high-throughput technologies to extend our analysis of the 16S gene in order to increment the resolution of the characterization of semen microbiome in infertile men.

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## Controlled release tablets of a water-soluble drug with Carbopol® 974 prepared by wet granulation

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Formulation and manufacturing of oral controlled release matrix tablets are well known and established processes that result in highly reproducible controlled drug delivery [1]. Hydrophilic matrices are one of the most interesting drug delivery systems, and they are currently some of the most widely used to control drug release [2].

The purpose of this work was to develop and characterize tablets with thiamine hydrochloride (vitamin B1), used as a model of a soluble drug, containing Carbopol® 974 NF polymer (hydrophilic matrix) to obtain a sustained release.

A specific formulation (batch of 100 g) was developed, containing thiamine hydrochloride (10 mg/tablet), Carbopol® 974 NF polymer (51 %, w/w), Avicel® PH-102 (microcrystalline cellulose), Emcompress® (dibasic calcium phosphate dihydrate), talc and magnesium stearate [3]. Tablets were prepared by wet granulation using a single punch compression machine (Korsch 9048-71). As granulating liquid, a polyvinylpyrrolidone alcoholic solution was used (30 %, w/w). Afterwards, weight uniformity (mean  $\pm$  standard deviation (SD), n = 20, analytical balance Mettler AE 200, Mettler Toledo), hardness (mean  $\pm$  SD, n = 10, tablet hardness tester Erweka TBH 28, Erweka GmbH) and friability (n = 12, 25 rpm/4 min, friabilator EF-1W, Electrolab) were evaluated in the obtained tablets [4]. Moreover, the “in vitro” drug release study (mean  $\pm$  SD, n = 2) was performed in a dissolution apparatus Sotax (model AT7) according to the paddle method at 100 rpm [4]. The dissolution medium used was 500 mL of water at  $37.0 \pm 0.5$  °C. Aliquots of 10.0 mL were collected after 15, 30, 45, 60 and 90 minutes (without volume replacement). Samples were filtered and drug concentration was determined using an UV-VIS spectrophotometer Jasco (model V-650) at 273 nm (isosbestic point of thiamine hydrochloride). A vitamin B1 calibration curve was previously prepared ( $y = 24.627x - 0.020$ ;  $R^2 = 0.997$ ).

The outcomes showed that the produced batch presented uniformity of weight, a hardness average of 87 N and a friability percentage of 1.0 % [4]. *In vitro* dissolution test showed at 90 minutes a mean thiamine hydrochloride release of 45.2%.

Summarily, the present study demonstrates that the produced tablets showed suitable mechanical characteristics (hardness and friability), uniformity of weight and sustained release of vitamin B1.

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# Dasatinib as an option to treat P-cadherin overexpressing poor prognosis Breast Cancer

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The treatment of triple-negative breast cancer (TNBC) is particularly challenging because tumours lack validated molecular targets [1]. TNBC includes the worse prognosis basal-like subgroup, which is characterized by the overexpression of basal markers, like CK5 and P-cadherin. With the aim of studying if P-cadherin could be a molecular target in TNBC, we addressed its role in breast cancer. In fact, P-cadherin is overexpressed in more than 50% of TNBC, being significantly associated to poor patient survival. *In vitro*, it increases cell invasion capacity, cell migration, confers stem cell properties and promotes the metastatic ability of cancer cells [2]. Till now, there are no drugs targeting P-cadherin, and as so targeting its intracellular pathway can be an interesting therapeutic alternative. In fact, our group has recently described a downstream signalling activated by P-cadherin expression - Src family kinase (SFK). SFK activation is highly associated with cancer, responsible for potentiating proliferation, migration, and survival of neoplastic cells [3].

Taking these into account, our aim was to evaluate, the *in vivo* effect of an FDA approved SFK inhibitor, Dasatinib, for the treatment of P-cadherin-overexpressing TNBC cells.

For that, female nude mice were inoculated with P-cadherin overexpressing cells (SUM149PT, BT20 and MDA-MB-468) in the mammary fat pad. After tumors were visible, the mice were randomly divided in two groups (control and dasatinib treated). Dasatinib was orally administered, during 5 days per week, and primary tumours were removed by surgery when they reached an average volume of 750-1000 mm<sup>3</sup>. All mice from each experiment were euthanized as soon as they presented weight loss superior to 20%, any clinical sign of pain and stress, or if tumour growth was clearly impacting mice suffering.

Our preliminary data show that MDA-MB-468 tumors treated with Dasatinib presented a decrease in tumor growth. However, no differences were observed in the other cell models. Interestingly, a decrease tendency in the recurrence time was seen in Dasatinib treated animals. Moreover, macroscopic analysis of lungs from sacrificed mice show evident decrease in lung metastization in Dasatinib treated groups.

Although this is an ongoing study, our preliminary results point to a possible and important effect of Dasatinib in P-cadherin highly aggressive TNBC cells.

Acknowledgments: This work was supported by Laço's Grant 2014

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# Development and characterization of proliposomes as potential drug carriers

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Liposomes are one of the most studied drug carriers due to their biocompatibility and biodegradability. However, its potential application as therapeutic agent is still challenged by its physical and chemical instabilities in aqueous dispersions for long-term storage. Proliposomes are dry, free-flowing particles that can immediately form a liposomal suspension when in contact with water [1]. Compared with conventional liposomes, proliposomes exhibit higher physical stability and have a great potential to be exploited in different routes of administration [2]. Lyophilization is a promising approach to ensure the long-term stability of liposomes [3] and this technique also allows obtaining proliposomes.

In this work, proliposomes were produced by the method of film deposition on carrier [4], using formulations with different molar ratios of phosphatidylcholine and cholesterol. Mannitol was the carrier chosen in the formulation. The proliposomes were hydrated and the zeta potential and particle size of the resulting liposomes were evaluated. The formulation with a molar ratio of phosphatidylcholine and cholesterol 3:1 showed the best results, therefore was the formulation chosen for the production of proliposomes by lyophilization. The proliposomes produced by this methodology were analyzed by differential scanning calorimetry. After rehydration the zeta potential and particle size of the resulting liposomes were evaluated.

All the results showed the potential of proliposomes as drug carriers.

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ENCONTRO INVESTIGAÇÃO JOVEM  
DA UNIVERSIDADE DO PORTO

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14<sup>th</sup> **THURSDAY**  
POSTER SESSIONS

# A new event-specific PCR method to detect MON89788 soybean in food

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For the 19<sup>th</sup> consecutive year of commercialization, the global area of biotech crops has grown, reaching 181.5 million hectares in 2014, which means an increase of 6 million hectares from the previous year [1]. Soybean remains the most important genetically modified (GM) crop with about 111 million hectares of planted area [1]. The GM soybean MON89788 expresses the CP4 EPSPS protein, which confers tolerance to the glyphosate herbicide. Owing to this trait, the weeds, one of the major production problems in soybean cultivation, can be controlled by its increased herbicide tolerance. This soybean event, also known as Roundup Ready 2 (RR2), was approved in the EU in 2008 [2]. As such, under the Regulations (EC) No. 1829/2003 and 1830/2003 regarding authorization and traceability of food and feed derived from GMO, the labeling of food products containing more than 0.9% authorized GM material is mandatory. To verify labeling compliance, appropriate analytical methodologies are required. For GMO testing, the methods of choice are based on DNA analysis by means of polymerase chain reaction (PCR) due to their demonstrated effectiveness.

The aim of this work was to develop and apply a PCR assay for the detection of RR2 soybean in foodstuffs. For this study, DNA was extracted using two methods: Wizard [3] and/or NucleoSpin. The amplification targeting the *lectin* gene was performed as an endogenous control for soybean detection to produce a PCR product of 120 bp [3]. For the event-specific detection of RR2 soybean, specific primers were proposed using the Primer-Blast designing tool. The PCR conditions for the detection of RR2 soybean were optimized with reference materials containing 0, 0.1, 1 and 10% of RR soybean in order to produce fragments of 123 bp. The results showed that 78% of a total of 90 food samples were positive to *lectin* gene. The application of the RR2 specific assay showed that 7 samples were positive for RR2 soybean, which emphasizes the need for the effective control GMO in foods. Further research is being pursued to confirm and quantify results by real-time PCR.

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## Comparison of two anesthetic protocols for short duration surgical procedures in rabbits

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Rabbits are widely used as an experimental model in biomedical research, because of their easy handling and docile temperament, and also because they require little space for housing. However, their use in experimental surgical procedures is still limited by the difficulties related to anesthesia of such animals. [1]. The tracheal intubation is hampered by anatomic characteristics [1, 2], induction with isoflurane mask can lead to apnea and there is a narrow safety margin between surgical anesthesia and death [2]. Thus, the objective of this study was to evaluate two anesthetic protocols that allow the performance of a short duration surgical procedure that produces moderate degree of pain. In this experiment, 26 New Zealand rabbits weighing 3.2 kg were used. All animals received ketamine (25 mg / kg) and midazolam (1 mg / kg), administered intramuscularly, associated to an opioid: group I received morphine (2 mg / kg) and group II, tramadol (5mg / kg). Twenty minutes after premedication, induction of anesthesia was achieved through face mask and isoflurane. Immediately before the beginning of the surgery, the animals received 1,5µg/kg fentanyl intravenously. Latency and duration of the effect, in addition to physiological parameters, were evaluated. Results were submitted to ANOVA and comparisons through Student t test, with 5% significance. The onset of effect was seen at 5.5 and 4.4 minutes after injection in groups I and II, respectively. The total duration of anesthesia was 147.5 and 90 minutes, for groups I and II, respectively, with significant difference between them. Both protocols allowed the surgical procedure to happen, which consisted of making a bone defect on the medial surface of the tibia. Heart rate remained stable and within the physiological range for the species, with no difference between groups and along evaluation time. Significant respiratory depression was observed following premedication in group I (57 mpm). Nevertheless, in group II, late respiratory depression was observed, 45 minutes after application (75 mpm). However, in both groups, the values of heart rate remained within physiological range for the species throughout the procedure. In conclusion, ketamine and midazolam in combination with morphine or tramadol are secure protocols for anesthesia in rabbits, and that the association of tramadol is related to shorter duration of effect and less respiratory depression.

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# Dispersive liquid-liquid microextraction for the determination of nitrated polycyclic aromatic hydrocarbons in water samples

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Polycyclic aromatic hydrocarbons (PAHs) are a wide group of ubiquitous contaminants whose sources are mainly natural incomplete combustion processes and anthropogenic emissions. However, during biological and chemical degradation of PAHs other toxic compounds may be formed. The nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) belong to this family of substituted-PAHs. Actually, these compounds are considered a class of organic pollutants widely distributed in the environment [1]. The study of this class of organic compounds is an emerging task from scientific community due to their mutagenic and cancerogenic properties. Nevertheless, PAHs and nitro-PAHs are found in very low concentrations in the environmental matrices. Consequently, leads to a difficulty in accurate and reliable detection and quantification of these compounds. In order to overcome this problematic, a new, sensible, selective and efficient analytic methods should be considered and developed.

The main purpose of this work is a development of an innovative, ecologic and easy method of extraction: dispersive liquid-liquid microextraction (DLLME). This treatment is very useful in the pre-concentration of nitro-PAHs. The DLLME method is based on a ternary component solvent system, however, the partition of nitro-PAHs occurs between two immiscible phases: organic and aqueous phases. The advantage of this methodology is the introduction of a dispersive solvent, which is used to disperse the extractor solvent in numerous droplets, allowing a quick nitro-PAHs partition. For the optimization of the extraction parameters an experimental design approach was implemented [2].

Based on experimental results concerning the proposed DLLME method, suitable calibration curves for seven nitro PAHs (2-nitrofluorene, 9-nitroanthracene, 3-nitrofluoranthene, 1-nitropyrene, 6-nitrochrysene and 7-nitrobenzo(a)anthracene), were obtained. These results have shown linearity in the range of 5-150 ng/L with correlation coefficients ( $r^2$ ) ranging between 0.9987 and 0.9997. The limits of detection (LODs) for the seven nitro-PAHs studied ranged from 2.87 to 6.21 ng/L.

As evidenced from these experimental data, this methodology is very promising for the determination of ultra-trace levels of nitro-PAHs in environmental waters.

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## Initial growth of *Tabebuia rosea* (bertol.) Dc. seedlings in different containers in northern Brazil

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One of the difficulties in the production of seedlings of native species is the slow growth [1]. For these reasons, alternative substrates and suitable containers should be studied in order to reduce production costs and make the nurseries an affordable activity to all producers. The objective of this study was to evaluate the growth parameters (diameter, height and number of leaves) as well as the survival of seedlings of *Tabebuia rosea* in plastic bags, and cartridge subjected to the same condition of light. The experiment was conducted in the Forest Nursery at the Faculty of Agricultural Sciences – UFAM, North of Brazil. The seeds (n = 200) were placed on seedbed containing sand. After 20 days, with the seeds germinated and the seedlings were approximately 10 cm above the collar, they were picked and placed in containers containing as substrate (soil + clay) 2: 1, supplemented with NPK. The experimental design was completely randomized. The seedlings remained in a greenhouse with 50% shading. Later the collection of data growth was performed. The means were compared by Tukey test (P < 0.05). About the diameter, the Ipe seedlings showed higher increase in bag container (1,53mm), but there was no significant difference between the two containers used. The seedlings had greater height growth in cartridge container (9,34cm), the container bag has dual behavior, which may favor or not on height. Regarding the number of leaves, the use of the bag container showed the best result. Comparing the two containers used, the Ipe seedlings had higher survival when packed in tubes (100%). According Candid et. al, larger volumes of containers offer better conditions for the development of the seedlings, but these should only be used for species with slow development, needing to stay in the nursery for a long time, or when you want well-developed seedlings for planting on public roads for example [2]. Therefore, both containers have good packaging capacity for seedlings. Thus, the cartridge can be used initially, because it saves substrate, is reusable and with easy handling, despite the changes require more area for percolation root, a condition that the cartridge does not provide to the seedlings.

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## Nutritional value and fatty acid profile from acorns (*Quercus nigra*): intending new alternative applications

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The development of rural areas depends on sustainability policies to promote agricultural and production systems, in order to increase those populations' income. To attain this purpose, it is important to find new opportunities to valorize the available, but still underexploited, natural resources [1]. Therefore, the aim of this study was to compare the nutritional profile of kernel, shell and the whole fruit of *Quercus nigra*, a Portuguese autochthonous acorn species, intending new alternative applications (functional foods).

Nutritional parameters (ash, fat, protein and moisture) were measured according to AOAC methods [2]. Total carbohydrates were calculated by difference. The fatty acid profile was also evaluated by GC/FID [3].

It was verified that acorn presents a high content of carbohydrates (> 90%) and lower fat and protein contents (< 3% and < 6%, respectively). Some compositional differences were observed in the different parts of the fruit (kernel and shell). Acorn kernel presented the highest values of moisture (43%), ash (1.4%), protein (5.1%) and fat contents (2.5%). The carbohydrate/starch fraction constitutes 91.9% of the kernel. The shell (it by-product) proved to be a good source of carbohydrates/fibre (94.5%), with lower fat and protein contents (0.8% and 3.8%, respectively).

From a nutritional point of view, the results indicate that acorns can be used as a source of dietary energy, starch and fibre, proving to be an attractive low cost food. Moreover, acorns kernel provide essential fatty acids (table 1).

Table 1. Fatty acid profile (relative percentage).

	<b>Whole fruit</b>	<b>Kernel</b>	<b>Shell</b>
<b>Total SFA</b>	<b>18.53</b>	<b>17.89</b>	<b>25.06</b>
<b>Total MUFA</b>	<b>51.92</b>	<b>49.98</b>	<b>54.32</b>
<b>Total PUFA</b>	<b>29.28</b>	<b>32.23</b>	<b>18.86</b>
Total n-3 PUFA	0.55	0.55	0.26
Total n-6 PUFA	28.68	31.61	18.60
n-3/n-6	0.02	0.02	0.01

Acorn flour can be an interesting substitute of wheat flour in bread production, answering to innovation purposes and traditional products valorisation. The edible fruit and its waste (shell) could also be considered added-value ingredients for other purposes, including gluten-free formulations. Further studies are being conducted in order to better characterize these matrices regarding their chemical composition, as well as further potential applications in different industries.

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# Pesticide residues in olive oils from organic and conventional agriculture

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Olive oil is one of the most important agricultural commodities in Portugal. In the last years, the production of organic olive oils has been steadily increasing due to new consumer behavior and the nutritional value of these products. The claimed healthiness quality of the organic olive oils is related, among other parameters, with the absence of toxic compounds such as pesticide residues. Thus, its verification is essential to ensure the quality of the product and thereby protect consumer health.

In this work, a procedure based in QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction and gas chromatography-mass spectrometry (GC-MS) analysis was validated for the simultaneous analysis of eight pesticides (deltamethrin, dimethoate, diuron, fenthion, phosmet, chlorpyrifos-methyl, endosulfan sulfate, fenthion sulfoxide) in olive oils obtained from organic and conventional agriculture. A new sorbent (Z-Sep+) was compared with the usual sorbents (C18; primary and secondary amine-PSA) during the dispersive cleanup-step in QuEChERS procedure [1], being the best results obtained with Z-Sep+. The linearity of the method was checked for all compounds (always >0.99) with linear calibration in the range of 0.01-2 mg/l for most of them. Method validation experiments at three spiking levels yielded average recoveries in the range 70-120% with relative standard deviations (RSD) in the range 1-20% for the majority of the pesticide residues. The applicability of the proposed method has been demonstrated in the analysis of a total of 46 olive oils samples including 21 from organic origin.

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# SALTED CODFISH AUTHENTICATION BY FAST PCR-HRMA

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The most common forms of adulteration of fish-based products involve replacement for other species of lower economic value. Atlantic cod (*Gadus morhua*, L.) is one of the most consumed and exploited white fish with gastronomic relevance in several European countries assuming a particular importance in Portugal. As a consequence *G. morhua* is highly exploited provoking dramatic fish stock depletion. In this framework the commercialization of different fish species, presenting similar organoleptic and morphological characteristics, fraudulently substituting *G. morhua* is a very attractive practice for unscrupulous traders. Also, the increase in processed products and the existence of a global market can lead to substitutions of species liberally or accidentally. Some examples of substitutes are pacific cod (*G. macrocephalus*) and Alaska pollock (*Theragra chalcogramma*) [1,2].

In the present work the barcoding gene cytochrome oxidase subunit I (COI) was sequenced in specimens of the three referred species. Sequences were subsequently aligned with several others from public databases. SNPs were then identified and primers *de novo* designed for short amplicons targeting these regions aiming at the development of a High Resolution Melting Analysis (HRMA) method.

Commercialized samples of dried salted codfish (shredded, fillets and whole fish) from different origins were acquired. Small aliquots of each sample (100 mg) were subjected to DNA extraction and the COI gene sequenced in all samples as a confirmation of the species identity. HRMA was carried out for the COI gene using standard primers as well as the newly designed ones. The technique proved to be very efficient in species discrimination using selected *de novo* designed primers targeting *ca.* 100 bp fragments which performed far more efficiently than the direct analysis with the standard COI primers targeting 600 bp amplicons (Figure 1). PCR and HRMA was performed in fast PCR mode allowing very short analysis time, i.e. PCR and melting in less than 1 hour.

Species substitutions can be assessed by the present methodology in a very specific and fast methodology permitting high sample throughput.

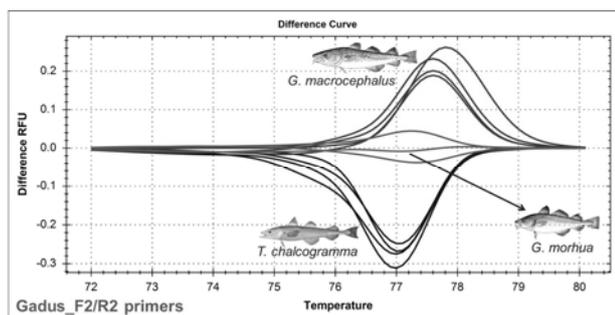


Figure 1. Discrimination of the three species in study by HRMA using the primers Gadus\_F2/R2

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## **STRUCTURES PARASITIC CARRIED BY FLIES IN FOUR BIOTOPE IN TEODORO SAMPAIO, SAO PAULO, BRAZIL**

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The flies, due to their synanthropic characteristics are mechanical vectors of important parasites in animal production and public and indicators of environmental quality health, highlighting muscidae and blowflies.[1-2] This study aimed to evaluate the presence of parasites on the outer and inner portion of Calliphoridae and Muscidae flies caught in four biotope Teodoro Sampaio, Brazil (Ribeirão Bonito settlement, urban center, Morro do Diabo Park and sugarcane fields). To capture the flies were used in environment, five traps baited with beef liver and fish. Collections were carried out at the beginning, middle and end of each season (March / 2012 to February / 2013). The flies were identified taxonomically and washed in sterile saline solution (SFE) and dissected. For recovery of parasitic structures, 10 mL of fluid obtained from external and internal washing were centrifuged (2500 rpm) for five minutes. Three aliquots of 50mL of sediment obtained were evaluated under microscopy (10X and 40X).[3] 3000 flies were captured, 3000 (70%) and 13000 Calliphoridae (70%) Muscidae. The parasitic structures were observed only in flies caught in the inner city and rural settlement, and the most common Giardia spp. (45 cysts, 23 in town and 22 on seating) and Entamoeba spp. (28 cysts, 25 in the city and 3 in the settlement). The Calliphoridae showed the greatest number of structures (41 Giardia spp. And 22 Entamoeba spp.), While muscideos broadcasted 4 Giardia spp. 6 and Entamoeba spp. There are also two non-sporulated oocysts from protozoa in Calliphoridae, one for each target environment. There was a significant difference between the number of cysts retrieved from the external portion in relation to the intestine ( $p < 0.05$  t-test). The results show that blowflies and muscideos can carry parasitic agents with zoonotic potential, especially by contact with food, carcasses and feces.[1]

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# Level dehydration in simulated combat jiu-jitsu.

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## Introduction

The practice of jiu-jitsu requires clothes as the kimono and demands various physical capacities. Because of that, it is one of the physical activities that most dehydrate the athlete, which can affect its performance.[1]

## Methodology

The study is composed by 12 athletes of jiu- jitsu , male , average age of 25 ( $\pm$  5), above blue belt graduation and who are training and joining competitions regularly.

Initially, all athletes were applied to a physical evaluation, in which it was measured: body mass and skinfold. Then, two simulated fighting of ten minutes each were played, with a five minutes break after the first fight. Right after the fights, body weight was taken again in order to apply the values in the equation proposed by [3] where sweating rate = Start Weight - Final Weight ( Kg ) . 1000 / Activity Time (min).

## Results and discussion

For the body weight measured before the fighting, it was registered an average value of 87.20kg (  $\pm$  13.17kg) and after the two simulated combats it was registered an average of 86.04kg (  $\pm$  13.16kg) . From these values, sweating rate was calculated , obtaining an average of 57.92  $\pm$  28.24 . ml / min . The reduction in body weight as indicative of fluid loss of athletes, according to [2], is one of the best reviews. Most athletes lost up to 2 % of their body weight. By [4], it provides significant dewatering and physiological effects that reduce performance.

## Conclusion

In two simulated combat jiu-jitsu lasting ten minutes each, most of the athletes had significant weight loss , showing that the jiu- jitsu is a sport that has a high dehydration rate.

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## The Construction of the meanings of the *cartoon* genre, by studying the re-categorization process

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Re-categorization studies have focused on the analysis of verbal texts, thus following the natural path of Textual Linguistics. This area has broadened its frontiers in line with the processing of multi-modal texts of socio-cognitive origin, in which it is possible to view a text beyond its verbal materiality, putting in place its other semiotics. Within those semiotics, we believe that the image may carry out the role of validating re-categorizations that are operated by the speaker. This project aims to (i) investigate the linguistic mechanism of re-categorization in the construction of multi-modal cartoon senses, (ii) describe which types of re-categorization are used as linguistic mechanisms in the construction of twenty multi-modal texts belonging to the cartoon genre, and (iii) analyse the types of re-categorization that exist in the construction of examples of the cartoon genre, focusing on the cognitive discursive functions of that linguistic mechanism. Amongst the vast bibliography that portrays the construction process of meanings and its re-categorization, we will be studying some theorists who cover this theme, such as Mondada and Dubois (1995), Lima (2003), Cavalcante (2003), Kress and Van Leeuwen (2006). Although this study is still in progress and in its initial stage, its full research has been methodically distributed in (i) bibliographic survey to enable the reading and discussion of its theoretical prerequisites, (ii) collection and organisation of the sample of investigation of the genre in study, (iii) identification and description of re-categorization occurrences, and (iv) systematization of the data under analysis; but in this study we shall particularly be focussing on data collection that broaches re-categorization as a process of the construction of meanings for the aforementioned genre. The results are not yet concluded, as the research is still in its initial phase; however, we hope to raise a few different types of re-categorization and their cognitive and discursive functions in the construction of multi-modal meaning of the cartoon genre.

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## National Centre for Immigrant: heterotopia the OPorto Society

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Immigration in Portugal reaches new dimensions and greater diversity. The scene brought challenges to existing policies and the need to create the National Center for Immigrant Support (CNAI). Inspired by the citizen Store, where in the same building are available different departments of the administration, CNAI-OPORTO, since 2004, the immigrant support within the social structure that formed. The objective of this study was to describe the CNAI space. The research had a theoretical, descriptive and reflective character. It was used free observation and document analysis available in the literature on the CNAI-OPORTO space. The reflection was based on the work "Des Espace Autres" [1], describing the concepts of heterotopia, the place of the other and the place out of place. The heterotopia is capable of juxtaposing in a single real place several spaces, several sites that are themselves incompatible. The Place of other can be understood as a physical and locatable space, belonging or that meets a certain group can be identified and described within a society. Think of a place within a geographical territory, which employs sometimes other languages, spoken or written, is to think of something out of place. The CNAI born with the intention of cooperation and congregation where they were gathered in same place services for foreigners. By type of service and the public attended, the CNAI is configured as an example of Place of other. Taking into consideration the environment and the society in which it operates the object of study, it was created for this scenario a perspective in which the "I" is recognized by Porto citizen and foreign citizen assumes the identity of the "Other." In order to promote better communication between the CNAI and foreigners who use this service, the socio-cultural mediators are an innovative focus on momentum to overcome difficulties encountered, whether linguistic or cultural, often themselves from different backgrounds and cultures, to recreate different scenarios of the world within the Porto society, not only the language of their country of origin, but also customs and ways to meet that are characteristic of other cultures..

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# **Copper based metallurgy of the fortified settlements of Cávado's middle basin, in the I millennium B.C.**

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The fortified settlements, commonly known as “castros”, in the Cávado's middle basin (Entre-Douro-e-Minho, NW Portugal), from the final stages of the Bronze Age (c. 1200 – 1000 BC) to the roman conquest of the NW Peninsula, provided a considerable amount of metallurgical related artifacts, some of which are stored today in D. Diogo de Sousa Museum. The presence of an archaeological context and the artifacts' condition was considered as a solid source for a master's thesis, still in the early stages of development. The purpose of this abstract is to present the said thesis, which in turn is meant to contribute to the progressive archaeological knowledge of the societies that once inhabited this region, specifically in the field of the copper based metallurgy.

With this thesis it's intended to better understand the artisanal specialization as an indicator of social-economic complexity within the referred societies. The study of the origin or provenance of raw material and metals, as an approach to the mechanisms of exchange and cultural relations as well as mutational agents of metallurgical technology, is also envisioned as a main purpose.

The preliminary structure of the thesis includes 6 chapters: Introduction, Physiographic Context, Historical Context, Fortified Settlements, Artifacts and Conclusions. The Physiographic Context will comprise a description of the region in question and its characteristics at various scales, including its geography, geology, hydrology, climatology, among others. The Historical Context will comprehend the State of the Art, namely what there is to know about the subject and what are the basic and the specialized bibliographic sources. In the fourth chapter, the fortified settlements will be systematized and correlated with the physiographic context, previously scrutinized in the second chapter. The artifacts will be systematized as well, in the subsequent chapter.

Two principal types of methodologies are meant to be used, first an analytic, individual study of the artifacts, and later an interpretive study, integrated with their physical, historical and archaeological context, bounded by the referred temporal and spatial limits. These will be combined with other sciences, such as experimental archaeology, history, geosciences and cartography. The analytic studies are thought to include from optical studies at macro and micro scale, elemental composition analyses, to impurity models and lead isotopes analyses, while the interpretive studies shall comprise style and decoration, morphology, function, symbology and excavation context.

The work here presented aims to be innovative for its regional and individual dichotomy, starting with the analysis of an artifact as a single entity and extending it to the whole regional context. The combination of various scales of analysis as well as multidisciplinary composes the great fundament of the thesis. In conclusion, it's purposed to bring new data to the understanding of copper based metallurgy of the Bronze and Iron Age of Northwest Portugal.

# Roman oil lamps of local production in northern Portugal: a collection of the *Necropole da Via XVII*

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In Roman period were used oil lamps with the function of illuminating the population in its various activities, allowed them to enjoy the evening for the various spectacles, illuminate the interior of their homes [1], and even account the time of mining operations by the intensity of the flame [2]. The existence of oil lamps of clear local manufacturing throughout the North of Portugal, is already documented. However, the analysis of its diffusion at regional level in the North Portugal remains to be done. The main goal of this work, is to develop a plural approach in order to establish a dispersion of oil lamps locally produced in the north of Portugal and to understand its importance for the population of its time. With this research, we are interested in understanding “Bracara Augusta” as an important producing center of oil lamps in the north of Portugal, and to understand the marks and inscriptions shown by the oil lamps and their molds. [3]

After the literature review, the study of each Lucerne was based on descriptive, photographic and graphic recording (drawing). The distribution analysis of these lighting objects will be illustrated on maps, created using the software for execution and processing of images. In this study it will be presented the local oil lamps’ collection of the “Necrópole da Via XII” an important necropolis of the roman city “Bracara Augusta”. [3].

These are preliminary results of a larger study which illustrates the main functions of the lamps in the within the Roman culture, specifically in the rituals related to death. [4] The oil lamps are a reflection of his time, showing artistic trends in their own decoration, symbolism and form. [5]

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# Development and validation of a method for the simultaneous determination of two piperazines used as drugs of abuse

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1-Benzylpiperazine (BZP) and 1-(3-trifluoromethylphenyl)piperazine (TFMPP) are piperazine drugs of abuse often taken in combination, either deliberately to mimic the effects of 3,4-methylenedioxy-methamphetamine (MDMA), or inadvertently as a result of the presence of this combination in the party pills ingested by abusers [1,2]. Considering the frequency of the co-administration of BZP and TFMPP in the forensic context and its toxicological impact, it is of utmost relevance to develop and validate a method that enables the simultaneous determination of these drugs. Thus, the objective of this work was to optimize and validate a gas chromatography-mass spectrometric (GC-MS) method for the simultaneous quantification of BZP and TFMPP in different matrices (urine, plasma and culture medium). Standard samples for each matrix were prepared by adding to the blank the required amount of the standard solution of each analyte (BZP and TFMPP) and of 4-hydroxy-3-methoxybenzylamine, used as internal standard. The standard samples preparation involved an enzymatic hydrolysis, a solid phase extraction (SPE), and a perfluoroacylation derivatization process. Before proceeding with the validation, the method was optimized. To this end, we tested the protein precipitation in plasma and the chromatogram baseline was greatly improved. In the culture medium, liquid-liquid extraction before the SPE was tested. However, there was no improvement in the baseline. For the three matrices four different extractions were tested and the yield of each extraction was evaluated with recovery tests. We opted for the extraction that had the highest recovery. When testing the different calibration curves a significant matrix effect was observed, so we opted to validate the method for each matrix separately. Linear calibration curves were obtained by using the selected ion monitoring mode for all analytes in the concentration range from 0.016µg/mL to 10µg/mL with six calibration points. The preliminary validation data show good recovery and precision of the method for all matrices. Limits of detection and quantification, accuracy, interference and stability are currently being evaluated. This GC-MS method has so far provided high sensitivity and adequate performance characteristics for the simultaneous quantification of BZP and TFMPP in the studied matrices.

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## Development lozenges (soft tablets) containing clindamycin hydrochloride for pediatric use

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Drug therapy plays an important role in the overall management of the patient. Many of drugs used frequently in all pediatric age groups are approved for adults; however lack indications for pediatric patients [1]. So, the use of extemporaneous preparations is often required in order to obtain dosages suitable for pediatric applications.

In this study, we developed and optimized a solid dosage form, lozenge containing clindamycin and comercial Royal flavored gelatin with strawberry flavor, aiming to use in children in hospital.

The lozenges were formulated according to the methodology applied in hospital pharmacy, molding method, described by Prista et al. [2]. Pharmaceutical stability studies were performed to ensure the safety, efficacy and quality of clindamycin lozenges, after 3 and 7 days of storage at  $5^{\circ}\text{C} \pm 2^{\circ}\text{C}$ .

Compatibility study between drug and excipient in the ratio of 1:1 was carried out using differential scanning calorimetry method (DSC). The interactions between clindamycin and gelatin occurred above  $120^{\circ}\text{C}$ . This temperature is higher than the temperature of manufactured and storage of solid dosage forms.

In visual examination, the lozenges were homogenous. The formulation presented a content of clindamycin hydrochloride of  $92.97 \pm 2.00\%$ , which is in accordance with the specification of USP (90 - 100%) [3], and in in vitro dissolution study, the lozenges disaggregated in the first 20 minutes, releasing  $92.87 \pm 2.00\%$  of clindamycin hydrochloride in this period.

Microbiologically the formulation was not contaminated with *E. coli*, total bacteria count was less than 102 CFU/g and fungal contamination was also less than 102 CFU/g on day 3 and 7 of the study.

To increase patient compliance, the administration of attractive, taste masking formulations are helpful. This study demonstrated that the flavored gelatin based medicated lozenges will be a promising formulation for clindamycin administration to pediatric patients in hospital. The microbiological stability proved that the prepared lozenges were stable during 7 days when stored at  $5^{\circ}\text{C}$ .

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## Development of lipid nanoparticles containing paclitaxel

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Glioma is the most frequent adult primary brain tumor. The current clinical standard for glioma treatment involves surgical debulking. But the proximity to critical regions of brain function makes the complete removal of tumor very difficult. Consequently, chemotherapy or radiotherapy is indispensable for glioma treatment after the surgery [1]. Although their efficiency, they present problems such as rapid release of the therapeutic agent, and consequently a low bioavailability, and lack of specificity resulting in adverse side effects. In addition, in treatment of brain tumors, the drug must overcome the blood-brain barrier (BBB), which can be a difficult process when it is in the free form [2]. Paclitaxel (PTX) is an effective anti-cancer drug against a wide spectrum of solid tumors, which promotes polymerization of tubulin dimers to form microtubules and stabilizes microtubules by preventing depolymerisation. The PTX presents limitations as, low therapeutic index and reduced bioavailability due to its low solubility in aqueous medium. To overcome these problems, nanoparticles (NPs) delivery systems were selectively and efficiently transport PTX to the target organ/cells, increasing the bioavailability [3]. The aim of this project was to develop and to optimize lipid NPs (SLN and NLC) containing PTX and to evaluate which is the best system.

SLN and NLC were prepared by high shear homogenization and hot high pressure homogenization techniques at concentrations of 5% and 10% (w/w) of lipid (cetyl palmitate and miglyol (in the NLC case), 2% (w/w) of surfactant (polysorbate 80), and 0.1 % (w/w) of PTX. The mixture of the drug, surfactant and lipid was melted at 70°C. The water was heated at the same temperature and added to the lipid phase and homogenized in an ultra-turrax followed by homogenization in an ultrasonication probe or by high pressure homogenization. The hot nanoemulsion was cooled down to room temperature.

NPs stability was determined by assessing the particle size, size distribution and zeta potential of NPs on the day of production and 1 month after storage at 4°C.

The NPs prepared by hot high pressure homogenization technique had a lower particle size than NPs prepared by ultrasonication. The NPs prepared with 5% (w/w) of lipid presented a lower particle size than NPs prepared with 10% (w/w) of lipid. The mean diameter, polydispersity index and zeta potential of empty SLN and NLC, and loaded-PTX NLC was similar between the day of NPs production and 1 month after. However, 1 month after production loaded- PTX SLN had a lower zeta potential than empty SLN.

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## Diabetic enteric neuropathy is associated with neuronal loss and reduced facilitation of acetylcholine release by prejunctional adenosine A<sub>2A</sub> receptors

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Enteric dysmotility is a long-term complication of *Diabetes mellitus* causing significant discomfort and disability in 76% of diabetic outpatients [1]. Diabetes may affect almost every part of gastrointestinal (GI) tract leading to nausea, vomiting, constipation, diarrhea and fecal incontinence. The mechanism(s) underlying enteric neuropathy in type I diabetes is relatively unknown, but evidences have been gathered suggesting the purines may be involved in synaptic transmission modifications in the central nervous system of diabetics. This prompted us to investigate if purinergic dysfunction could also play a role in enteric neuropathy of type I diabetic rats.

Adult male Wistar rats (300-500g) were injected once with streptozotocin (STZ rats, 55mg/kg, IP). Forty eight hours after STZ injection rats became hyperglycemic (404.11±56.01 md/dL, *n*=9) and blood glucose was kept at high levels until experimental day 14 (497.33 ± 47.17 md/dL, *n*=9) [2]. Although STZ rats presented obvious polydipsia/polyuria, they presented a minimal loss of body weight (-3.51±4.35%, *n*=9). *Post-mortem* observation of the GI tract of STZ rats revealed an increase in caecum size, which was significantly (*P*<0.05) heavier (2.18±0.17%, *n*=4) than control animals (5.33±0.55%, *n*=9) when normalized by the total body weight of each animal. Experiments were performed at day 14 on longitudinal muscle-myenteric plexus (LM-MP) of the ileum of control and STZ rats.

Confocal microscopy studies using whole-mount LM-MP preparations suggest a decrease in the immunoreactive staining for protein gene product 9.5 (a pan-neuronal marker), neuronal nitric oxide synthase (nNOS) and vesicular acetylcholine transporter (VACHT) in STZ compared to control animals. Data from enzymatic kinetic HPLC experiments demonstrate that the extracellular catabolism of ATP (30 μM) was faster (*t*<sub>1/2</sub> 4.33±0.45 min, *n*=3) in STZ-injected rats than in control animals (*t*<sub>1/2</sub> 7.18±1.14 min, *n*=6). Formation of adenosine from the extracellular ATP catabolism reached a maximum of 12.18 μM at 10 min incubation with the substrate in STZ rats, but a similar concentration (12.81 μM) was only observed 30 min after ATP application in the control group. Despite the faster formation of adenosine from ATP hydrolysis in STZ animals, the nucleoside levels hardly accumulate at the LM-MP because adenosine (30 μM) is inactivated into inosine more rapidly in STZ rats (*t*<sub>1/2</sub> 13±3 min, *n*=4) than in control animals (*t*<sub>1/2</sub> 34±1, *n*=4) [3]. The inhibitory effect of the A<sub>1</sub> receptor agonist, R-PIA (300 nM), on [<sup>3</sup>H]-acetylcholine ([<sup>3</sup>H]-ACh) release evoked from stimulated myenteric motoneurons (EFS, 5 Hz, 200 pulses, 1 ms) was similar in control (-36±4%, *n*=4) and STZ (-45±8%, *n*=3) rats. Conversely, the adenosine A<sub>2A</sub> receptor-mediated facilitation of evoked [<sup>3</sup>H]-ACh release caused by CGS 21680C (3 nM, 53±10%, *n*=4) was abrogated in diabetic animals (-19±7%, *n*=3).

Data suggest that although adenosine formation is faster in the LM-MP of diabetic rats the nucleoside is rapidly inactivated, thus preventing its extracellular accumulation. This situation together with the neuronal loss and, consequently, the reduction in A<sub>2A</sub>-receptor-mediated facilitatory tonus observed in diabetic rats may contribute to constipation, the most common GI complaint of diabetic patients.

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# Different Evaluation Methods of Resting Energy Expenditure in Obese Women Before and After Bariatric Surgery

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**Background:** Obesity has been increasing on a global scale in recent decades, making it important to properly evaluate the energy balance so that energy needs in these subjects are established as accurately as possible. The main objective of this research was to study the suitability of using various equations used to estimate resting energy expenditure with the results of indirect calorimetry (used as the reference method) in women undergoing bariatric surgery.

**Methodology:** A total of 17 women undergoing bariatric surgery in three stages: pre-surgery, 1 and 3 months after surgery were evaluated. In all evaluations, it was made anthropometric measurements, body composition and it was estimated resting energy expenditure by the method of indirect calorimetry. Several predictive equations of resting/ basal energy expenditure daily, used in clinical practice were used.

**Results:** At first assessment, equations of MifflinMLG, OwenMLG and Bernstein showed no significant differences when compared with the average of indirect calorimetry. In the first month, in addition to the three above, the Cunningham equation and BIA method also did not differ significantly from the results of indirect calorimetry. In the third evaluation Owen's equation and the above obtained the previous results. Analyzing the deviation from the average in the three assessments, Bernstein's equation showed the greatest similarity with the values of calorimetry.

**Conclusions:** Further studies are needed to establish the clinical practice a credible, fast and accurate method to calculate the resting energy expenditure in this population.

**Keywords:** obesity, bariatric surgery, resting energy expenditure, indirect calorimetry, predictive equations

# Discovery of new modulators of caspases 3 and 7 using a yeast-based screening assay

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Caspases are a family of proteases with a crucial role in the initiation and regulation of apoptosis [1]. At the core of the execution phase of apoptosis are the executioner caspases 3 and 7. Like most proteases, these caspases are stored as procaspases that once activated by proteolysis, cleave a large set of substrates, ultimately resulting in the characteristic morphological and biochemical hallmarks of apoptosis. Therefore, the search for activators of these procaspases has deserved particular attention in the field of anticancer drug discovery [1]. Nevertheless, to date, only few small molecule caspase activators have been reported [1].

In a previous work from our group, the use of a yeast screening assay, based on the heterologous expression of human caspases 3 or 7 in the yeast *Saccharomyces cerevisiae*, led us to the identification of two activators of caspase 7: 5,6-dihydroxy-7-prenyloxyflavone (**1a**) and 3-hydroxy-7-geranyloxyflavone (**2a**) [2]. These results prompted us to the present work aiming to discover new activators of caspases 3 and 7. For that, several analogues of compounds **1a** and **2a** were synthesized, and their activities on caspases 3 and 7 were evaluated, using the previously developed yeast-based screening assay [2]. Potential activators of caspases 3 and/or 7 were therefore identified with improved pharmacological properties compared to their starting analogues. Further work is underway to confirm their molecular mechanisms of action in human tumor cells.

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## Dissecting Macrophage-Treg Crosstalk as a Therapeutic Approach for Human Colorectal Cancer

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In colorectal cancer (CRC) the role of tumour-associated macrophages (TAMs) and regulatory T cells (Tregs) is controversial since, contrarily to most tumours, their intratumoral infiltration is often correlated with good prognosis. Depending on the tumour microenvironment, TAMs may either exhibit pro- or anti-inflammatory properties, modulating cancer cell activities and dictating disease outcome. Also, Tregs may steer macrophage differentiation and thus modulate macrophage function.

This study aims to elucidate how TAM-Treg crosstalk is established in the CRC context, clarifying the role of Treg-educated macrophages on cancer cell invasion. Macrophage precursors and Tregs were co-cultured for 3 or 7 days and their impact on CRC cell invasion assessed by 3D-Matrigel-invasion-assays. For control purposes, each cell population was cultured alone. Our results evidenced that CRC cell invasion was significantly promoted by macrophages and macrophage-Treg co-cultures, but not by Tregs alone. After 3 days of co-culture, Tregs partially inhibited macrophage-mediated cancer cell invasion, whereas at day 7 Tregs potentiated macrophage pro-invasive activity. This suggests that at early stages, Tregs can modulate macrophage activity, reducing their capacity to promote cancer cell invasion. However, continuous interaction between macrophages and Tregs seems to favour macrophage pro-invasive properties. To further confirm this, it is now crucial to evaluate the inflammatory profile of Treg educated macrophages at different time points.

Additionally, the expression of FoxP3 (the most specific Treg marker) on human CRC specimens was characterized by immunohistochemistry at three distinct regions: normal mucosa, intratumoral region and tumor invasive front. Interestingly, a significantly high number of FoxP3-positive cells was found at both intratumoral region and tumour invasive front, compared to the adjacent normal mucosa. To assess the prognostic value of these findings, our results will be crossed with patients' clinicopathological data.

In conclusion, our *in vitro* results suggest that, in a CRC context, Tregs may promote macrophage-mediated invasion. Furthermore, the higher infiltration of FoxP3-positive cells, observed at the invasive front of CRC patients surgical resections, likely corroborates this hypothesis.

# Effect of encapsulation on the photoprotection effectiveness of the UV filter 2-ethylhexyl 4-methoxycinnamate

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With the increasing incidence of melanoma, the protection against solar radiation has become a growing concern and hence the widespread use of sunscreens. 2-ethylhexyl 4-methoxycinnamate (EHMC) is a chemical filter that exerts its action by absorbing ultraviolet radiation. However, when exposed to solar radiation, it undergoes isomerization/degradation processes with consequent reduction of effectiveness [1, 2]. Thus, the cosmetic industry has developed various strategies such as encapsulation in order to increase the EHMC photostability [3]. A previous work from our group suggested that sodium alginate could be used as a coating material for UV filters stabilization and so the main goal of this study was to prepare sodium alginate microparticles (MP) containing EHMC and to evaluate the photoprotection effectiveness by measuring the sun protection factor (SPF). EHMC-MP and empty-MP were prepared by an extrusion process with aerodynamic jet assisted methodology. To this end, the sodium alginate grade was selected according to flow properties and a HPLC method for EHMC quantification was developed. This method was optimized for the analysis of EHMC incorporated in a semisolid formulation and its ability to detect EHMC isomers was evaluated. The MP were evaluated in terms of EHMC-loading, moisture content, morphology and size (using laser diffractometry technique). To assess the photoprotection properties, a semisolid formulation was prepared (containing free EHMC or EHMC-MP) and applied in PMMA plates at an application rate of 1 mg/cm<sup>2</sup>. The plates' absorbance spectrum was measured from 290 nm to 400nm and the SPF was calculated. All MP showed a homogeneous size distribution with a Dv [50] of 81.10±3.80 µm for EHMC-MP and 54.38±18.46 µm for empty-MP. The EHMC loading was 25.62±4.27%. EHMC-MP were larger in size, but had a lower moisture content when compared with empty-MP (69.16 % and 92.80 %, respectively). EHMC-MP were successfully incorporated into a semisolid formulation, with no evidence of phase separation or colour modification. Sun protection effectiveness was maintained after encapsulation of the UV filter since SPF for free EHMC was 6.45±0.92 and for EHMC-MP was 7.62±1.94. In summary, the incorporation of EHMC in MP maintained the filter's photoprotection properties as examined by the SPF evaluation. As the filter is retained within the MP, microencapsulation can represent an alternative to decrease the exposure of individuals to toxic products formed by the filter photodegradation and to reduce EHMC environmental levels.

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## Effects on Endothelial Cells and Angiogenesis of Structurally-Diverse Xanthenes

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In the last years, natural prenylated and glycosylated flavonoids have been emerging as promising anti-angiogenic agents. Nevertheless, different results were obtained concerning hydrophobic and hydrophilic derivatives. We have been investigating xanthenes with antitumor and antiplatelet properties. The aim of this work was to investigate if several synthesized xanthenes with prenyl (XP13), epoxy (12EPOXI), glycosyl (XG and M) and sulfate (XGS and MS) substituents are able to modulate angiogenesis.

Endothelial cells (HUVEC) were incubated with 0-500 $\mu$ M of XG, XGS, M, MS, XP13 and 12EPOXI for 24h. Cell's viability was assessed by MTS. Apoptosis and necrosis were evaluated measuring caspase-3 and lactate dehydrogenase by spectrophotometry. Proliferation was quantified by BrdU incorporation. Migration capacity was measured by wound-healing assay. Angiogenic properties were confirmed by quantification of tubular structures formed on matrigel. Variables are expressed as mean  $\pm$  SEM and were evaluated by T-test and Mann-Whitney test. Results were considered significant when  $p < 0.05$ .

HUVEC exposure to 500 $\mu$ M of XG, XGS, M and MS decreased viability by 10%, and increased apoptosis by 28-36%. 100 $\mu$ M of 12EPOXI and XP13 decreased viability (30% and 70%) and increased apoptosis (76% and 43%) and necrosis (58% for XP13). XG, XGS, M and MS decreased HUVEC proliferation (30-44%, for 500 $\mu$ M) and migration (25-42%, for 100 $\mu$ M and 200 $\mu$ M). HUVEC exposure to 100 $\mu$ M XP13 and 12EPOXI decreased proliferation (69% and 35%) and migration (37% and 29%). All compounds tested decreased formation of tubular-like structures on matrigel by 31-60% for 100-200  $\mu$ M, being 12EPOXI the most potent, inhibiting the formation of these structures by 70%.

All tested compounds seem to manifest anti-angiogenic properties, affecting several steps of the angiogenic process. XP13 and 12EPOXI are more toxic for HUVEC at higher concentrations but seem to decrease tubular-like structures formed on matrigel more efficiently. The anti-angiogenic properties of these xanthenes deserve to be better studied as they may be interesting regarding therapeutic targets in pathologies where angiogenesis is deregulated.

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# Estimating the lipophilicity of chiral derivatives of xanthenes by RP-HPTLC

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Lipophilicity is one of many parameters that are involved in biological activity of drugs being a key parameter involved mainly in pharmacokinetic processes such as absorption, distribution, metabolism, excretion, and toxicity (ADMET), and in ligand-target interactions [1,2]. Generally, this physicochemical property is defined by the partitioning of a compound between a nonaqueous (nonpolar phase) and an aqueous phase (polar phase) and is expressed as P (partition coefficient) or its decimal logarithm (logP) [2]. The partition coefficient can be experimentally determined by a classical shake-flask method in a *n*-octanol-water system and by chromatographic methods [3,4]. In this work the lipophilicity of twelve chiral derivatives of xanthenes was estimated by the use of reversed-phase high performance thin layer chromatography (RP-HPTLC). Experiments were performed using a non-polar stationary phase of precoated HPTLC silica gel HF<sub>254</sub> plates impregnated with a solution of *n*-octanol (5% in diethyl ether). Mixtures of methanol:water were used as the mobile phases. Amounts of methanol as organic modifier were in the range 30–60% (v:v) in 5% increments. For each solute, three chromatograms were performed and R<sub>f</sub> values were averaged. The R<sub>M</sub> values were calculated and extrapolated to pure water as mobile phase (R<sub>MW</sub>). Linear correlations were found between the parameter R<sub>M</sub> and the concentration of methanol in the mobile phase. The results of lipophilicity measurements obtained by experimental method were compared with the theoretical values of partition coefficients estimated with two different computer programs.

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## Evaluation of neem leaves by high pressure extraction

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Neem is a commonly used medicinal plant in India, was characterized as a valuable source of natural products for nature's drugstore with applications such as antimicrobial, analgesic, immunomodulator and anti-cancer [1]. The new strategies used to obtain alternative drugs include studies of natural products used in folk medicine [2]. These compounds can be extracted selectively by extraction under high pressure using solvents which interact with the substances of interest to the scientific community [3]. It was used the technique that makes use of pressurized fluid with a pressure of 100 bar and a temperature of 25 °C and 50 °C by 25 minutes or 12,30 minutes, with flow of 1 mL/minute or 2 mL/minute using the solvent hexane, ethyl acetate and ethanol/water (80:20, v:v). The figure 1 show the results achieved in study.

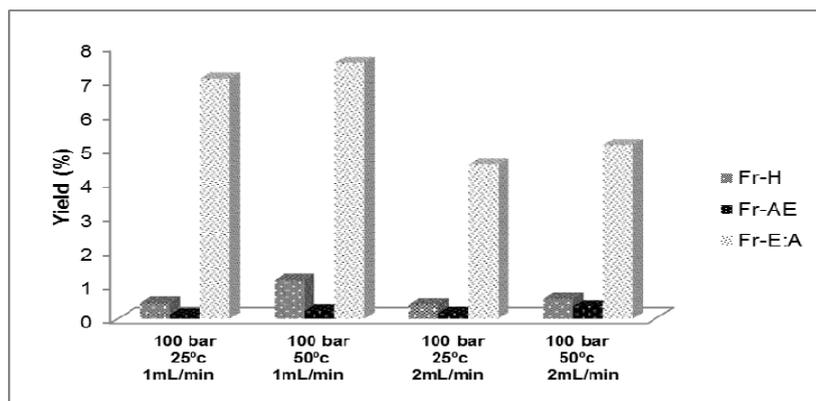


Fig. 1- Extraction in high pressure of extracts neem by solvents hexane Fr-H, ethyl acetate Fr-AE and ethanol/water 80/20 Fr-EA with changes of temperature and flow.

The main conclusions show that selective extraction and not by drag of the compounds, considering that the temperature increase from 25 °C to 50 °C increases the yield of extraction and of flow rate of 1mL/min to 2 mL/min decreases the extraction efficiency, showing solvents with solubility of the extracted compounds.

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# Evolution of body composition and anemia indicators in obese patients submitted to bariatric surgery

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**Background:** Evaluating body composition of obese patients makes possible for the health professional to realize about possible disturbances related to the regional accumulation of fat, to warn patients about the risks associated to excessive or decreased corporal fat levels and to verify the treatment efficiency. There are a lot of treatments for obesity and surgical treatment is one of them. Some population groups are more likely to develop nutritional deficiencies after bariatric surgery.

**Aim:** Study the evaluation of body composition, B12 vitamin and iron in patients submitted to bariatric surgery, during 24 months.

**Methodology:** In this retrospective study, patients who attend nutrition consultations in HSJ were evaluated. Anthropometric and biochemical data were collected before and after surgery - at 1, 3, 6, 12, 18 and 24 months.

**Results:** The sample consisted of 189 patients (163 females and 26 males) with a mean age of 41 years. The average height was 1.61m (dp = 0.079m). BMI decreased from 44,3kg / m<sup>2</sup> to 29.9kg / m<sup>2</sup>. Fat mass decreased 18.2%, free-fat mass decreased 6,4kg and the percentage of water increased 12.3% in women undergoing gastric bypass. There was an increase in the deficit of B12 vitamin from pre-surgery up to 12 months after surgery, contrary to Iron where there was a reduction of the deficit.

**Conclusion:** The study of body composition wins relevance each day in clinical practice. It is essential for proper action and in many cases can make a difference between success or failure. Assessing biochemistry is essential to ensure nutritional balance and prevent nutritional deficit or excess.

**Keywords:** Obesity, body composition, bariatric surgery, B12 vitamin and iron

## Formulation of extended release tablets: Thiamine Hydrochloride with Cutina® HR

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The development of sustained release formulations is currently one of the most important challenges in pharmaceutical research, because these delivery systems offer several advantages over immediate release formulations [1, 2]. Lipid excipients are very attracting for drug developers due to their performance, ease of use, versatility and potential to generate intellectual property through innovation in drug delivery, particularly in the case of modifying drug release systems [3].

The objective of the present work was to develop and characterize tablets with thiamine hydrochloride (vitamin B1), used as a model of a soluble drug, containing Cutina® HR (lipid matrix) to obtain a sustained release.

For this purpose, a specific formulation (batch of 100 g) was developed, containing thiamine hydrochloride (10 mg/tablet), Cutina® HR (hydrogenated castor oil; 48 % w/w), Avicel® PH-102 (microcrystalline cellulose), talc, Aerosil® (colloidal silicon dioxide) and magnesium stearate. For its preparation, drug, matrix excipient and filler were blended in a Turbula WAB mixer (model T2F) for 10 minutes. Afterwards, the lubricants were added and blended for 3 minutes in the same mixer. Tablets, with a target weight of 500 mg ( $\pm$  25 mg), were prepared by direct compression using a single punch compression machine (Korsch 9048-71). Then, weight uniformity (mean  $\pm$  standard deviation (SD), n = 20, analytical balance Mettler AE 200, Mettler Toledo), hardness (mean  $\pm$  SD, n = 10, tablet hardness tester Erweka TBH 28, Erweka GmbH) and friability (n = 15, 25 rpm/4 min, friabilator EF-1W, Electrolab) were evaluated in the obtained tablets [4]. In addition, the “in vitro” drug release study (mean  $\pm$  SD, n = 2) was performed in a dissolution apparatus Sotax (model AT7) according to the paddle method at 100 rpm [4]. The dissolution medium used was 500 mL of water at  $37.0 \pm 0.5$  °C. Aliquots of 10.0 mL were collected after 15, 30, 45, 60 and 90 minutes (without volume replacement). Samples were filtered and drug concentration was determined using a UV-VIS spectrophotometer Jasco (model V-650) at 273 nm (isosbestic point of thiamine hydrochloride). A vitamin B1 calibration curve was previously prepared ( $y = 24.627x - 0.020$ ;  $R^2 = 0.997$ ).

The outcomes showed that the produced batch presented uniformity of weight, a hardness average of 81N and a friability percentage of 0.94% ( $< 1.0\%$ ) [4]. *In vitro* dissolution test showed at 90 minutes a mean thiamine hydrochloride release of 45.2%.

Briefly, it can be concluded that the produced tablets showed good mechanical characteristics (hardness and friability), uniformity of weight and sustained release of vitamin B1.

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## Hepatitis E virus: How broad is the animal reservoir?

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Hepatitis E virus (HEV) is the most common cause of human acute viral hepatitis worldwide. Originally considered to be restricted to humans it is now clear that HEV and HEV-like viruses have a lot of animal reservoirs. The zoonotic foodborne transmission of HEV is nowadays well established and the increasing host range and identification of new reservoir species poses a purposeful public health concern [1]. HEV detection have been reported both in developed and developing countries in many animal species, such as domestic pigs, wild boar, deer, rabbit, and mongoose [2].

The aim of the present work was to search for the presence of HEV in stools of a variety of both domestic and wild animals, from different regions in Portugal.

Between 2007 and 2014, a total of 233 stools from different animals (68 dogs, 51 cats, 98 wolves, 11 rats, 1 horse, 1 mongoose, 1 gineta, 1 otter and 1 badger) were collected and screened for the presence of HEV RNA. Nucleic acid was isolated from stool suspensions (10%) using QIAamp Viral RNA Mini Kit (Qiagen, Germany). The detection of HEV RNA was performed using two reverse transcription real-time PCR (qRT-PCR) approaches, namely sybr green (KAPA™ SYBR® FAST Universal, KAPA Biosystems, USA) and probe (KAPA™ PROBE® FAST Universal, KAPA Biosystems, USA), using primers targeting a conserved region within open reading frame (ORF) 3 [3]. Subsequently, HEV RNA positive samples were tested by a nested RT-PCR (nRT-PCR) with two degenerated primer pairs constructed with binding sites within ORF1 region [4]. The amplified products were separated by electrophoresis using a 2% agarose gel (SeaKem LE Agarose, Lonza, USA).

The presence of HEV RNA was detected in 4 (5.88%) dogs, 9 (4.75%) cats and 6 (6.12%) wolves by qRT-PCR. No HEV RNA was detected in the stools of the other animal species. However, the 19 positive samples did not present the expected amplified product (330bp) when tested by nRT-PCR, which could indicate a different sequence in the ORF1 region of the HEV RNA detected in these animals.

In conclusion, this ongoing study has uncovered the first evidence about HEV circulation in domestic and wild animals in Portugal. Hence, novel animal reservoirs of this virus would certainly imply a greater risk of transmission to humans, requiring more attention to the public health.

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# Hospitalizations due to burns in Portugal: 2000 to 2010

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Burn injuries are, to patients and families, a traumatic event that will affect their lives afterwards. The quality of life maintenance is harder due to the associated morbidity [1,2]. Recent studies in Norway [3] and Sweden [4] showed a decrease of length of hospitalization stay. In Portugal, a study performed by *Natividade da Silva et al.*, did not show a significant difference in mortality during the studied time period [5]. However, there is a lack of analyses over the recent past years. This study aims to characterize hospitalizations due to burn injuries in Portugal.

We used the national hospitalization database provided by the Central Administration of Health System of the Portuguese Ministry of Health. This database includes 21,741 discharges in public hospitals from 2000 to 2010, in mainland Portugal. We considered only in-patients episodes with a principal or secondary diagnosis of burn injury, specifically the range of codes 940-949.x from the International Classification of Diseases - 9th revision - Clinical Modification (ICD-9-CM). The mean age over the years varied from 34.5 in 2000 to 42.1 in 2010, with a total age average of 37.3 years old (34.6 for men and 41.0 for women). Across the study period, mean charges varied between 5,666 and 7,915 Euros, with the total average of 6,482 Euros. In this decade, the lowest in-hospital mortality occurred in 2010 (3.4%), while the highest occurred in the years 2000 and 2005, with 5.2%. Considering the etiology of the injury, the most current were burns due to hot liquids/objects (43.0%), followed by burns due to fire/flames (33.3%).

Burns injuries represent an important burden and require an expensive care, as reflected in the increased mean charges. In the last years, in-hospital mortality has decreased from 5.2% to 3.4%, possibly indicating an improvement of health care in these patients.

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## Cell-free DNA levels in obese pediatric patients

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The prevalence of overweight and obesity in children and adolescents is increasing worldwide at an alarming rate, initially in developed countries but now also in many developing countries. Studies have shown that obesity in children and adolescents is strongly correlated with increased morbidity and mortality, reflecting a variety of pathological conditions with risk of persistence in the adult.

Adipose tissue produces and releases a number of mediators with an important role in the development of chronic low-grade inflammation state present in obese individuals. Circulating cell-free DNA (cfDNA) appears following cell damage and DNA release, and its levels are increased in several pathologic processes characterized by marked inflammation, namely cancer, psoriasis, preeclampsia and chronic kidney disease.

Our aim was to evaluate and compare the cfDNA levels in obese pediatric patients and study their association with systemic inflammation (cross-sectional study).

In this work, the study population consisted of obese children group (n=52) and a non-obese control group (n=10). Anthropometric parameters were measured, such as the weight, BMI z-score or waist circumference, and also biochemical parameters such as tumour necrosis factor – alpha (TNF-  $\alpha$ ), C-reactive protein (CRP) and cfDNA.

The results show a positive association between obesity and increased anthropometric values with high levels of biochemical markers, except for TNF- $\alpha$ , comparatively with a non-obese control group. In obese patients, CRP levels were significantly and positively correlated with cfDNA (n = 51, r = 0.311, p = 0.027).

Our results demonstrated in obese patients aged 6-12 years, a significant change in the CRP and in cfDNA levels; in obesity, cfDNA correlated with obesity markers and with inflammation level.

# Implementation of a methodology for the evaluation of skin penetration

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The skin is the first and the main barrier of the human body. *Stratum corneum* is the outermost layer and the rate-limiting step to skin penetration [1]. Knowledge about dermal permeability is important not only for pharmaceutical and cosmetic products but also for all chemical compounds that may come in contact with the skin, intentionally or accidentally. Skin absorption studies can be performed using animal or human models (*in vivo* or *in vitro*) or synthetic membranes (*in vitro* studies) [1]. The goal of this experiment is to implement a skin penetration evaluation method with human skin (*in vitro* study). To this end, some methods and processes must be validated, namely: skin membrane preparation, which includes the hypodermis removal and epidermis isolation; skin integrity evaluation by measuring Transepidermal Water Loss (TEWL); and evaluation of the penetration of a marker compound (caffeine). After obtaining approval of the Ethics Committee, skin samples were collected from women who were submitted to an abdominoplasty. Hypodermis was removed and the samples were frozen at -20°C. For each experiment, biopsies were obtained from frozen skin and then, the epidermis layer was obtained with a thermal method. The skin was heated at 60-65°C from 60 until 90 seconds and then peeled off with forceps. Epidermis was placed in the Franz cells of a 6-cell manual test system (Hanson Research). A gel with caffeine was added to the donor chamber and samples were collected from the receptor chamber (filled with PBS, pH 7.4). The epidermal integrity was evaluated for 24 h by measuring TEWL (Tewameter® TM210). During the *in vitro* test the stability of the temperature (32°C) was checked with periodic readings. Caffeine quantification was performed in a HPLC system equipped with a Brisa LC<sup>2</sup> C18 column, with particle size of 3 µm and a dimension of 10x0.46 cm (Teknokroma) with a pre-column (TR-C-160K1, Teknokroma) with the following conditions: flow rate of 1.0 mL/min, 20 µL injection volume, 10 minutes run time and detection at 273 nm. Several proportions of the eluent mixture (methanol:water) were tested in order to optimize the separation. In the epidermis isolation method, the samples that were heated during 60 or 70 seconds did not detach completely from the dermis, while for the samples heated during 80 or 90 seconds epidermis was easily peeled. The temperature was stable for 24h. The best eluent proportion for the quantification of caffeine was 25:75 (methanol:water) with a retention time around 6 minutes. The results of the skin integrity test were not conclusive, probably due to the high influence of ambient conditions. Until now, we were able to establish the conditions for epidermis isolation and the HPLC methodology for quantification of a marker compound. Further tests are needed to confirm the suitability of the proposed methodology for the evaluation of skin penetration.

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# **Inhibition of histone deacetylases with entinostat (MS-275) rehabilitates neuromuscular transmission deficits in autoimmune *Myasthenia gravis***

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Neuromuscular transmission deficits in *Myasthenia gravis* (MG) are attributed to autoantibodies raised against muscle-type nicotinic acetylcholine receptors (nAChR) [1]. Therefore, the therapeutic strategies to control muscle weakness and fatigability in myasthenic patients are mainly devoted to counteract excessive immune responses. Inhibitors of histone deacetylases (HDACIs) have recently received attention as important new tools for the treatment of autoimmune diseases [2]. Therefore, we decided to evaluate the therapeutic impact of HDACIs on neuromuscular transmission deficits in an experimental animal model of autoimmune *Myasthenia Gravis* (EAMG).

In this study, we assessed tetanic fading (fatigue) of diaphragm muscle contractions in response to high-frequency (50 Hz-bursts) nerve stimulation in EAMG animals treated with the selective inhibitor of HDAC1 and HDAC3, entinostat (MS-275, 3.5 mg/kg, i.p. q48h); controls received only vehicle (DMSO in PBS). Experimental autoimmune myasthenia gravis (EAMG), 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)[3]. 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.

Compared to control animals, EAMG rats exhibited a more intense ( $P<0.05$ ) tetanic failure (fatigue) of diaphragm muscle contractions induced by phrenic nerve stimulation with 50 Hz intermittent bursts (17 pulses per second). The initial tetanic peak tension rise ( $123\pm 6\%$  above baseline,  $n=8$ ) observed during the first 30 seconds of stimulation was absent in EAMG rats. Fifteen-day treatment with entinostat (MS-275) restored the magnitude of the initial facilitatory component to values observed in control animals. Tetanic tension decline (fatigue) reached a minimum of  $14\pm 5\%$  ( $n=8$ ) of the initial tetanic peak tension in the diaphragm of EAMG animals, but this value recovered significantly ( $P<0.05$ ) to  $34\pm 4\%$  ( $n=4$ ) after treatment with entinostat (MS-275).

Data show here for the first time that inhibition of HDAC1 and/or HDAC3 by entinostat (MS-275) can rehabilitate neuromuscular transmission failure in EAMG animals. Further studies are required to investigate if these findings are also observed with distinct HDACIs (e.g. tubastatin A) and whether these drugs target preferentially the pre- or the post-synaptic component of the myasthenic neuromuscular junction.

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## Insulin exhibits an antiproliferative effect in first trimester human extravillous trophoblasts

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In the early weeks of human pregnancy the embryo implants completely into the uterine wall, surrounded by trophoblast cells [1]. A lineage of extravillous trophoblast (EVT) detach from the placental villi and invade directly through the decidual stroma or advance along the luminal endothelium of decidual spiral arteries, resulting in increased blood flow to the growing fetoplacental unit. An inadequate EVT invasion can result in preeclampsia, fetal growth restriction or abortion [2]. Therefore, it is important to understand the factors that control EVT invasion capacity. In this context, our study aimed to investigate the influence of diabetes mellitus (DM) in the placentation process. For this purpose, we investigated the effect of insulin (a hallmark of both type 2 DM and gestational DM) upon cell proliferation (<sup>3</sup>H-thymidine incorporation assay, which measures mitosis index), culture growth (sulforhodamine B assay, which measures total protein content), apoptosis (TUNEL assay) and migration (injury assay) of a human first trimester EVT cell line (HTR8/SVneo cells). In addition, we also investigated the intracellular mechanisms involved in the effect of insulin on these parameters.

HTR8/SVneo cells were exposed to insulin (10 nM) for 24, 48 and 72hrs. A 48h-exposure to insulin caused 18% decrease in cell proliferation in 4-day old cell cultures. No significant effect was observed after 24h and 72h exposure to insulin. A similar (13%) inhibitory effect of 48h insulin was observed in 7-day old cultures. Curiously, a 20% increase was observed in culture growth after a 48h exposure of 4-day old cultures to insulin. Again, 24h and 72h insulin was devoid of effect, and the effect of 48h-insulin was also observed in 7-day old cultures (12% increase). Exposure to insulin (10 nM) for 48h did not show a significant effect upon the apoptosis index and the migration capacity. Moreover, we verified that the antiproliferative effect of insulin was reversed by simultaneous (48h) exposure to either the specific inhibitor of the mammalian target of rapamycin (mTOR) (rapamycin; 100 nM) or the specific inhibitor of phosphoinositide 3-kinase (PI3K) (LY 294002; 1 μM).

Taken together, these data suggest that insulin causes hyperplasia of HTR8svNeo cells, as it decreases the cellular mitosis index (as assessed with the <sup>3</sup>H-thymidine assay) while simultaneously increasing culture protein content (as assessed with the SRB assay). Moreover, our data suggest that the antiproliferative effect of insulin involves activation of mTOR and PI3K intracellular pathways.

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# Insulinresistance in patients undergoing bariatric surgery

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**Background:** Obesity has become an epidemic worldwide and is associated to the development of various diseases such as diabetes mellitus type 2. In 2014, the overall prevalence of DM2 was estimated at 8.3% of the adult population and 23% of patients morbidly obese have DM2 associated. Compared to conventional treatment, bariatric surgery appears to be a viable option for the treatment of severe morbidities associated with obesity, such as type 2 diabetes.

**Aim:** To evaluate the evolution of Insulin Resistance in patients undergoing bariatric surgery.

**Methods:** Retrospective analysis of medical records and collection of demographic, anthropometric and clinical and 145 patients who attended the Multidisciplinary Assessment Consultation for the Surgical Treatment of Obesity of Hospital de São João, E.P.E., with primary diagnosis of obesity submitted to gastric banding, Sleeve or Bypass. Patients were split by groups of diabetic and without diabetes. Insulin resistance was measured with Quiki, Homa-IR and Glucose/ Insulin ratio.

**Results and conclusions:** In the pre-surgery time the patients median of Body Mass Index was 44,8 kg/m<sup>2</sup> for diabetic patients and 42,9 kg/m<sup>2</sup> for non diabetic. There is a steady decrease in this parameter up to 18 months after surgery. After the surgery, the proportion of patients with IR decreased, as well as the proportion of patients taking diabetes medication.

**Keywords:** Insulin Resistance, Bariatric Surgery, type 2 diabetes, HOMA-IR, Quiki, Glucose / Insulin ratio.

# Klinefelter Syndrome – a cause of male infertility

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The WHO defines infertility as “absence of pregnancy after two years of regular sexual intercourse without contraception use”. Several studies indicate that infertility is expressed in 14% of the western population [1]. We are currently faced with a decline in sperm count and motility, with azoospermia responsible for 10-15% of cases of male infertility [2]. The causes of genetic azoospermia can be divided into non- obstructive and obstructive [3].

Klinefelter syndrome is the most common genetic cause of male infertility (1:500). This syndrome results in reduced growth of the penis and testicles which will be responsible for the failure of spermatogenesis, which explains azoospermia detection in 74% of men with this syndrome [3].

A case of infertility in men who suspected genetic cause was studied. We started with blood collection that will be processed in cytogenetics laboratory. In the laboratory begins with short-term culture of cells, a period of cell growth and multiplication, cells are arrested in metaphase and its handling and spreading technique and GTL bandage which allow obtaining chromosomes with bands for subsequent optical microscopy analysis.

After this observation we noticed the existence of 47 chromosomes, 22 pairs of autosomes and three sex chromosomes, XXY. This observation confirms the diagnosis of Klinefelter Syndrome (47, XXY) and its etiology of couple's infertility.

In the past, men with Klinefelter Syndrome had no hope of having children. Today, with the advent of IVF and ICSI technique in combination with TESE can help more than 50% of men [4].

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# Lifelong musculoskeletal injuries in competitive rowers

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Competitive rowing has been associated to traumatic and overuse injuries that may lead to long periods of inactivity or retirement. There are several studies [1,2] on injury occurrence, but none about lifelong injuries and none in Portugal. The objective of this study was the assessment of the injuries occurred over the lifetime of Portuguese senior competitive rowers.

We sent a questionnaire to all Portuguese senior rowers medalled in the national championships during the 2013-14 season. We analysed biometric variables, anatomic location, type and circumstances of injury occurrence, type of treatment provided and time of inactivity. For statistical analysis we used parametric and non-parametric statistics with 95% confidence levels ( $p < 0,05$ ).

The questionnaire was answered by 18 of the 18 female and 74 of the 77 male rowers. Females had significantly lower ages, heights and weights and rowed more often sculling boats ( $p < 0,05$ ). The number of years of practice, as well as the number of injuries per rower was similar, for both females and males. The mean age at the first injury was significantly lower in females ( $p < 0,001$ ), who also had more overuse lesions than traumatic ones, although not significantly. In both, the most frequent anatomical location and type of injury were the lumbar region and muscular pathology, with a higher occurrence in winter and spring, during land training. Females sustained longer periods of inactivity, but not significantly. Surgical treatment was rare, more associated to males and to knee injuries. The time spent by the teams in injury preventive sessions was very scarce.

The lifelong injuries sustained by Portuguese competitive rowers were similar to those reported by other authors [1,2] regarding anatomical location, type of injury and circumstances of occurrence. However, in our study, females presented a risk of injury occurrence at significantly lower ages than males. The differences in results between our and other studies may be related with differences in the studied populations, study time span or other factors that need to be clarified.

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## Lithium levels in the human brain: A post-mortem study of anatomical region differences and age-related changes

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Lithium (Li) has long been used as a treatment for some psychiatric diseases, in particular bipolar disorder, a serious medical illness characterized by episodes of depression and mania [1]. Although it is well known that Li improves mood and reduces aggression potential, its exact biological mechanism of action in the brain is not clearly known. On the other hand, recent studies also suggest that Li provides neuroprotective effects in neurodegenerative diseases (ND) such as Alzheimer's and Parkinson's disease [2], opening the possibility of an alternative use of Li as a novel treatment for ND, but the exact mechanism by which it exerts these effects is also unclear. Furthermore, the brain is a highly heterogeneous organ with anatomically and physiologically different areas that may be selectively affected by the aging and neurodegenerative processes. Therefore, a detailed study of the anatomical distribution of Li in the "normal" brain is essential to clarify its role in the human brain physiology and ND.

Based on this background, we conducted a work aiming to study, directly in human brain samples: 1) the regional anatomic differences of Li levels within the brain tissue and 2) the changes on Li levels in relation to age.

From neurologically and psychiatrically healthy individuals submitted to autopsy (n=42; 71±12, range: 50–101 years old) the following 14 brain areas were sampled: frontal cortex, superior and middle temporal, caudate nucleus, putamen, globus pallidus, cingulate gyrus, hippocampus, inferior parietal lobule, occipital lobe, midbrain, pons, medulla and cerebellum. After samples microwave-assisted acid digestion in closed vessels, Li were determined by inductively coupled plasma-mass spectrometry.

Considering the whole data set (n=588; 42 individuals x 14 brain areas), Li was not detected (< 0.05 ng/g) in 32.7% of the samples. Globally (i.e., considering the mean value of the results obtained for the 14 regions in each individual with measurable levels), mean Li content was 5.38±3.43 ng/g, ranging 1.35–28.8 ng/g (on a dry weight basis). Lithium distribution within the brain showed to be heterogeneous: highest levels were found in the caudate nucleus (9.80±10.6; range: 0.05-47.4 ng/g), a brain region related to declarative (or cognitive) memory and learning, and the lowest in the cerebellum (3.65±5.90; range: 0.05-27.18 ng/g). No significant gender- and age-related changes were found.

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## Modulation of Macrophage Motility and Differentiation by Human Colorectal Cancer Cells

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Tumours are complex microecosystems composed of cancer cells, extracellular matrix components and other cell types. Tumour-associated macrophages (TAMs) constitute, in many tumours, the major inflammatory cellular component of the stroma. Depending on the surrounding tumour microenvironment, TAMs may express distinct functional profiles, harbouring tumour-preventing (M1-like pro-inflammatory macrophages) or tumour-promoting (M2-like anti-inflammatory macrophages) activities. Tumour cells could take advantage of these macrophage functional heterogeneity and plasticity to shape the local inflammatory milieu, promoting tumour progression.

The aim of this project is to unravel the impact of colorectal cancer cells on macrophage motility and inflammatory profile. At first, monocytes isolated from healthy blood donors, were allowed to differentiate, during ten days, in the absence of exogenous stimuli. After this, macrophages remained unstimulated or were stimulated with pro-inflammatory (LPS, bacterial lipopolysaccharide) or anti-inflammatory (IL-10, interleukin-10) factors for additional 72 hours. During this period, macrophages were maintained in the presence or absence of cancer cell conditioned medium (CM).

Through immunocytochemistry analysis, our results demonstrated that cancer cell CM affects macrophage motility and their actin-tubulin structural organization. This effect was more evident on macrophages differentiated in the presence of LPS- and IL-10 than on their unstimulated counterparts. Interestingly, we observed that, in the presence of cancer cell CM, both the elongated pro-inflammatory or the rounded anti-inflammatory macrophages became smaller and acquired a rounded-shape, with alterations of their actin-tubulin distribution. In addition, we observed that soluble factors released by the cancer cells abolished LPS-stimulated macrophage expression of CD80, a pro-inflammatory cell surface receptor. In contrast, no alterations were induced by such soluble factors on IL-10-stimulated macrophages, regarding the expression of CD163, an anti-inflammatory cell surface receptor. Altogether, our results suggest that cancer cells are modulating macrophage differentiation towards an anti-inflammatory profile.

To confirm this hypothesis, indirect co-cultures between macrophages and colorectal cancer cells, on static Transwell-filters or dynamic microfluidic chambers, are being established. The effect of colorectal cancer cells on macrophage surface receptor expression and cytokine profile is currently under investigation.

# Neonatal cardiac injury: To regenerate or not to regenerate, that is the question

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Cardiovascular diseases are the leading cause of death in the modern World [1] and until the past century the mammalian heart was considered to be a non-regenerative organ, displaying no proliferative capacity shortly after birth [2]. However, Porrello *et al.* recently reported that 1-day-old neonatal mice successfully regenerate the heart after apex surgical resection, forming new muscle without generating a scar. In contrast, after post-natal day (P)7, this phenomenon no longer occurs and extensive fibrosis is observed [3]. These findings were considered a milestone in cardiovascular field. Though, in 2014, Andersen *et al.* refuted this model by reporting that neonatal murine heart is incapable to recover after resection and developed a non-functional fibrotic scar [4].

This work aims to characterize the neonatal regenerative response through the evaluation of the dynamics of cardiac fibroblasts and extracellular matrix (ECM). Moreover, given the controversy surrounding the concept of cardiac regeneration, a deeper characterization on the neomyogenic potential after injury was performed.

Herein, we showed that fibroblasts, defined as the CD31<sup>+</sup>CD45<sup>-</sup>Ter119<sup>-</sup>CD90<sup>+</sup> population, colonize the heart following birth and that the cell-surface signature of this compartment changes throughout post-natal life. By reproducing the neonatal heart injury model we were able to identify that the response to injury involves the recruitment of inflammatory cells, fibroblast activation, ECM production and neo-vascularization. Importantly, High Content Analysis (HCA) revealed that the removed tissue was, at least in part, re-established by the proliferation of resident cardiomyocytes and that, 60 days after resection, no elicited signs of hypertrophy were detected. Despite formation of scarring tissue (inner core of the injured area) and incomplete histological restoration, resected hearts were functionally competent at 21 days post-lesion.

Overall this work describes the microenvironmental alterations, with particular emphasis on fibroblasts and ECM, triggered following neonatal apex resection and that culminate on partial restoration of the organ.

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# Characterization of Proteolysis in two types of barley, *Quench* and *Tipple*

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Barley malt is used mainly as a raw material in beer. The variety of barley and conditions malting process have an important role in malt composition and consequently in quality of beer. Malting is the controlled germination of the seed and the malting process has three main steps: steeping, germination, and kilning [1]. Extensive proteolysis occurs during malting and the condition of this process, like temperature and humidity, will determine the qualitatively and quantitatively protein composition. Protein content malt has a great influence in quality of beer, particular in flavour, taste and mouthfeel [1]. Hordeins are the most abundant proteins, but albumins and glutelins (globulins) are also relevant proteins [2]. The aim of this study was evaluate influence of kilning temperature in protein contend and it was used two variety of barley, *Quench* and *Tipple*.

Hordeins, albumins and other soluble proteins were characterized by size-exclusion high performance liquid chromatography (SE-HPLC) and the protein profiles of the different malts were compared. Free amino acids were quantified by GC–MS followed derivatization with MTBSTFA [3].

The SE-HPLC analysis enabled the quantification and identification of proteins with different molecular weights to comparison of varieties of barley. Typical chromatograms peaks were assigned based on their retention times (RT). With RT between 2-15min belongs to 205-51 kDa, peaks from RT 15-26min corresponds to proteins and polypeptides >6.6 kDa and < 51 KDa. Peaks between TR 26 to 38 min are from polypeptides <6.6kDa. When compared different varieties of barley, protein contend in malt was different, but when compared different kilning temperatures for same variety of barley, protein contend in malt was similar.

Composition in free amino acids is strongly influence by variety of barley. *Tipple* barley has a lower concentration in free amino acids that result in malt poor contend in free amino acids. In other hand, in *Quench* barley, free amino acids contend in malt differs according the kilning temperature was used.

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# Characterization of the Glutamine synthetase (GS) gene family in *Solanum lycopersicum* (cv. Micro-Tom)

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*Solanum lycopersicum* (L.), also known as tomato plant, is a member of the SOLANACEÆ family. Its fruit, present in almost every world diet, may be eaten in its raw state, or it can be processed into sauces, juices, ketchup and pasta. In a nutritionist view, tomato and its products, are a good source of vitamins, minerals and fibers, are low in calories and have high contents of carotenoids, beta-carotene and lycopene, which confers the red color and is also a great antioxidant [1].

Currently, and accordingly to FAOSTAT and WPCT 2015, the tomato world production is growing, predicting it will reach 41 602 000 metric tons this year, which represents a growth of 4,3 % relatively to last year [2]. In Portugal this crop is gaining economic importance, since our country has become the eighth larger producer of this fruit in 2013 [3], and is also a leading exporter of tomato concentrate. We also have in our lands very suitable conditions for the production of tomato, fact reflected in 2011, when we had the second more productive tomato culture with 87 t/ha, being only surpassed by California [4].

Tomato culture is made mainly as a protected culture whereby the environmental conditions could be adjusted to improve its productivity. Glutamine synthetase (GS) is a key enzyme in the improvement of plant yield and N-use efficiency and the study of the environmental conditions more suitable to increase its activity are of pivotal importance [5].

This work aimed to identify the whole *S. lycopersicum* GS gene family and to characterize the expression of the different GS genes all through the plant. A search on genome databases showed the existence of 5 GS genes in *S. lycopersicum*, four encoding cytosolic isoforms (collectively termed GS1) and one encoding a chloroplastic isoform (termed GS2). We collected samples from different organs of *S. lycopersicum* cv. Micro-Tom (leaves, roots, flowers, fruits, senescent leaves, leaf blades and ribs), extracted mRNAs and produced cDNAs from the different samples, and GS gene expression was analysed by RT-PCR by using primers designed specifically for each of the genes. GS expression patterns reveal a differential expression of the five genes in *S. lycopersicum* plant, disclosing divergent roles of the different GS isoforms in plant metabolism. Underpinning this previous findings further work will be done to pinpoint environmental conditions like N-feeding availability, stress situations and developmental stages, with differential expression of the GS genes. This work will contribute to a better understanding of nitrogen metabolism and its regulation in *S. lycopersicum* and will open new ways to conveniently explore the involvement of GS in nitrogen use efficiency and plant productivity in this important crop plant.

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# Cobalamin receptors in vertebrates: transitions for novel functions?

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Cobalamin (Cbl), or Vitamin B<sub>12</sub>, is a vital cofactor that mediates the reaction of methylation of homocysteine to methionine and the isomerization of L-methylmalonyl CoA to succinyl CoA [1]. In humans, after intestinal absorption, Cbl binds to the carrier protein transcobalamin (TCN2) in the blood. The transcobalamin receptor (TCbIR), encoded by the CD320 gene, is responsible for the internalization of the complex TC-Cbl in target cells [2]. A knock-out mouse model suggests that this receptor is essential for TC-Cbl internalization in the central nervous system; however, in other tissues, additional, yet unidentified mechanisms seem to compensate for the lack of CD320: for instance, the large spectrum receptor, belonging to the low density lipoprotein receptor (LDLR) family [3].

The TCbIR is composed of two LDLR-A domains, typical of lipoprotein receptors, which suggests that they might be evolutionarily related. After examining the “*locus of origin*” of the CD320 gene, we found that it is well conserved in mammals, birds, reptiles, amphibians and fish. However, while mammals exhibit a highly conserved two-domain receptor, fishes and amphibians retain a gene coding for a longer receptor, with additional LDLR-A domains, similar to LDLR/VLDLRs. Birds and reptiles, present a shorter receptor yet, with lower sequence and domain conservation when compared to mammals. These observations put forward a truncation event of a LDLR/VLDLR-like gene, at the base of amniotes, which gave origin to the Cbl-specific receptor.

Thus, combining synteny, phylogenetic and gene expression analyses, we propose to elucidate the evolutionary origin of the CD320/LDLR/VLDLR-like gene. Also, using cell-based internalization assays, we will functionally characterize the affinity of LDLR/VLDLR-like receptor towards TC-bound Cbl and LDLs. Together these results should contribute for a better understanding of vertebrate Cbl metabolism evolution.

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## **Cyanobacteria from the McMurdo Dry Valleys (Victoria Land, East Antarctica): Their Biodiversity and Chemodiversity**

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Cyanobacteria constitute one of the largest, most diverse and widely distributed group of prokaryotes. In fact, these photosynthetic microorganisms are present even in extreme environments such as those from cold Polar Regions, where they play an important ecological role as primary producers and nitrogen fixers. Moreover, cyanobacteria are well recognized as a prolific source of complex bioactive secondary metabolites. However, little is known about the potential of Antarctica's cyanobacterial strains for the production of those bioactive molecules.

Here, we present a project that aims to study the diversity of cyanobacteria from the Dry Valleys - the largest ice free zone in continental Antarctica and one of the coldest and driest terrestrial environmental on Earth, with the objective of searching for new secondary metabolites produced by Antarctica's cyanobacteria isolates. So far, two endolithic strains have been successfully isolated, from a sandstone sample. Additional samples collected from soil are in isolation process. Preliminary data derived from the morphological and molecular characterization (16S rRNA gene sequencing; detection of polyketide synthase genes (PKS) and nonribosomal peptide synthase genes (NRPS) by PCR) indicate that two of the strains exhibits high similarity with *Leptolyngbya antarctica* (and fits within the clade harboring *Nodosilinea* strains) and possess genes involved in the PKS biosynthetic machinery, and thus has the genetic potential to produce bioactive compounds of polyketide nature. In order to isolate secondary metabolites produced by this strain (and from others that may eventually be isolated), large-scale cultivation followed by organic extraction of the biomass has been performed. Bioassays will then serve as a guide for isolation. An overview of the whole experimental approach is conveyed.

# Detection of antibiotic resistant coliforms on recreational coastal sands in North of Africa and South of Portugal

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Bacterial resistance to antibiotics is a growing medical and ecological problem worldwide, generated by the selection processes following the massive use of antibiotics. The dissemination of multidrug resistant bacteria and their reservoirs represents a huge preoccupation in terms of public health. In our previous work the presence of antibiotic resistant bacteria in sands in the north of Portugal was evident with special focus on Extended Spectrum Beta Lactamase (ESBL) producing coliforms. [1,2] To improve our research we collected new samples from beaches with different characteristics of those of previous works in terms of putative biological pollution. We collected samples from south of Portugal and from Morocco beaches.

Dry and wet sand was collected in five beaches in the summer of 2014, one in Morocco (bathing area and fishing area) and four in south of Portugal. Four grams of each sample were suspended in 40 mL of Tryptic Soy Broth (TSB) and incubated overnight at 37°C. Isolates were selected by spreading 200µl of broth on MacConkey agar and MacConkey agar with ampicillin. Representatives of different colony morphotypes that grew on MacConkey agar with ampicillin were randomly selected and tested for antimicrobial susceptibility by agar diffusion method according to the Clinical Laboratory Standards Institute (CLSI).

All selected isolates from Algarve sand were lactose non-fermentative and showed resistance to amoxicillin with clavulanic acid. In Morocco sand isolates showed sensitive to all of the antibiotics tested and just one lactose fermentative isolate, resistant to ampicillin, amoxicillin with clavulanic acid and cefoxitin. No cephalosporin, carbapenem and fluoroquinolone resistance was detected in Algarve and Morocco sand.

According to our knowledge, this is the first study in Portuguese and Morocco beaches. South of Portugal has very prestigious and crowded beaches, and the analyzed sand is from beaches distinguished by Quercus with golden quality. Morocco beach was a very calm and clean beach.

The detection of antibiotic resistant coliforms and their occurrence in the sand of recreational beaches can possibly be used as an alternative bioindicator of beach sand contamination by anthropogenic activities and of the returning rate of resistant isolates and resistance genes to the human population through the seawater and the marine coastal sand beach use [3].

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# Development of extraction methodology for simultaneous determination of bisphenol A and tetrabromobisphenol A in seafood by LC-MS/MS

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In last years a significant increase in public concerns about the adverse effects of substances with endocrine disrupting properties, like bisphenol A (BPA) and its analogous tetrabromobisphenol A (TBBPA) has been observed. BPA is a synthetic chemical highly used in the production of polycarbonate plastics and epoxy resins used in several food containers such as baby bottles and drink cans [1]. TBBPA is a known brominated flame retardant (BFR) present in epoxy, polycarbonate and phenolic resins used in printed circuit boards and [2]. When conditions that trigger it occur, these chemicals may leach into the ambient and accumulate in the environment. Both compounds can act as estrogenic agonists, in animal and human organisms with harmful effects even in small quantities [3].

The aim of this work was the optimization of an analytical procedure based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the simultaneous determination of BPA and TBBPA on seafood samples. Initially, three extraction procedures described in literature were compared. Best results were achieved with the use of QuEChERS (quick, easy, cheap, rugged, safety) procedure followed by liquid-liquid cleanup. The optimized method was validated concerning to linearity, repeatability (intraday precision and interday precision) and recovery.

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## Effects of $\Delta 9$ -tetrahydrocannabinol in human endometrial stromal cells

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The main psychoactive component of *Cannabis sativa* is  $\Delta 9$ -tetrahydrocannabinol (THC), which exert its effects by binding to specific plasma-membrane receptors (CB1 and CB2). These are G protein-coupled receptors, their activation diminishes cAMP levels and both are coupled to intracellular signaling pathways related to activation of mitogen-activated protein kinases (MAPK) [1]. CB1 receptors are distributed extensively in neural tissues, where their distribution has been well characterized. In addition, CB1 has been localized to ovary, uterine endometrium, urinary bladder and other peripheral endocrine and neurological tissues. CB2 receptors, in contrast, have a fairly limited distribution, being found predominantly in immune cells [2].

Human endometrium regeneration, which occurs throughout a woman's lifetime, is crucial for embryo implantation and pregnancy establishment. Smoking marijuana has always represented a warning for the long lasting effects not only on physical and mental performances but also on the reproductive events and several studies have correlated *Cannabis sativa* consume with deregulated ovulation and endometrium regeneration [2]. In addition, it has been shown that cannabinoids induce apoptosis in various cell types [3]. However, the mechanisms behind these effects are not fully understood. We have previously shown that primary human endometrial stromal cells (HESCs) and the endometrial stromal cell line (St-T1b) express both cannabinoid receptors [4]. Then, this study intends to evaluate the effects THC may have on both HESCs and St-T1b.

Cell viability was accessed by MTT assay, whereas cell morphology was evaluated by phase contrast microscopy, Giemsa and Hoechst staining. In addition to these experiments we intend to carry out Western Blots to evaluate the effect on p38 and p42/44 expression. Concentration of THC between 0,1-50  $\mu$ M have not revealed cell viability reduction or altered morphology.

Although THC does not affect cell viability, it may interfere with endometrial stromal cell function through the activation of the proteins p38 and p42/44. Thus, further studies are required in order to understand the mechanisms behind the effects of THC on infertility.

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## Effects of 24-epibrassinolide in *Solanum lycopersicum* L.'s tolerance to aluminum toxicity

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Brassinosteroids (BRs) are a class of steroid phytohormones vital for growth and development of higher plants. Besides their regulatory functions in cellular metabolism and homeostasis, BRs are involved in plant responses against biotic and abiotic stresses, namely in heavy metal (HM) toxicity [1]. Prevalence of soluble aluminum ( $Al^{3+}$ ) ions in soils represents the main growth-limiting factor for plant crop cultivation. Soil acidification by Al affects 40% of arable land throughout the world. *Solanum lycopersicum* L. is a plant species with great economic importance, particularly by the worldwide rate consumption of its fruits. Until now very few information is described in literature regarding the mechanisms of Al tolerance in tomato plant. Therefore, the aim of this study was to evaluate the responses of *S. lycopersicum* to Al exposure and the possible ameliorative role of 24-epibrassinolide (EBL) against Al-induced phytotoxicity.

Studies regarding seedling germination in medium supplemented with different concentrations of Al (0, 50, 100 150 and 200 ppm) and biometric evaluations were performed to determine the Al concentration to be used in further studies. Then, *S. lycopersicum* plants were grown hydroponically for 30 days under four situations: control plants, watered with Hoagland solution (HS); Al-exposed plants, watered with HS+Al 100 ppm; Al/EBL-treated plants, watered with HS+Al 100 ppm, but previously sprayed with EBL 1  $\mu$ M and EBL-treated plants, watered with HS and sprayed with EBL 1  $\mu$ M. Collected plants were separated into shoots and roots, and biometric parameters, lipid peroxidation (LP) and free proline (Pro) content were analyzed.

Phytotoxicity studies regarding seedling germination in medium supplemented with the different concentrations of Al demonstrated that this HM had negative effect at 150 and 200 ppm, since the rate of germination decreased 29 and 43%, respectively. Primary root length significantly decreased in all Al treatments when compared to control situation. At harvest, no significant differences were found between the four groups of plants in shoot fresh weight, however, plant exposure to Al caused a reduction of 41% in root fresh weight when compared to control. The pre-treatment with EBL significantly alleviated the negative effect of Al. LP levels in shoots were similar in the all groups of plants. The evaluation of Pro content showed that the level of this antioxidant increased in both Al- and EBL-treated plants, by about 54% compared to control situation, while Al/EBL-treated plants exhibited similar values of Pro to those of the control plants.

These preliminary results suggest that Al provoked toxicity at germination level and in primary root length. Also, we observed that root fresh weight was negatively affected by Al treatment, although no deleterious effects of this HM were detected in shoots of Al-treated plants. Evaluation of LP and Pro content in roots, as well as other parameters of the antioxidant system are in progress.

\*These authors contributed equally to this work

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# Effects of temperature and light on the development and productivity of *Laminaria ochroleuca* gametophyte cultures

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Kelps forests are one of the most diverse and productive ecosystems worldwide. Global climate change and human exploitation threaten the stability of many of these ecosystems. In this study we analysed the effects of temperature and light on meiospore germination and growth of gametophytes of *Laminaria ochroleuca*. Germination were measured in culture for two types of light and under two different temperatures (10°C and 15°C). Biomass and pigments were measured in culture for two types of light under 15°C.

In this study, the highest germination percentages (83% and 86%) were found at the highest temperatures employed in the experiment, 15°C, these were similar to the original temperature at their site of origin and were lower (74% and 79%) at colder temperatures (10°C). The experiment undertaken with *L. ochroleuca* gametophytes showed higher values of germination, biomass and gametophytes pigments under white light conditions compared to red light. At red light meiospores had lower germination percentages (74% and 83%) than meiospores cultivated at white light (79% and 86%). Relatively biomass, highest values were obtained for cultures exposed to white light and in gametophytes pigments, the higher values were always obtained for the cultures exposed to white light too.

In conclusion, maximum percentages of germination were obtained at 15°C and white light. Relatively biomass and pigments the higher values were obtained at white light and according to the results obtained the best conditions for culturing gametophytes of *Laminaria ochroleuca* are at white light and 15°C.

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# Efficiency of different enrichment media in the detection of gram negative bacilli in human, animal and environmental samples for antimicrobial susceptibility studies

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Gram-negative bacteria of *Enterobacteriaceae* family are important causing agents of urinary tract and bloodstream infections, pneumonia and various intra-abdominal infections. Antibiotic resistance in *Enterobacteriaceae* is a significant problem as they are present in different ecological niches<sup>[1]</sup>. Once the literature for this subject is scarce, this study developed a methodology for the culture and isolation of gram negative bacteria by comparison of performance of five enrichment broths: tryptic soy broth (TSB), brain heart infusion broth (BHI), brilliant green, peptone water and buffered peptone water, for the detection of gram negative bacteria, namely coliforms, in 35 samples of different origins: human, animal (domestic and bovine) and environmental (water, sand and mud). All samples were measured (100mL of water by membrane filtration, 3g for sands, 1g for the other ones), incubated at 37° C overnight with 40 mL of enrichment broth. Cultures were diluted from 1:10 to 1:100 000 and spread in a solid selective for Gram negatives and differential culture media (MacConkey), to detect different colony types. Colony-forming units were analysed in order to differentiate lactose fermenting and non-fermenting bacteria morphologies, from the MacConkey agar plates with better colony dispersion. Although all enrichment media demonstrated capacity of increasing the bacterial mass of the samples, it was found that TSB is an efficient enrichment medium without favouring the growth of lactose fermenting or non-fermenting gram negative bacilli; Brilliant green showed a prevalent enrichment of lactose fermenting gram negative bacilli; BHI, peptone water and buffered peptone water provided a significant increasing of lactose non-fermenting gram negative bacilli. It was also found that environmental samples are those who most need to be enriched, unlike human and animal samples, whose enrichment may be dispensed due to its huge natural flora. As environmental (sands) and animal (cats and dogs) isolates are part of dynamic niches that have a correlation with each other, we thought that it would be important to test isolates resistance to some antibiotics to search for connections in terms of antibiotic resistance dissemination. Samples were suspended in TSB and inoculated in MacConkey agar with ciprofloxacin, cefotaxime and meropenem. Colonies were randomly selected and tested for antimicrobial susceptibility according to the Clinical Laboratory Standards Institute. Animal samples showed the presence of multi-resistant lactose fermenting *Enterobacteriaceae*, which is a worrying question in terms of public health<sup>[1]</sup>. These results showed the effectiveness of TSB for detection of isolates resistant to antibiotics towards other enrichment broths in this specific approach.

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# Encapsulation of a Natural Bioactive Compound for Industrial Applications

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Natural matrices represent a rich source of biologically active compounds, such as antioxidants, vitamins and minerals, having a recognized potential for the development of pharmaceutical, cosmetic and food products [1]. Bioactive compounds commonly found in plants and herbs have been shown to have beneficial properties, including antioxidant, photo-protective, anti-inflammatory, anti-allergic, antimicrobial and anti-carcinogenic. They also demonstrated to have mild-features, low side effects, non-toxicity and strong activities [2]. In particular rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis* L.), thyme (*Thymus vulgaris* L.) and mint (*Mentha sp.*), native to the Mediterranean region in countries including Portugal, possess very useful antioxidant and anti-carcinogenic properties. This appears to be related to their content in phenolic compounds, amongst which rosmarinic acid was found to be one of the most important [3]. Unfortunately these natural bioactive compounds are often unstable and sensitive to temperature, pH, light and oxidation, and may undergo reactions that lead to the reduction or loss of its effectiveness, or even to the degradation of the product. Moreover, they often present a poor bioavailability mainly due to low water solubility, which limits their transport across biological barriers. Stability and solubility of the bioactive ingredients is also essential during formulation, storage and application of the final product. To circumvent these drawbacks delivery systems have been proposed to increase stability, to protect against degradation, and also to direct and control the release of natural bioactive ingredients. Microencapsulation offers an ideal and unique carrier system for bioactive ingredients, as it has the potential to respond to all of these requirements. Many encapsulation methods are described in the literature, among which some have been successfully applied to natural bioactive compounds. The spray-drying method is one of the most used due to its low cost, availability of equipment and efficiency [4]. Biopolymers and biodegradable polymers are the encapsulating materials with greater interest for pharmaceutical, cosmetic and food applications [5]. The purpose of this study was to prepare and characterize biopolymer-based microparticles encapsulating rosmarinic acid (RA), a natural antioxidant, by a spray drying technique, as means to overcome its limitation in industrial formulations. A satisfactory product yield was obtained. An analytical method for RA determination was developed and validated.

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## Establishing lung cancer drug resistant cell lines

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More than one-quarter of all cancer deaths are due to lung cancer [1], which constitutes a significant public health problem. Drugs currently used in the treatment of lung cancer may consist of traditional chemotherapeutical drugs (e.g. doxorubicin) [2] or the more modern molecular-targeted drugs (e.g. erlotinib) [3]. Nonetheless, lung cancer patients often relapse due to intrinsic or acquired drug resistance. Drug resistance may be due to several causes such as low intracellular drug concentrations caused by the overexpression of drug-efflux pumps (e.g. P-glycoprotein or multidrug resistance associated protein), increased DNA repair, defects in apoptosis, mutations in drug targets, amongst others [4].

The identification of causes of drug resistance is crucial for the development of new drugs to overcome this problem. One approach to identify causes of drug resistance, and thus new therapeutical targets, is to compare drug resistant cells with their normal counterparts. For such, the creation of drug resistant cell lines from parental drug sensitive cell lines is imperative. Therefore, the objective of this work is to establish drug resistant lung cancer cell lines.

Cells from the A549 epithelial cell line (human lung carcinoma) are currently being cultured in RPMI medium supplemented with 10% FBS and with increasing concentrations of doxorubicin. Morphological cellular alterations are being recorded throughout treatment.

The initial results indicate that following an initial selection process with doxorubicin, in which many cells did not survive, the remaining cells present drastic alterations in their morphology while they adapt to the new growing conditions. Nonetheless, initial results indicate that these alterations in morphology seem to depend on the drug concentration used. It is not known if the observed alterations are caused by DNA damaging agents only or if selection with a molecular-targeted drug also induces such morphological alterations.

Further continuation of the selection process should provide drug resistant cells. Future work will also include selecting cells with erlotinib treatment.

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## Evolution of eye size and shape between *Drosophila novamexicana* and *Drosophila Americana*

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The insect's compound eye is essential to key processes such as feeding and reproduction. Diversity in *Drosophila*'s eye morphology is thus likely related to the different environmental conditions experienced by different species. Here we show that the closely related species *Drosophila novamexicana* and *D. americana* (*virilis* group) show differences in eye size, having the former smaller eyes than the latter. This result holds even after correcting for differences in body size. We also performed a detailed morphological characterization of the hybrids between these two species. Hybrids have smaller eyes, but bigger wings and tibiae than *D. americana*. When corrected for body size the hybrids have even smaller eyes than *D. americana*. This means that the growth of the different body parts is, at least, independently regulated. As a first step to find out the genes responsible for this difference, presently we are performing an association study involving the progeny of the cross between *D. novamexicana*/*D. americana* hybrid males and *D. novamexicana* females to determine the chromosomal region responsible for the observed differences.

## Exploring sea anemones: *Actinia equina* metabolome and bioactivity

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Oceans consist on extremely rich and complex ecosystems, exhibiting greater diversity than terrestrial environments. Therefore, the search for biologically active compounds has grown exponentially in recent years, particularly those from marine invertebrates. *Actinia equina* L. is a cnidarian, preferentially found in the intertidal area, in the North Atlantic Ocean and Mediterranean Sea, British islands and African Atlantic coast. This work intended to explore the metabolic profile and the antioxidant and antimicrobial activity of the ethanol extract of *A. equine* and to establish possible relationships.

The HPLC-DAD analysis revealed the presence of four carotenoids, while fatty acids were determined by GC-FID (Fig.1). Scavenging capacity was observed against both DPPH• radical and nitric oxide, but no antimicrobial activity against a set of Gram+ and Gram- species was noticed.

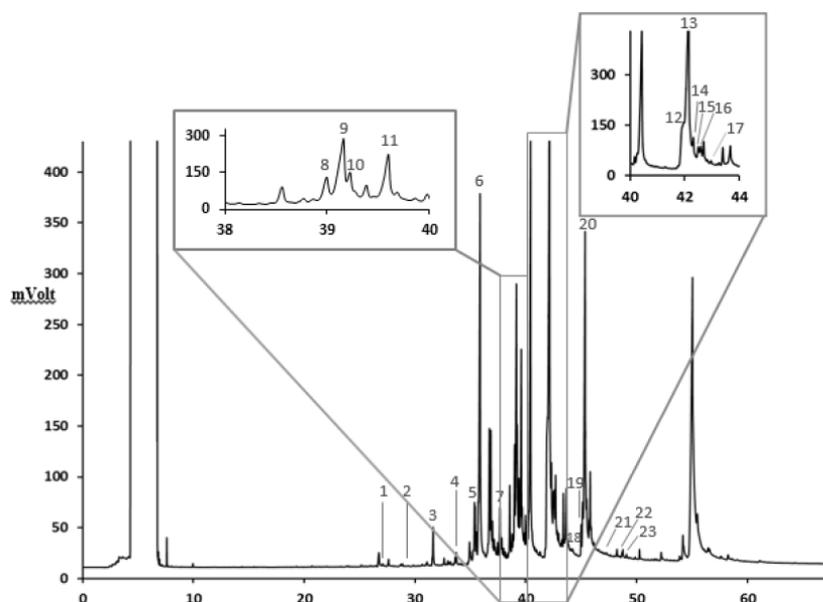


Fig. 1. GC-FID chromatogram of the ethanol extract of *A. equina*. Fatty acids were determined as methyl esters.

This work was developed within the optional curricular unit “**Bioactivity of Natural Matrices**” of the 5th 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 David Pereira.

## Expression of Galectin-3 and Nitrotyrosine in a Type-2 *Diabetic Mellitus* mouse model.

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*Diabetes mellitus* (DM) is a complex metabolic disease of multiple etiologies with a high prevalence worldwide. Type 2 diabetes (T2DM) is a type of DM, which has become a global concern, given its association with the lifestyle of the XXI century. It is the result of a sedentary life, poor and inadequate diet and overall stress. One of the consequences of DM is vascular complications, which is the major cause of mortality and morbidity. The Galectin-3 (Gal3) is a lectin, a member of a highly conserved family of  $\beta$ -galactosidase binding proteins, featuring different metabolic actions depending on other factors on its location and the target tissue. Overexpression of Gal3 is associated with predisposition to cardiovascular disease and follows the strong state of inflammation and oxidative stress [1]. In order to verify the correlation between the expression of Gal3 and the oxidative stress state, the localization of 3-Nitrotyrosine (3NT) was evaluated. The presence of nitrotyrosine has been observed in numerous pathological conditions such as inflammation. It is a result of Nitric Oxide binding with superoxide radical, which results in peroxynitrite, a powerful nitrating and oxidation agent. Peroxynitrite can be cytotoxic by itself or may be decomposed in other products such as hydroxyl radical or nitronium ion, which may lead to the nitration of aromatic amino acid residues such as tyrosine [2].

We assessed the differences in 3NT and Gal3 expression in the liver, kidney, left ventricle and gastrocnemius muscle in C57BI/6 mice submitted to different types of diets: Standard diet as control and High Fat Diet as a study model of T2DM. Both expressions were detected by immunohistochemistry in tissue sections and the location was considered separately.

Overall, we found that there is a positive correlation in the expression of Gal3 and 3NT with T2DM. Both in liver and kidney, the localization of Gal3 is almost coincident with the expression of 3NT, although 3NT exhibited a larger extent. In the left ventricle and gastrocnemius muscle, the localization is not coincident. We found that the location of 3NT is dispersed and extensive, while the expression of GAL3 is punctual and sharp.

The obtained results indicate that further studies are required in order to better understand the association between Gal3 and 3NT, so that Gal3 may be considered as a therapeutic target in diabetic complications.

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## Extraction of bioactive compounds to develop effective functional foods

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The World Health Organization (WHO) predicts that 80% of the world population depends on traditional medicine as primary health care, mainly through the use of plant extracts and their bioactive compounds [1]. The high diversity of bioactive compounds demands the development of standard approaches for their extraction. In fact, the quality of the further steps of separation, identification and characterization of bioactive compounds is highly dependent on the suitability of the extraction process. Herein, different extraction techniques, namely i) Soxhlet extraction, ii) ultrasound-assisted extraction, iii) supercritical fluid extraction, iv) accelerated solvent extraction, and v) shake extraction were comprehensively compared considering different aspects such as their cost, required technical skills, integrity of the obtained extracts, green chemistry principles, type of solvent, sample size, pH of extract, temperature and pressure [2]. The performed screening allowed inferring important conclusions towards the extraction effectiveness of target bioactive compounds from plant samples, selectivity of analytical methods, sensibility of the forwardly performed bioassay, conversion of the bioactive compounds into more suitable forms for detection and separation and development of strong and reproducible methods. Furthermore, the different types of extraction procedures were evaluated regarding their suitability to be incorporated in functional foods.

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# Hunting, distribution and Red Fox (*Vulpes vulpes*) population characteristics in the north and center of Portugal

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The Red Fox (*Vulpes vulpes*, Linnaeus, 1758) is a member of the Mammalian order Carnivora present across the entire northern hemisphere [1]. It is an abundant species in Portugal [2]. Although being a game species, most fox kills result from a regulated hunting activity, they are not considered under any immediate threat and is included in the category Least Concern [3]. The current study aims to characterize the hunting activity and some aspects of the Red fox biology (distribution, sex ratio and biometric characteristics) in Portugal.

Several hunting clubs and associations were contacted throughout the 2014/2015 hunting season and 70 specimens (36 males and 34 vixens) were collected for our study, mainly in northern and central hunting zones. Fox hunting offer and accomplished battues were accounted for. The geographic origin of each fox obtained was plotted in a map using Google Earth v7. All specimens were measured (body and tail length, head and chest perimeter, forelimbs spread, head width, hind limbs length, height and ear length) to the nearest mm, weighted to the nearest decigram. Damaged carcasses did not allow to obtain all biometric parameters. IBM SPSS v23 was used to do the biometrical analysis and one-way ANOVA to test sexual dimorphism.

Most hunting zones included fox battues in their annual plan. From the 300 hunting zones with planned battues contacted only 23% were accomplished; weather conditions and lack of hunters were the main justifications for not carrying out the battues. Battues success, meaning fox kills, was 61%, with a wide distribution in the two regions. Sex ratio is near the equilibrium (1♂:0.94♀). Sexual dimorphism is obvious from the comparison of the biometric characteristics, males having a more robust and strong body type.

Our data confirms the wide distribution of the Red Fox in Portugal. Hunting pressure was smaller than in previous years; weather, hunter involvement and probably the actual economic situation are probable determining factors. The population parameters (sex ratio and biometrical characteristics, good indicator of physique and overall species fitness, are within its known limits. Further research shall be directed to the age/growth estimation (through teeth analysis) and to the reproductive cycle (fecundation and birth dates, fecundity and embryo characteristics).

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## ***In vitro* neuroprotection of *Annona muricata* L. leaves**

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Fruits and leaves of *Annona muricata* L. (Annonaceae) are used in traditional medicine as tranquillizer and sedative. It is already known that the isoquinoline alkaloids isolated from the fruits exert antidepressant activity, by acting as 5-HT<sub>1A</sub> receptor agonist [1]. The knowledge on the neuroprotection provided by *A. muricata* leaves has been further investigated in this study, by evaluating the capacity to inhibit the enzymes acetyl- and butyrylcholinesterase (AChE and BuChE) and monoamine oxidase A (MAO-A), which are implicated in the development of Alzheimer's disease and depression, respectively.

The decoction from *A. muricata* leaves displayed weaker inhibition of AChE (IC<sub>25</sub> = 1743.7 µg/mL) than of BuChE (IC<sub>50</sub> = 1355.8 µg/mL). On the other hand, stronger activities were observed against MAO-A using two distinct methods (Fig. 1). In the horseradish peroxidase-linked MAO-A inhibition assay, using tyramine as substrate, an IC<sub>50</sub> value of 19.3 µg/mL was obtained, whereas in the assay based on the rate of disappearance of kynuramine, a higher IC<sub>50</sub> value was found (IC<sub>50</sub> = 160.8 µg/mL). Concerning the chemical composition of this decoction, five phenolic compounds were identified and quantified, namely 5-*O*-caffeoylquinic acid, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-rutinoside and kaempferol-3-*O*-rutinoside.

These results show that not only the alkaloids, but also the phenolic compounds present in this species, are responsible for its antidepressant activity.

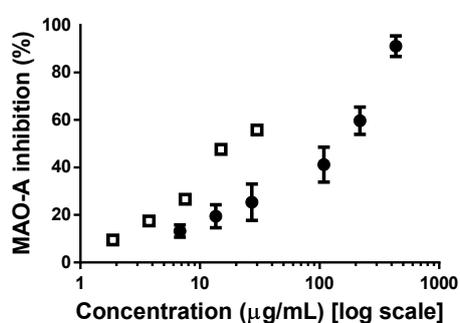


Fig. 1 – Inhibition of MAO-A by *A. muricata* decoction: □ – horseradish peroxidase-linked MAO-A inhibition assay; ● – MAO-A inhibition assay based on kynuramine disappearance.

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## Inflammation and flavonoids: influence of the structure on cell effects

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Inflammation is a complex process caused by endogenous and exogenous factors. The recurrent use of anti-inflammatory drugs and their side effects led to a growing demand for viable and safer alternatives. In this context, natural products arise, playing an important role in the treatment of this pathology. Flavonoids, polyphenolic plant secondary metabolites ubiquitously present in fruits and vegetables, possess interesting anti-inflammatory actions. The human being is exposed to a huge variety of flavonoids, rendering difficult to know which one is the most effective. In this work, the capacity of four flavonols (kaempferol, kaempferol-3-*O*-rutinoside, quercetin and quercetin-3-*O*-rutinoside) and two flavones (apigenin and luteolin) to reduce the levels of the pro-inflammatory mediator nitric oxide (NO) in RAW 264.7 macrophage cells was assessed. In addition, structure-activity relationships related with the effect of the catechol group in ring B, the presence of the hydroxyl group at C-3 and the effect of glycosylation were established.

Regarding cell's viability, the flavonols tested had no effect, at the tested concentrations. All compounds showed ability to decrease NO in a concentration-dependent way, quercetin and kaempferol being the most effective ones (IC<sub>50</sub> values of 7.47 and 15.07  $\mu$ M, respectively; Fig. 1). The effect of these compounds in other enzymes involved in the inflammatory process, is currently being tested.

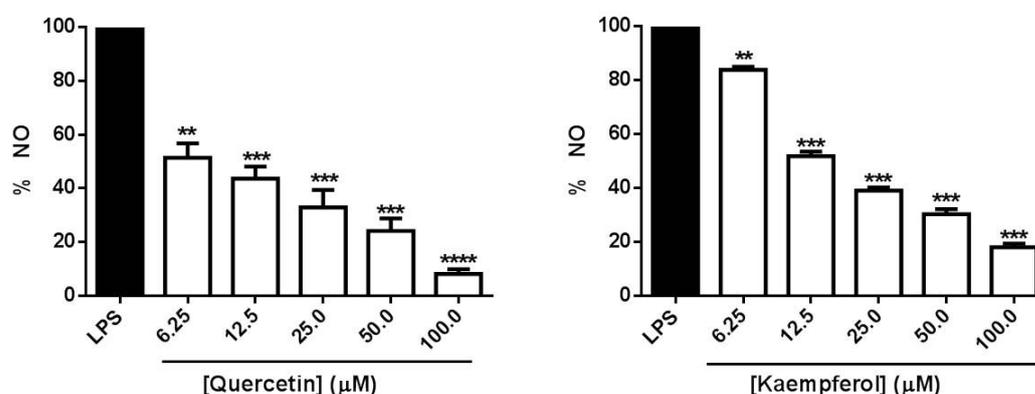


Fig. 1. (%) NO levels in RAW 264.7 cells pre-treated for 2 h with quercetin and kaempferol, followed by 22 h co-treatment with LPS (1 $\mu$ g/mL) or vehicle (culture medium). Results represent the mean  $\pm$  standard deviation of four independent experiments, performed in triplicate. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  \*\*\*\*  $p < 0.0001$  compared with control with LPS.

Acknowledgments: The authors are grateful for the financial support from the European Union (FEDER funds through COMPETE) and National Funds (FCT, Fundação para a Ciência e Tecnologia) through project Pest-C/EQB/LA0006/2013 and from the European Union (FEDER funds) under the framework of QREN through Project NORTE-07-0124-FEDER-000069. Andreia P. Oliveira (SFRH/BPD/96819/2013) is indebted to FCT for the grant.

## Insights on the fungus *Neurospora crassa*

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Due to their great diversity, easy growth and wide distribution, fungi are microorganisms with interest for the search of new molecules with potential application by the pharmaceutical industry. *Neurospora crassa*, a eukaryotic multicellular fungus, is a central organism in the history of twentieth-century genetics, biochemistry and molecular biology. In the latter half of the century, it had a central role as a model organism, contributing to the fundamental understanding of genome defense systems, DNA methylation, mitochondrial protein import, circadian rhythms, post-transcriptional gene silencing and DNA repair. Here, we report a survey on the ethanolic extract of this fungus.

The analysis by HPLC-DAD allowed detecting several carotenoids (Fig.1) and the first precursor of such compounds, phytoene. By GC-FID, nine fatty acids were determined, which included saturated and unsaturated molecules.

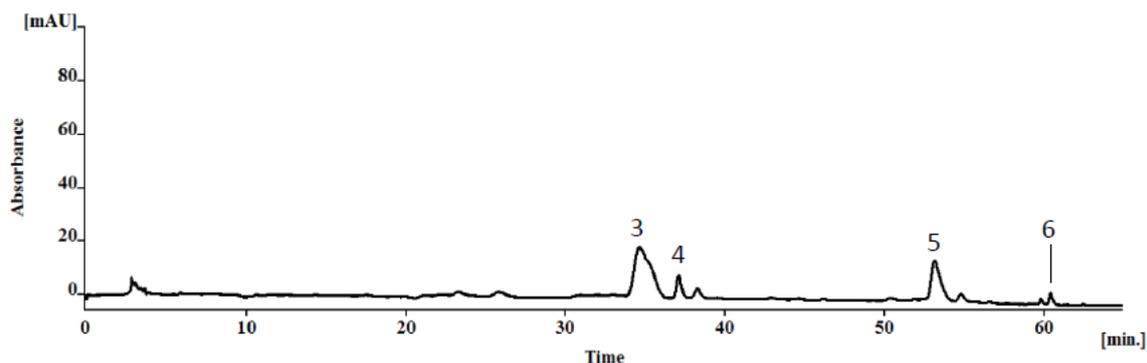


Fig. 1. HPLC-DAD chromatogram of the ethanol extract of *N. crassa*, registered at 450 nm. Compounds: (3) unidentified carotenoid; (4)  $\beta$ -carotene; (5)  $\gamma$ -carotene; (6) lycopene.

The extract was further studied regarding its antiradical properties, though no effect was observed against DPPH and nitric oxide radicals. Antimicrobial activity was checked against *Staphylococcus aureus* and *Escherichia coli*, but it failed to inhibit the growth of these bacterial strains.

Other extracts prepared from this fungus may provide distinct molecules, to which other bioactivities may be attributed.

This work was developed within the optional curricular unit “**Bioactivity of Natural Matrices**” of the 5th 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 David Pereira.

## Isoflavone content in two alfalfa-derived products used for the preparation of nutritional beverages

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There is a growing recognition that isoflavones are the most common phytoestrogens having some pharmacologic effects related to menopause [1]. Currently alfalfa cultivated in Mexico is used to manufacture two alimentary products, freeze-dried juice (FDJ) and dehydrated powder (DP), used for beverage preparations. Since alfalfa is considered as a source of isoflavones [2], the quantification of those compounds in these new ingredients are needed as well as the evaluation of changes in isoflavones content during different months of harvest [3]. HPLC-DAD analysis was carried out to detect and quantify seven isoflavones (glycitein, formononetin, biochanin A, genistein, daidzein, genistin and daidzin). Also, five different solvents (water, methanol, water-methanol 1:1, ethanol and water-methanol-formic acid) were employed to evaluate isoflavones extraction. Results demonstrated that only genistein, daidzein and their glycosides (genistin and daidzin) were found in the two products, being genistein and daidzein the most abundant. Significant difference was found between extractions: while genistein was better extracted using water (0.13-0.36 mg/g and 0.48- 1.99 mg/g for DP and FDJ, respectively), daidzein showed better results with methanol-water-formic acid mixture (0.22-0.34 mg/g and 0.35- 1.05 mg/g for DP and FDJ, respectively). Isoflavone content of different harvest were also statistically different. However, in all cases, total isoflavone content was higher in FDJ than DP. Further studies are needed to evaluate the pharmacologic and nutritional value of the two alfalfa-derived products.

### *Acknowledgements:*

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# Kelps from Portuguese Coast: the key for neurodegenerative disorders management?

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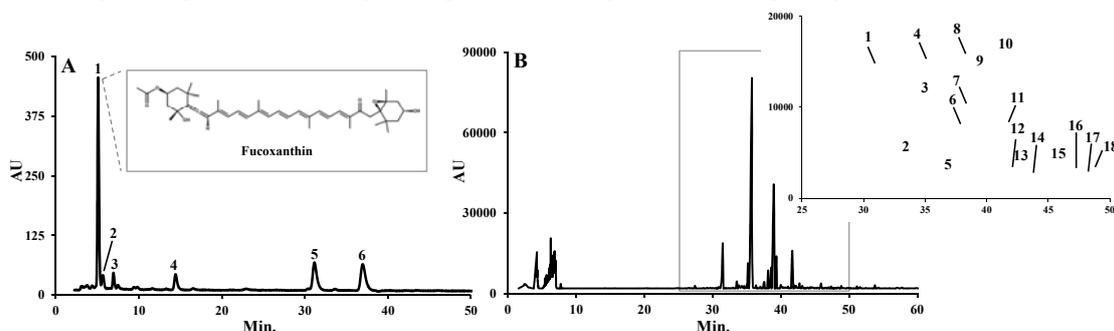
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Brown macroalgae of the order Laminariales, commonly known as kelps, are key components of cold-temperate coastal ecosystems and economically important sources of prominent compounds. Among the great chemical diversity, fatty acids and carotenoids are highlighted. This work aimed to evaluate both fatty acids and carotenoids profiles of *Laminaria ochroleuca* Bachelot de la Pylaie, *Saccharina latissima* (Linnaeus) J.V. Lamouroux, and *Saccorhiza polyschides* (Lightfoot) Batters, collected along the Portuguese Northern Coast, by GC-FID and HPLC-DAD, respectively.

The qualitative profiles of the studied kelps species were very similar. As expected, fucoxanthin was the most representative carotenoid in all samples (Fig. 1A). Concerning fatty acids composition, palmitic acid (C16:0) was the dominant one, followed by oleic acid (C18:1, *cis* 9) (Fig. 1B).

Preliminary *in vitro* assays using the characterized extracts revealed a mild inhibitory activity against AChE and BuChE, important enzymes related to the etiology of numerous neurodegenerative conditions. Moreover, the most promising extracts are currently being evaluated regarding their neuroprotective capacity, in SH-SY5Y cells.



**Fig. 1.** (A) HPLC-DAD chromatogram of the acetone extract of *L. ochroleuca*. Detection at 450 nm. (1) Fucoxanthin; (2) Fucoxanthin *cis* isomer; (3) Violaxanthin; (4) Chlorophyll *a*; (5 and 6) Chlorophyll *a* derivatives. (B) GC-FID chromatogram of the chloroform:methanol (2:1) extract of *L. ochroleuca*. (1) C14:0; (2) C15:0; (3) C16:1 *cis* 9; (4) C16:0; (5) C17:0; (6) C18:3 *cis*-6, 9, 12; (7) C18:2 *cis*-9, 12; (8) C18:1 *cis*-9; (9) C18:0; (10) C20:4 *cis*-5, 8, 11, 14; (11) C20:5 *cis*-5, 8, 11, 14, 17; (12) C20:3 *cis*-8, 11, 14; (13) C20:0; (14) C21:0; (15) C22:0; (16) C23:0; (17) C24:1 *cis*-15; (18) C24:0.

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## Limpet (*Patella* sp.): what can be further expected?

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In the last years, as consequence of an increasing search for new natural products, there is a raising interest in marine organisms. The goal is to find new molecules with biological activity, which could be applied in the future, in areas like health and environment. *Patella* sp., commonly known as limpet, is a marine animal that is widely distributed in the Portuguese shore. Besides being a much appreciated food in some regions, it can also be used as biomarker for pollution.

*Patella* sp. ethanol extract was analyzed by HPLC-DAD, several carotenoids being identified (Fig.1). Furthermore, fatty acids were determined by GC-FID, methyl palmitate, methyl *cis*-9-octadecenoate, methyl *trans*-9-octadecenoate and methyl laurate being found in higher concentrations.

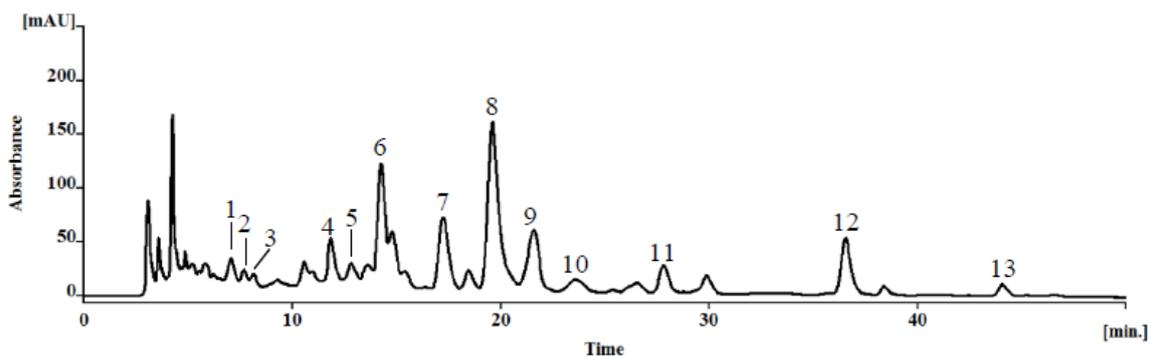


Fig. 1. HPLC-DAD chromatogram of the ethanol extract of *Patella* sp. (450 nm). Compounds: (1, 2 and 8) unidentified carotenoids; (3) neoxanthin; (4, 9, 10 and 13) chlorophyll *a* isomers; (5) astaxanthin; (6) lutein; (7) zeaxanthin; (11) echinenone; (12)  $\beta$ -carotene.

The antioxidant activity of the extract was also evaluated, using DPPH and NO microassays, a reduced capacity being observed. In addition, the ethanol extract revealed no antibacterial activity towards selected Gram<sup>+</sup> and Gram<sup>-</sup> species.

This work was developed within the optional curricular unit “**Bioactivity of Natural Matrices**” of the 5th 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 David Pereira.

## Marine epiphytic bacteria associated with macroalgae

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In recent decades new studies have emerged in order to understand the relationship between the communities of bacteria and algae. Algae are key components of the aquatic environments which represent not only habitats for many macroorganisms, but also for millions of microorganisms waiting to be discovered. Although different macroalgae may exert the same important ecological role as primary producers, each single species represents a different microenvironment occupied by rich and diverse bacterial communities. The present study focuses on the bacteria-macroalgae association of three different species of macroalgae, *Porphyra dioica*, *Ulva* sp. and *Sargassum muticum*. Bacterial isolation and molecular analysis of the 16S rRNA gene of the epiphytic bacterial community diversity were performed. In eight marine media, 227 bacteria were isolated from *Porphyra dioica* (53), *Ulva* sp (95) and *Sargassum muticum* (79) sampled in autumn, in Porto, Portugal (41.153680 N, -8.679332 W 41° 18' North, 8° 44' West 41°09'N, 8°40'W). Denaturing gradient gel electrophoresis (DGGE) fingerprinting profiles provided the comparative analyses of the communities of the 3 algae in autumn and winter.

## Marine *Planctomycetes*: isolation and bioactivity studies

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Planctomycetes is a unique, still unexplored phylum of bacteria. They comprehend peculiar characteristics such as peptidoglycan-less cell wall of proteic nature [1] and a compartmentalized cell structure through a complex system of membranes [1]. Phylogenetically, this phylum is part of the PVC super- phylum [2]. They can colonize variable ecosystems, including terrestrial, aquatic and even extreme environments [3]. They also live associated to organisms, like macroalgae [3]. These can potentially benefit of the presence of *Planctomycetes* by using growth factors or antimicrobial molecules produced by them. The search of the production of bioactive compounds was initially made by genome mining in *R. baltica* [4] and subsequently in 13 genomes [5], revealing the still uncharacterized antimicrobial potential of planctomycetes. The aims of this study were the isolation of the planctomycetes and their subsequent study regarding bioactivity potential. Planctomycetes (nine colonies) have been retrieved in selective medium from the biofilm of macroalgae (*Porphyra dioica* and *Ulva* sp.) sampled in autumn in the north of Portugal (no isolation was achieved in the winter sample). A potential new selective medium with incorporated sodium azide was assayed without good results. The new isolates were identified based on the analysis of the 16S rRNA gene. Bioactivity studies included the analysis of nonribosomal peptides synthetases (NRPS) and polyketide synthases (PKS-I) genes which are involved in the production of secondary metabolites. The nine planctomycetes plus other seventeen previously isolated were screened for the presence of these genes. Amplification of bands corresponding to PKS-I and NRPS genes were respectively eighteen and fourteen. Screening assays against several human pathogens are envisaged.

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# Multiple antibiotic-resistant *Enterobacteriaceae* in the Douro river

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Despite their vital role on reducing mortality and morbidity associated to infectious diseases, antimicrobial agents have been the major driving force in the emergence and spreading of drug resistance traits among pathogenic and commensal bacteria [1]. Surface waters, mainly those integrating the urban water cycle, represent one of the most important vehicles of dissemination of human-associated microorganisms. The ability of resistant microorganisms to spread from these surface waters, soil and sewage through various mechanisms is a potential threat to public health [2].

The aim of our work was to detect antimicrobial-resistant gram-negative bacteria in water samples from the Douro River, with special focus in Extended Spectrum Beta Lactamase (ESBL) producing *Enterobacteriaceae*.

The water samples from the Douro River were collected in three different spots along the riverbank at places targeted for recreational activities like swimming and fishing. The water samples were analyzed by the membrane filtration method on MacConkey agar and MacConkey agar with cefotaxime, ciprofloxacin and meropenem (2mg/L). Representatives of different colony morphologies were tested for antimicrobial susceptibility, according to CLSI and EUCAST. ESBL producers were confirmed by the double-disk-synergy-test and the addition of clavulanic acid, according to the CLSI guidelines.

A total of 46 isolates showed resistance to at least 2 of the antibiotics tested. The majority of the isolates were resistant to amoxicillin, amoxicillin with clavulanic acid, ciprofloxacin, cefotaxime and ceftazidime. Resistance to cefepime, ceftazidime and aztreonam was also evident. Twenty three of the isolates showed reduced susceptibility to at least one carbapenem (meropenem and imipenem). Eight of the isolates were resistant to at least one antibiotic of all the antibiotic families tested, showing a multidrug-resistant phenotype. In the examined water samples 7 ESBL-producing lactose fermentative isolates were found.

The results indicate that river waters in the North of Portugal are reservoirs of ESBL producing *Enterobacteriaceae* and non-fermentative-gram-negatives with reduced susceptibility to carbapenems. The impact of polluted river waters on human health depends mainly on the water usages as well as on the antibiotic-resistance profiles of pathogens inhabiting these surface waters, hinting at a public health threat.

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## **Plant-derived and synthetic compounds affect the proliferation and metabolic activity of 3T3-L1 preadipocytes**

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The prevalence of obesity has steadily increased over the past three decades. Obesity is defined by excess adipose mass and adipose tissue expansion, which occurs through adipocyte hypertrophy and hyperplasia. Adipose tissue is an important energy storage organ and has been progressively recognized as a key endocrine organ which function is critical to overall energy balance and homeostasis. Hypertrophic obesity is correlated with metabolic complications as insulin resistance, type II diabetes, hypertension, inflammation or excess deposition of lipids in other organs. A reduction of adipocyte number in surplus energy settings may not be favourable. Instead the accommodation of surplus energy may be a key feature to reduce metabolic complications of obesity.

In this work, we tested the effect of plant-derived and synthetic compounds from a library from the Group of Natural Products and Medicinal Chemistry of CIIMAR on the proliferation of the 3T3-L1 preadipocyte cell line. The tested samples compounds consisted of a library of 88 compounds including polyphenols and flavonoids. Proliferation was assessed by two methodologies: (1) MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay was used to analyze the mitochondrial metabolic activity and to estimate the toxicity of compounds (number of viable cells); (2) SRB (sulforhodamine B) assay was used to measure the whole cellular protein content as indicator for cell proliferation. Both assays were performed in 96-well plates and effects were analyzed in a spectrophotometer after 24h and 48h exposure to the compounds. In our preliminary results, we observed promising activity of some compounds on the proliferation in preadipocytes. Some compounds decreased the proliferation, and others increased the proliferation in comparison to the solvent control. MTT and SRB demonstrated consisting effects in some cases, but also contrary results. MTT positive, SRB negative results may indicate increased mitochondrial metabolic activity, while MTT negative and SRB positive results may indicate proliferation. Generally, effects were more pronounced after 48h exposure.

Future works will focus on the analysis of dose-response curves from selected compounds in order to determine the effective concentration EC50. Additionally, it will be important to decipher their mode of action by using a combination of biochemical and molecular methods, which is important to understand how these compounds affect cellular pathways.

Acknowledgements: This research was developed under the Project PEst-OE/SAU/UI4040/2014, and partially supported by the ERDF through COMPETE and FCT (PEst-C/MAR/LA0015/2013); This work was partially funded by the Project MARBIOTECH (reference NORTE-07-0124-FEDER-000047) within the SR&TD Integrated Program MARVALOR - Building research and innovation capacity for improved management and valorization of marine resources, supported by the Programa Operacional Regional do Norte (ON.2 – O Novo Norte) and by the European Regional Development Fund.

# Red fox, *Vulpes vulpes* L., skin trophies as a potential tool for the species management

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Hunters frequently keep some form of trophy from their hunting journeys: a simple photograph and/or some part of the animal. In the case of big animals trophies might be the antlers (case of the red deer, fallow deer, roebuck and mouflon), the teeth (case of the wild boar), the skull and/or the skin (case of the carnivores - wolf and red fox) in Portugal [1, 2]. The importance of trophies is well recognized worldwide and Portugal is not an exception, a database being maintained by a trophy evaluation committee (Comissão Nacional de Homologação de Troféus-CNHT) [3]. The red fox trophies (skull and skins) are not considered at present, a lacuna that we consider that should be quickly overcome, as trophies data analysis can and should be used as a tools in game management. Our main goal is therefore to analyse a sample of red fox skins trophies in order to evaluate their importance as a tools for red fox population management.

A sample of 24 fox skins was used to obtain a set of measurements: body and tail length, head and chest perimeter and front limbs spread (all measurements to the nearest mm). In a sample of 70 foxes (36 males and 34 females) obtained during the 2014-15 hunting season (offered by the hunters) the same set of measures was also obtained. The analysis of each sample included the determination of several statistical parameters (mean, S.D., variance, minimum and maximum) and the comparison of such results by a one-way ANOVA using the program IBM SPSS V23; a multivariate analysis was also performed using the program Statistica V12.

The size of the skins (body and tail length) clearly show that the skins measured were obtained mostly from big fox males (result later confirmed by the hunter) as compared with the biometric characteristics of the 2014-15 fox sample. The other three biometrical parameters are smaller than those obtained from the 2014-15 male sample; however the difference is more pronounced for the head and chest perimeter; this results may be a consequence of the techniques applied during the skinning (eventually with some skin cutout) and pelt tanning processes. Multivariate analysis confirms this outcome.

Our results show that at least body and tail length are not affected by the tanning process and so such biometrical data can be valuable in population management. In what concerns the other parameters further research is needed on the effects of skinning and tanning in the dimensions and characteristics of the skins, so that future analysis can be as objective and accurate as possible.

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## Rediscovering the silkworm

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Since the beginning of times, humanity searched in nature for new sources of medicines with diverse practical applicability. Natural matrices are an interesting target for analysis, for their chemical properties and potential therapeutic properties. The aims of this work were to perform the chemical characterization and the evaluation of the bioactivity of the chrysalides of *Bombyx mori* L., commonly known as silkworm.

Phenolic compounds and carotenoids were characterized by HPLC-DAD (Fig.1), fatty acids by GC-FID and the presence of alkaloids was checked by proper precipitation reactions. The results allowed the identification of methyl *cis*-9-octadecanoate, lutein and quercetin as major fatty acid, carotenoid and phenolic compound, respectively.

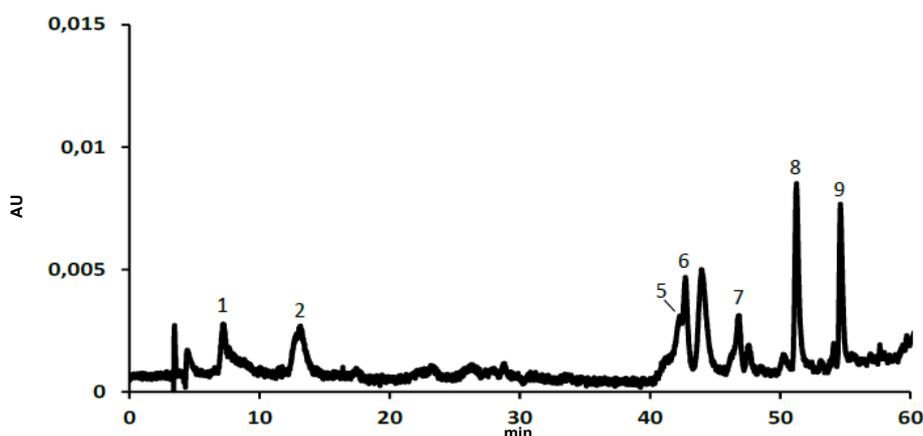


Fig. 1. HPLC-DAD chromatogram obtained with the ethanol extract of *B. mori* (350 nm). Compounds: unidentified flavonoid (1, 2 and 6); quercetin-3-*O*-sulfate (4); quercetin-3-*O*-glucuronide or quercetin-3-*O*-galactoside (5); kaempferol-3-*O*-glucoside (7); quercetin (8); kaempferol (9).

Additionally, the antioxidant potential was assessed by scavenging assays for the radicals DPPH and NO and the effect against *Staphylococcus aureus* and *Escherichia coli* was evaluated. A slight activity was observed only against DPPH. No microbial inhibitory capacity was noticed.

It is worth to consider that the nature of the identified compounds and the evaluated activities are strongly dependent on the extractor solvent used.

This work was developed within the optional curricular unit “**Bioactivity of Natural Matrices**” of the 5th 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 David Pereira.

# RPS4 paralogues in mammalian spermatogenesis: gains and losses in translation

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## Abstract

Ribosomal protein S4e is a highly conserved protein present in all Eukarya.[1] The *RPS4* gene is found on autosomes in all vertebrates except mammals, in which it is X-linked, having also retained an ancestral Y-linked copy in some lineages.[2] In primates the ancestral *RPS4Y* gene duplicated and originated a second Y-linked copy (*RPS4Y2*) in Old World Monkeys[3], while in the mouse *RPS4X* acquired an intronless paralogue on chromosome 6 encoding a highly identical protein.[4] The primate-specific *RPS4Y2* as well as mouse *Rps4L* are expressed during spermatogenesis and may compensate for the transcriptional silencing of the X-linked copy during meiotic sex chromosome inactivation (MSCI).[5]

In this study we aim to characterize lineage-specific potentially functional copies of *RPS4*. By exploiting the available draft genome sequences of several species we have found *RPS4* autosomal retrogenes with conserved open reading frames in dog, cow and rabbit. Interestingly, in those species which retained a Y-linked *RPS4* gene (pig and cat) we did not succeed in finding potentially functional duplicates in other chromosomes. We will combine phylogenetic analysis and functional genomics tools in order to address the role of *RPS4* duplication in spermatogenesis, throughout mammalian evolution.

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ENCONTRO INVESTIGAÇÃO JOVEM  
DA UNIVERSIDADE DO PORTO

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15<sup>th</sup> **FRIDAY**  
POSTER SESSIONS

# Analysis of reflectance spectra of minerals using Hapke's radiative transfer model

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Hapke's radiative transfer model [1] provides a way for modelling reflectance spectra of minerals and intimate mixtures of minerals. Reflectance, however, depends on a considerable number of unknown parameters and wavelength-dependent variables, such as optical constants (also termed the complex indices of refraction) and grain sizes [2].

In this study, we estimated wavelength dependence of the imaginary coefficient of the complex index of refraction ( $k(\lambda)$ ) of some orthopyroxene mineral assemblages. For this purpose, we analysed reflectance spectra from RELAB database [3] as a function of grain size and optimized  $k(\lambda)$  using the equivalent slab model [1, 4] to calculate single-scattering albedo.

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# **The importance of the Exchange System of Automatic Data Of Tax Havens in relation to Drug Trafficking**

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In 2013 the G20 countries, a group formed by the finance ministers and heads of central banks of the 19 largest economies of the European Union over the world, supported as new global model automatic exchange of account information in tax matters, established by the Organization for Economic Cooperation and Development - OECD, highlighting the need for change on the level of fiscal transparency.

The new global standard was approved in July 2014 by the Council of the OECD and provides for the annual automatic switching between governments from the financial account, including balances, interest, dividends and sale of financial assets revenues.

In September 2014 the Global Forum on Transparency and Tax Purposes for Information Exchange delivered a script to the Working Group and the G20 Development, referring to the participation of countries in the new standard strategy. This script on the efforts to contain the multinational tax evasion and offshore tax evasion in developing countries.

The crisis of the European Union countries and the United States of America, is certainly encouraging in a way that strategic change coming standardize this policy exchange of tax information, which is influencing developing countries, such as Brazil, to adopt such measures.

It is in this work to analyze the situation in developed countries shaken by epic economic crisis, as well as developing countries, noting the relationship of economic and social facts with the global initiative that is trying to achieve in line in this group of countries.

Keywords: Offshore; Political and economic; Drugs.

# CONSTITUTIONAL PROTECTION OF THE CONSUMER: The Omission of the Internal Law and the e-Commerce.

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In these past decades, the associated evolution between the consumption ratio and the means of communication became notorious, however the Brazilian law did not follow them. The present is about to discuss the gaps presents in the Brazilian law about e-commerce, mostly at the international scope.

The "e-commerce" as it is known in Brazil, began in the 90s when the first sites on the internet in order to conduct business transactions were created. Like any start of a new concept, transactions with the e-commerce were still very small. But with the change in the world market profile, did not take long for this new concept of buying and selling to become the largest and most massive way to market products or services.

Through the actions of the market, companies around the world have signed the e-commerce and more and more people gained access to both computers and connectivity to global network, the Internet. It was these actions and other variables that caused the e-commerce where he came and conquered more and more customers worldwide.

Over the years, changes had to be made in companies that could meet great growing demand of the public who adhered to this new way of buying and one of the most significant changes were with respect to delivery logistics. However, despite technological advances, this has not shaken conflicts and imbroglios that are significantly more complex when the relationship is established virtually, which are even lower than the levels consumption relations given over the internet and other sites.

Well, as these imbroglios were becoming judicial demands, legislators gradually been taking care to urgent cases, such as computer crime, stealing the focus of digital rights, which should not be limited in this endeavor. In turn, consumer relations in the foreign sites are left without strong legislative support, are only treated with constitutional guarantees and also by the Consumer Protection Code.

The overall objective is to analyze the demand, specific data and examples on the subject of national and international, to find the methodology used in cases of this issue and on what basis this way of dealing with the case was created. Emerge, then, the following problem: What security must be guaranteed for the parsimony of a purchase relationship and online international sale?

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# Social Harm Approach and Scientific Fraud

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In this presentation we will use the Social Harm Approach (SHA) to understand scientific fraud and to establish a connection between both. The scientific fraud topic has gained more and more relevance and attention since the 70's and 80's, including in the news media.

Some authors suggest the need to look beyond what is defined as crime because not always what law defines as crime are the most dangerous anti-social behaviours [1]. This happens because power makes harmful acts visible and defines them as crime, keeping other dangerous acts invisible. This is the problem of Criminology working with the concept of crime and, thus, some authors replace it with the concept of harm [2]. It has a global nature and can be divided into four categories: (1) physical, (2) economic/financial, (3) emotional/psychological and (4) cultural security.

Researchers had already established a relationship between SHA and scientific fraud [3]. However, currently there is no consensus on the definition of scientific fraud, which leads to some difficulties in measuring the phenomenon itself [4]. Although it is widely accepted that falsification, fabrication and plagiarism (FFP) are behaviours that deeply undermine the integrity and correction guiding scientific research, other behaviours exist that, despite their seriousness, may or may not be considered scientific fraud [5].

There are several negative consequences in a scientific fraud case, not only for the individual or the institution involved, but also for the general public. The harm associated to reputation, the constraints that may appear in the professional career and the funding allocated to the fraudulent project, correspond to some of the costs that may be related to scientific fraud, and that can result in a negative impact in the public trust in science and between scientists [6]. Despite that, very few efforts have been undertaken to estimate these costs or harms, and, in particular, social costs. This paper will reflect on what is yet to be done.

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## **Development and optimization of printed heating circuits.**

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The goal of a heating system is to maintain pleasant thermal conditions within the home without spending a lot of electric power. An underfloor heating is a perfect solution.

Radiant floor is the underfloor heating system which heats the air upwards, where the heating element is integrated in the floor structure itself. The popularity of this type of heating system has been increasing, because of high energy efficiency and fast response [1]. Nowadays there are two systems of radiant floors: electrical and hydraulic.

The present study will be focusing on electrical heating systems by using printed carbon tracks. For this purpose printed heating circuits will be produced using the screen-printing technique. It will be discussed their morphological, micro structural and electrical study considering the printing parameters and curing conditions. Moreover, it will be done the study of degradation of materials by combining the thermal and electrical current effects. Important is the methodological studied of different ink compositions, which allow us to plot a graph of the resistivity as function of the conducting ink proportion in the mixture composition. Finally, for one particular composition it was studied the cure parameters on the electrical resistivity.

Finally, numerical simulation of heat transfer phenomena generated on the heater tracks and different structures with integrated heating tracks using the software Comsol Multiphysics® will be presented and compared them with real experimental data obtained in a chamber using thermocouples as temperature sensors.

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# Incorporation of magnetic nanoparticles through textile coating

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It is possible a t-shirt could charge the battery of your smartphone? And is a simple cotton sweater able to measure the heart beats?

The magnetic properties of the materials are different at nano scale what makes emerge a huge set of applications for magnetic nanoparticles. Combining knowledge and research to these on textiles is only one of the applications that has great potential.

After the magnetic nanoparticles synthesis through not very expensive chemical process is necessary measure the concentration of particles existing in the solution and their properties using a vibrating sample magnetometer or VSM. Here a little sample is enough to obtain results. So, using a micro pipette to collect a few milliliters of the solution. Then it is placed in a capsule which is then placed on the tip of the sample holder of VSM.

It isn't necessary a wide range of variation of the magnetic field to calculate the concentration and to find that the particles are paramagnetic. But it is also possible to confirm this fact by approaching an imam close to the container where the solution is. The nanoparticles are attracted by an imam, but in the absence of an external field these atomic dipoles do not interact with each other and are randomly oriented.

Then has to find a way to incorporate them in textiles. The coating is a process already used in the textile industry and that allows incorporate nanoparticles in cotton, for example. By repeating several times the coating follows by drying and after each one measure is possible to draw a graph as a function of coatings number and optimize this process.

# Modelling and mining of characteristic product attributes in the context of Consumer Analytics

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It is a general belief that our characteristics (gender, marital status, socio-economic group,...) influence our shopping habits. In that sense, marketing departments may rely on available personal data from their clients and then predictive statistical analyses can determine intervention strategies and effectively plan new campaigns. Over the last years, there have been many studies which have researched further on consumers' knowledge structures and types of product knowledge. As a results several different concepts have emerged, which only agree that the concrete product features form the basis for that structure (Lawson, 1998).

This work follows from an internship carried out at adidas, in Germany, at the ProductDNA Department. The main goal was the identification of a predictive model for the sales of running shoes. As too many variables and product related to the shoes were available, a secondary question related to the clustering of variables and products.

The data concerned sales of running shoes in the United-Kingdom e-commerce since Spring/Summer of 2012 untill Fall/Winter of 2014. It consisted of 381 articles and 774 variables, representing each part of the shoe and the associated material, and 3 variables related with the sales. As all variables, but those sales-related, were binary, the clustering algorithm used a dissimilarity matrix (Jaccard index seemed to be appropriate, Hastie, 2008). The obtained clusters are described and interpreted. A linear regression on sales will determine appropriate marketing plans of action.

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# Computer Intensive methods in Statistics Teaching

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Even if “Resampling” was mentioned for the first time in the XIX century as a statistical methodology, it was published in an operational way by Bradley Efron as "Bootstrap methods: another look at the jackknife" in 1979. This event marks the beginning of computer intensive techniques that even now are very far from being used for teaching and for the exploration of experimental data.

All these approaches in what concerns teaching as also the exploration of experimental data have been putted aside in favor of a “scientific corpus” that it is not only boring as an old priest but also mind-boggling and not even formative where it counts the most – real world applications.

As citizens of our time we show how to produce appealing and meaningful results using dedicated statistical software like MiniTab as also MatLab routines and going from the simple approaches of Efron and Tibshirani, accessible to High School students, to the more elaborate ones at PhD level devised by Von Neuman

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# Characterization of diatexites and metatexites from Madalena-Lavadores (Vila Nova de Gaia)

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The gneiss-migmatite rocks that outcrop in Madalena-Lavadores coast line (Vila Nova de Gaia) belong to a metamorphic belt, the Porto-Viseu belt, which was later intruded by the post-tectonic granite of Lavadores. The gneiss-migmatite rocks display two different lithologies: diatexites and metatexites, predominant in Lavadores and Madalena respectively, both showing evidences of variscan deformation. In this study both lithological and petrographic analyses were developed, resulting in a detailed description of the gneiss-migmatite rocks, at macro and micro scale.

In Lavadores it's possible to distinguish leucocratic diatexites from mesocratic diatexites, all usually displaying compositional banding. Darker nodular zones are evident in the leucocratic rocks and the mesocratic diatexites are intrusive in the leucocratic ones. Microscopically, they both show a heterogranular texture, but the leucocratic diatexites are rather isotropic while the mesocratic ones have a more or less incipient orientation manifested by cordierite and some plagioclase megacrystals. The nodular zones have a fine texture and are composed by quartz, k-feldspar, plagioclase and cordierite.

The Madalena's diatexites are leucocratic to mesocratic, more porphyritic and coarser than Lavadores's diatexites. They appear as intrusive lenses and veins in the metatexites. The diatexites are heterogranular and isotropic, with schlieren, visible both at macro and micro scale. They exhibit microveins composed of plagioclase, k-feldspar and scarce quartz, one of the typical microstructures of migmatites.

The metatexites display an irregular compositional banding and the darker biotite-rich layers preserve the protolith foliation. Microscopically, nodular structures are composed of felsic minerals and poikilitic garnet that can be considered a peritectic mineral that result of the biotite incongruent melting reaction in pelitic and / or quartz-pelitic rocks.

Common to all the studied migmatites, there are two generations of quartz, plagioclase, k-feldspar, biotite and a late muscovite in large crystals interpreted as being caused by back-reaction promoted by the fluid resulting from the crystallization. Andalusite is also present but only in the mesocratic diatexites. Quartz crystals occur as aggregates and as inclusions in almost all minerals, including quartz, which is a typical microstructural feature of migmatites.

The diatexites' orientation both in the leucocratic and the mesocratic is acquired by magmatic flow, evident in the preferential orientation of quartz and feldspar and in schlieren structures.

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# Antibiotic resistant bacteria in sludge of municipal sewage

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The development of bacterial resistance to antibiotics is a major public health challenge recognized by the medical and public health communities and governments.

Recent studies show that incomplete metabolism in humans and improper disposal of antibiotics to sewage treatment plants has been a main source of antibiotic release into the environment. This gives bacteria enough time and sufficient contact to shield themselves by altering genes and cellular mechanisms, favoring growth.

Sewage sludge is one of the major sources of release of antibiotic resistant bacteria and antibiotic resistance genes in the environment. *Enterobacteriaceae* present in natural microbiota of the human and animal gastrointestinal tract, represent a large part of bacterial communities of effluents, and can be a source of genes encoding relevant antibiotic resistance mechanisms as are extended-spectrum-beta-lactamases (ESBLs). Those genes might be transmitted to other bacteria present in sewage and the environment<sup>[1]</sup>.

The aim of our work was the detection of antimicrobial resistant bacteria in sludge of municipal sewage. One gram of 18 different sludge samples were suspended separately in 10 ml of saline solution, spread on MacConkey agar and incubated at 37°C overnight. Isolates were selected by spreading 100 µl of suspension on MacConkey agar and MacConkey agar with cefotaxime and meropenem (2mg/L). Colony forming units were randomly selected and susceptibility to antimicrobial agents was determined by the agar diffusion method, according to the Clinical Laboratory Standards Institute (CLSI). Identification of the selected strains was achieved by Chromagar Orientation, API 20E and ID 32 GN.

A total of 56 isolates presented relevant resistance to the antibiotic families tested. The majority of the isolates were resistant to the β-lactam antibiotic family. Twenty three isolates presented reduced sensibility to imipenem, which were identified as *Escherichia coli* (2), *Stenotrophomonas maltophilia* (5), *Pseudomonas* spp.(1) and 15 of KESC group (*Klebsiella* spp., *Enterobacter* spp., *Serratia marscescens*, and *Citrobacter* spp.). Six isolates were ESBL producers, showing positive for bla<sub>CTX-M-group-1</sub> and identified as *Escherichia coli* (4) and *Klebsiella pneumoniae* (2).

Our study showed the dissemination of antibiotic resistant bacteria in the sewage environment. The wide spread of multi-resistant *Enterobacteriaceae* may be related with their spread from sewage to the environment. Municipal sewages may contribute to the dissemination of antibiotic resistant bacteria in natural environments. This represents a public health risk, which needs future evaluation and control. Agricultural use of sludge might contribute to dissemination of resistant bacteria and resistance genes to soils contributing for relevant ecological changes.

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# **Benthic macrofaunal communities differences between oligotrophic and eutrophic abyssal habitats: a case study of the West Pacific**

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The deep seafloor ecosystem is the most extensive ecosystem on Earth and about 60% of the globe lies beneath water depths exceeding 1000 m. Yet, only <1% of this area has been fully explored, especially in terms of biota. Deep benthic and pelagic communities tend to feed off organic matter sinking through the ocean interior from the euphotic zone. Recent evidences suggest that the structure and dynamics of deep-sea ecosystems can change radically and deep-sea ecosystem functioning may change in relation to biodiversity. The content of available organic matter controls the benthic communities, working as a major environmental driver. In this context, the benthic macrofaunal communities of two different habitats of the western Pacific were analysed: one eutrophic (high organic matter content; 39°00N, 146 °00E, water depth: 5256 m) and another oligotrophic (low organic matter content; 1°15N, 163°15E, water depth: 4277 m). Macrofaunal samples were taken on the submarine Shinkai 6500, carried by the Yokosuka vessel, at a depth of 5270 meters. A total of four cores were collected at each habitat and each core were fractioned in six depth layers, namely 0-1, 1-2, 2-3, 3-5, 5-10 and 10-15 cm. Samples were sieved under 300 µm and stored in 4% formaldehyde stained with Rose Bengal. In the laboratory, macrofaunal specimens were identified until family level. Differences between the two habitats were investigated based on structural (abundance and diversity) and functional (trophic guilds) attributes of the benthic macrofaunal community. Mean abundance ranged between 83±61 individuals m<sup>-2</sup> in the oligotrophic habitat and 1320±277 individuals m<sup>-2</sup> in the eutrophic habitat. Species richness also varied, with 23 taxa observed at the eutrophic habitat and only 6 taxa in the oligotrophic habitat. Diversity was generally low for both habitats: H´=1.14 at the eutrophic and H´=0.90 for the oligotrophic habitat. Macrofaunal communities tended to be numerically dominated by few abundant taxa, although at the eutrophic habitat Nematoda dominated the community (74%), while Arthropoda comprised 30% of the total oligotrophic community. In terms of trophic guilds, macrofauna comprised predators and species that rely on organic detritus for food, namely filter feeders and deposit feeders. Results indicate that eutrophic habitat supported a more abundant and diverse community in comparison with the oligotrophic habitat, reflecting the higher content of available organic matter. This study supports the importance of food to determine the carrying capacity of habitats, highlighting the importance of available organic matter as a major control of abyssal macrofauna.

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# BIODEGRADATION OF ENROFLOXACIN BY A FLUOROAROMATICS-DEGRADING BACTERIAL STRAIN

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Antibiotherapy was a milestone in modern Medicine, revolutionizing the treatment and prevention of numeral infectious diseases. However, the extensive use of antibiotics, either in human and veterinary applications, has led to their emergence in the environment.

Quinolones are among the most used antibiotics in Europe, with fluoroquinolones (FQ) constituting the fourth largest class of antibiotics used in human and veterinary medicine, for the treatment of several bacterial infections [1]. FQ are fluorinated aromatic compounds, effective against aerobic Gram-positive and Gram-negative bacteria and to several anaerobic strains [2]. They have been found in several environmental compartments, namely marine [3] and freshwater [4], and their genotoxicity and potential for ecosystem damage has been confirmed.

This work aimed to study the biodegradability of a widely used veterinary fluoroquinolone - enrofloxacin (ENR). The study was carried out in batch mode in aerobic conditions, using as inoculum a bacterial strain with a high versatility to degrade fluoroaromatic compounds (*Labrys portucalensis*, strain F11). Biodegradation of ENR was investigated in cometabolism with sodium acetate. Fluoride ion release was used as an indicator of the biological degradation of the compound and was measured with an ion-selective combination electrode.

The results obtained along a period of 70 days, indicate biodegradation rates of ENR by strain F11 higher than 60%. High cell densities have been observed throughout the experiment, which is expected due to the presence of acetate as an easily degradable carbon source.

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## Combined effects of SDS/DDAB nanovesicles and of chlorpyrifos pesticide on the reproduction of *Eisenia andrei*.

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Nowadays, with the advance of nanotechnology and corresponding applications, the levels of nanomaterials in the environment are expected to increase. The effects of these nanomaterials aren't yet well known for the soil biota. This is particularly concerning, because this compartment is also the sink of many other contaminants, like pesticides.

In this work, the sub-lethal toxicity of the nano-vesicles of SDS/DDAB (Sodium Dodecyl Sulphate/Didodecyl Dimethylammonium Bromide) was assessed for the earthworm *Eisenia andrei* either alone or in the presence of the pesticide chlorpyrifos. For this purpose, we have performed two reproduction assays with the species *Eisenia andrei*, according to the OECD 222 guideline. Chlorpyrifos is a large-spectrum organophosphorus insecticide [1] that is widely used in agriculture, and that affects acetylcholinesterase (AChE) activity. We tested 5 concentrations of SDS/DDAB (1000, 833.3, 555.6, 370.4, and 246.9 mg Kg<sup>-1</sup>) alone and in the presence of chlorpyrifos (0.5 mg Kg<sup>-1</sup>). The concentration of the pesticide corresponded to an EC<sub>10</sub> for reproduction in *Eisenia andrei* as described by Silva et al. [2]. Four replicates were prepared for each treatment. At the end of each assay, the number of juveniles hatched from the cocoons was counted, and the inhibition of AChE activity was determined.

No significant effects on reproduction were recorded for concentrations up to 1000 mg Kg<sup>-1</sup> of SDS/DDAB nano-vesicles. However, in the presence of chlorpyrifos significant effects on reproduction were recorded for the two highest concentrations of SDS/DDAB tested (833.3 and 1000 mg kg<sup>-1</sup>). An EC<sub>50</sub> of 724.7 [263.6-1185.7] mg SDS/DDAB Kg<sup>-1</sup> was recorded in the presence of the pesticide, thus indicating that the toxicity of the nano-vesicles was increased in the presence of the pesticide.

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# Effects of antidepressant fluoxetine in European sea bass juvenile's behavior

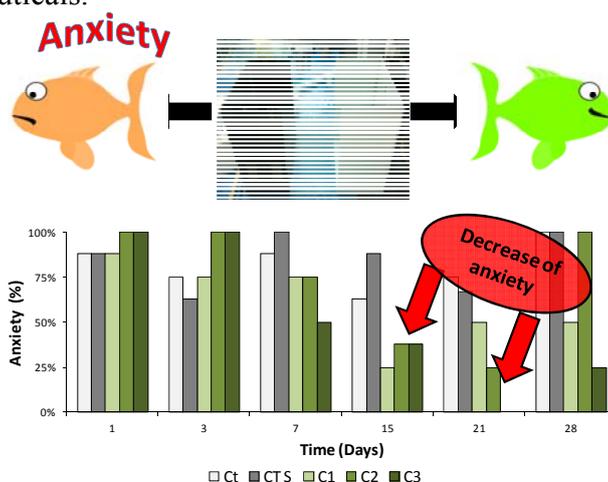
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Recently, the presence of several pharmaceuticals as the antidepressant fluoxetine (FX) and metabolites has been documented in aquatic ecosystems. FX is a selective serotonin reuptake inhibitor that operates in the serotonergic system of the central nervous system. These compounds are not always eliminated in wastewater treatment plants, reaching aquatic ecosystems as rivers and estuaries. This study aims to evaluate the effect of FX exposure in European sea bass (*Dicentrarchus labrax*) juveniles at the behavior level and also at the expression of associated specific brain genes. For this purpose, an exposure assay of 21 days plus a 7 days recuperation period was conducted, using 3 FX concentrations. To evaluate potential changes of juveniles behavior, points of analysis were selected (Day 1, 3, 7, 15, 21, 28). Swimming velocity was evaluated using a raceway device, registering the time that each individual took to swim through 3m of the raceway. Anxiety was also assessed by scototaxis (dark/light preference) using a specific aquarium containing a colorless central zone and two lateral dark and light compartments (Fig.1). The preference of each juvenile for the black or the white compartment was registered, using the preference of dark compartment as proxy of anxiety. Raceway results showed that only the fishes exposed to the highest FX concentrations (5 and 50  $\mu\text{g/L}$ ) swam through the raceway, with an average swimming velocity of  $0.26 \pm 0.18 \text{ m s}^{-1}$ . Scototaxis results denoted a decrease of anxiety in exposed fishes from the day 15 until the end of the exposure, with more than 50% of the juveniles avoiding the black compartment (Figure 1). Further studies will analyze the expression of seven associated genes from brain tissue samples collected on the 21th and 28th day. The behavioral and gene expression results will be interpreted and discussed in the context of potential contamination risk associated with contamination by psycho pharmaceuticals.



**Figure 1.** Scototaxis results showing a decreased of seabass juvenile's anxiety exposed to higher FX concentrations (C2 and C3) throughout the essay. Ct- Control; CtS- Control solvent; C1-0.5  $\mu\text{g/L}$  FX ; C2- 5  $\mu\text{g/L}$  FX; C3- 50  $\mu\text{g/L}$  FX.

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## Effects of two different concentrations of Ozone in *Salix atrocinerea* and *Plantago lanceolata* pollen

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Pollen can be modified by the increasing environmental pollution which can lead to a rising trend in pollen-induced respiratory allergies.

The aim of this work was to study the viability, protein profile and allergenicity of *Salix atrocinerea* and *Plantago lanceolata* pollen after exposure to two different concentrations of O<sub>3</sub>.

In this study, pollen collected directly from the anthers of *Salix atrocinerea* and *Plantago lanceolata* was exposed to O<sub>3</sub> in an environmental chamber with temperature and relative humidity controlled and artificial sunlight. Each sample of pollen was exposed during 6h to two different concentrations, two times and to the hour-limit value acceptable for human health protection in Europe (Directive 2008/50/EC).

After exposing the pollen to ozone, viability tests (Trypan Blue) and *in vitro* germination was performed. Protein extraction was made and total protein content was determined colorimetrically by the Bradford method. The proteins extracted from the exposed pollen of *S. atrocinerea* and *P. lanceolata* were separated by SDS-PAGE and the antigenic and allergenic properties were analysed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunological techniques using patients' sera.

It was observed that, the concentrations tested of O<sub>3</sub> can affect pollen fertility, indicating damage to the pollen membrane system. We also verified a decreasing trend in the total protein content of exposed samples comparatively with the control samples, but the SDS-PAGE protein profiles did not show differences. The patient sera tests revealed an increase in immunoglobulin E (IgE)-binding reactivity in the exposed pollen compared with the non-exposed pollen, suggesting alterations in the antigenic characteristics of the studied pollen types.

Our results showed that an increase in the atmospheric O<sub>3</sub> concentrations can have negative impact on the airborne pollen of the studied species, with impact on the reproductive process as well as on pollen related respiratory allergies.

# Evaluation of the ecological quality the Rivers Ferreira and Âncora by using biomarkers in benthonic macroinvertebrates

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Biomarkers are common tools used in environmental epidemiological studies in order to establish a relationship between the exposure to chemicals and the effects on the health of individuals exposed [1]. Thus their application could also be useful to find out if the deterioration of water quality can influence the activity of some enzyme in aquatic organisms. Following the Water Framework Directive (WFD), that pretends to achieve a good ecological status on all water bodies within in European territory [2], we have analysed several biomarkers in macroinvertebrates to test whether they are sensitive to the current state of the water quality in the rivers Ferreira and Âncora (Porto and Viana do Castelo districts).

The macroinvertebrates have been reported as ideal for the application of the biological markers because they are highly sensible to impacts on their habitat; this is the reason why their biotic indexes are the most used tool to define pollution rates [2]. Furthermore, they are ubiquitous and abundant [2].

First, we collected samples of various macroinvertebrate families (like Chironomidae, Baetidae, Simuliidae) in different points of each river and placed them in microtubes with a phosphate buffer solution. In the laboratory, the biological material was homogenized for the quantification of the total protein and for the analysis of two biomarkers: acetylcholinesterase (AChE) activity, catalase (CAT) and thiobarbituric acid reactive substances (TBARS). The protocols for these biomarkers were adapted from others originally developed for fishes and adapted for invertebrates [3].

More biological indicators will be applied in this study and date is being analysed to test if the classification of the rivers based on metrics recommended by the WFD, is coincident with the results provided by biomarkers measured in the most abundant families of macroinvertebrates.

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# Evaluation of the potential impacts of using olive oil mill effluent for ferti-irrigation on terrestrial plants and on soil edaphic fauna

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The olive oil production gives rise an extremely toxic and difficult to treat effluent. Since, we still lack an appropriate treatment method to deal with this effluent, its application for ferti-irrigation of agricultural soils has been recommended in Portugal (Despacho Conjunto 626/2000) to deal with the large volumes produced each year. The impacts of this application in soil, especially on soil fauna, are still poorly studied.

In this work, we aimed to study the impacts of soil irrigation / fertilization with olive oil mill wastewater (OOMW) on soil habitat and production functions. For this purpose, were conducted reproduction assays with invertebrates of the species *Folsomia candida*, and germination and growth assays with terrestrial plants, namely, *Brassica oleracea*, *Lactuca sativa*, and *Avena sativa*, according to OECD's 232 and 208 guidelines, respectively. For the reproduction assay, we used nine dilutions of OOMW [30%, 20%, 13.3%, 8.9%, 5.9%, 4.0%, 2.6%, 1.8%, 0%], with five replicates in the control and four for the treatments. The OOMW was applied to the soil, only once, in the beginning of the assay. In the germination and plant growth assays were tested five concentrations of the OOMW [30%, 23.1%, 17.8%, 13.7%, 10.5%, plus the control], each one with four replicates.

*F. candida* reproduction was not affected by OOWM, since no significant differences were recorded ( $p = 0.237$ ) in the number of juveniles produced, comparatively to the control. Emergence of *A. sativa* (oat) was significantly affected in 17.8% of OOMW ( $F = 7.3057$ ; d.f. = 23,18;  $p < 0.001$ ). However, no significant effects were observed for fresh and dry biomass. Thus we can not exclude that this effect has probably occurred by chance. In *Lactuca sativa* the dry biomass was significantly reduced for soils irrigated with 13.7, 17.8, 23.1 and 30% effluent dilutions ( $F = 4.844$ ; d.f. = 23,18;  $p = 0.006$ ). *B. oleracea* reveals to be the most sensitive species since all the parameters were significantly affected (emergence:  $F = 7.3224$ ; d.f. = 23,18;  $p < 0.001$ ; fresh biomass:  $F = 36.751$ ; d.f. = 23,18;  $p < 0.001$ ; dry biomass:  $F = 15.764$ ; d.f = 23,18;  $p < 0.001$ ). Although no meaningful effects were recorded on collembolans, the phytotoxic effects of OOMW were evident. Therefore, the results obtained encourage more testing to obtain information about the impacts of this procedure on soil health and functions.

# Improving risk assessment of the disinfectant Triclosan and its metabolite Methyl-triclosan using *Paracentrotus lividus* and *Danio rerio* embryos bioassays

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The presence of emerging compounds in the environment is a major concern, not only because of the potential negative impact in human health, but also due to potential toxicity to non-target organisms. The Personal and Care Products (PCPs) are referred as emerging pollutants since they encompass a major class of compounds detected in the waters, with limited available information on their environmental impact. Within the PCPs class, the disinfectant Triclosan (TCS) is one of the most concerning compounds. TCS is an antimicrobial used in many products of our daily life such as toothpastes, shampoos, deodorants or skin care products. It has the ability to block the fatty acid synthesis, inhibiting the cell growth [1]. Many studies have focused on the occurrence of this compound in the water systems and it has been detected in about 60% of the water analysis [2].

Regarding biotransformation, TCS is an organochloride, photodegradable that enters the environment in the parental form. Once in the WWTPs, it is biotransformed. A small part is methylated resulting in a more persistent by-product: Methyl-triclosan (M-TCS). Although not as prevalent as TCS, M-TCS has been raising concern about possible effects on organisms due to its high lipophilic characteristics [3]. Yet, the information on this subject is very scarce and so the toxicity of M-TCS is not well established.

In this work we aimed to improve the risk assessment of TCS and its metabolite M-TCS using sea urchin *Paracentrotus lividus* and the well-known model for toxicity assessment, zebrafish (*Danio rerio*). These species were chosen based on their ease obtaining, short embryonic development and finally due to their high sensitivity, as shown in previous works [4].

We performed bioassays with embryos exposed to TCS and M-TCS during 144h for zebrafish (early larva) and 48h for sea urchin (larva *pluteus* stage). Our preliminary results points to an impact of both chemicals, i.e., an increase in the abnormalities rates in zebrafish embryos and impact in the development of sea urchin larvae

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# Ingestion of microplastics by Portuguese estuarine fish fauna

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Microplastics (<5 mm) are widespread in marine and fresh water ecosystems. Concerns exist about potential physiological damage to a wide range of organisms and about bioaccumulation of toxic pollutants adsorbed on plastic. Unlike the marine environment, presence and effect of microplastics in freshwater ecosystems remain relatively unknown. This work aimed to study the ingestion of microplastics by fish fauna of the Douro, Lima and Minho estuaries. Several species were studied, including macrobenthic feeders (*Platichthys flesus*, *Dicentrarchus labrax*), detritivorous (*Chelon labrosus*, *Liza ramada*) and omnivorous (*Diplodus sargus*). A total of 145 individuals were collected, including 21 adults and 124 juveniles, by beam-trawl and pushing-nets. Microplastics ingestion was analyzed through visual observation of stomach contents, made under a stereoscope microscope. Prior to the opening, all of the stomachs were rinsed with filtered distilled water, as well as all of the instruments, to prevent external contamination. A control glass petri dish was placed near by the working area, to check for airborne contamination, which was negligible. Considering all stomachs, 29 (20%) contained microplastics, and the most abundant form were fibers. Adult fishes (33%) had a higher frequency of microplastic ingestion than juveniles (18%). Douro was the most impacted estuary, with an ingestion rate of 22%, compared with Lima (16%) and Minho (9%). Macrobenthic feeders were the most contaminated species (38%), above detritivorous (14%) and omnivorous (0%), pointing out to species-specific differences in terms of microplastic ingestion. This study includes the first analysis of contamination by microplastics in Portuguese estuarine fish fauna and results are discussed in the scope of environmental risk related with these emergent contaminants.

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# Microbiological quality of untreated water used for human consumption with special focus on antibiotic resistant bacteria

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The public water supply is a growing concern in terms of public health, due to the scarcity and decreasing quality of water resources. Untreated water can be improper for human consumption, may contain pathogenic microorganisms or chemicals, capable of causing waterborne diseases. Coliforms, microorganisms from fecal contamination are the main biological indicators of concern in terms of water quality. These microorganisms have been developing resistance mechanisms to antibiotics, such as to  $\beta$ -lactams, one of the most widely used antibiotic classes. This bacterial resistance problem has increased dramatically, putting the general population in serious health risk [1,2].

Our work aimed to verify the presence of antibiotic resistance in gram-negative bacilli present in untreated drinking water. A total of 32 water samples used in this study were collected from different locations: wells (47.62%), fountains (38.10%) and mines (14.29%). The water sample collection was performed according to the ERSAR recommendation 03/2010 [3]. Water samples were analyzed by the membrane filtration method on MacConkey agar and Mackonkey agar with ampicillin. Representatives of different colony morphologies were tested for antimicrobial susceptibility, according to CLSI. Identification of resistant bacteria was performed by API 20 E and ID 32 GN.

Thirty eight antibiotic resistant isolates were obtained, of which 15 were lactose fermentative and 23 non-fermentative. The presence of a *Shigella* spp. in a fountain water sample alerts for unknown risks of human consumption of this kind of waters. It was also identified a *Pseudomonas fluorescens* in a well, which has a huge relevance given the resistance shown to carbapenems. *Stenotrophomonas maltophilia* a multidrug resistant opportunistic pathogen was found in wells and shafts.

This work found antibiotic resistant isolates of lactose non-fermenters that are not considered as indicators of water quality but can be relevant in terms of water safety, creating reservoirs of bacterial resistance genes and antibiotic resistant bacteria able of intestinal colonization of the population that use these sources of untreated water.

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## Photocatalytic reduction of CO<sub>2</sub> into fuels

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In recent decades, rising atmospheric levels of carbon dioxide and depletion of fossil fuel reserves have raised considerable public concern. Utilizing renewable solar energy to convert CO<sub>2</sub> emissions into value added chemicals, would be a fascinating sustainable solution to solve both issues. Solar fuels derived from CO<sub>2</sub> can contribute to neutralize the carbon balance into the atmosphere, while CO<sub>2</sub> is converted into easily transportable liquid chemicals, such as methanol [1]. Graphene based composites prepared with TiO<sub>2</sub> have attracted enormous attention for photocatalysis, due to the unique properties of these materials.

In this work, reduced graphene oxide-titania composites (GO-TiO<sub>2</sub>) were prepared by liquid phase deposition followed by post-thermal reduction [2]. The composite materials were applied for the photocatalytic water reduction of CO<sub>2</sub> to renewable fuels (i.e. methanol) under UV/Vis light irradiation. The effect of initial pH as well as platinum and copper metals as co-catalysts on the photocatalytic CO<sub>2</sub> reduction were systematically investigated.

The photocatalytic runs were carried out in a cylindrical glass immersion photo-reactor (250 mL) with a Heraeus TQ 150 medium-pressure mercury vapor lamp located axially in the reactor and held in a quartz immersion tube. The experiments were performed at neutral pH (7.0) and also at lower (3.0) and higher (13.0) initial pH values. The photocatalyst load was typically 1 g L<sup>-1</sup>. The gaseous products were analyzed online by using gas chromatography (GC-TCD). Methanol and ethanol in liquid samples were also determined by gas chromatography using a different detector (GC-FID).

The presence of GO and metals in the composites increased the absorption in the visible spectral range, enhancing the CO<sub>2</sub> photoreduction in aqueous phase, with formation of both methanol and ethanol as main products under near-UV/Vis light irradiation. The effect of pH in the photoreduction process depended on the availability of dissolved CO<sub>2</sub>. The best performance was obtained at pH 13 where the dissolved CO<sub>2</sub> level in water was the highest.

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# Transcriptional level of genes involved in the neurotransmitter system of *Dicentrarchus labrax* in response to chronic exposure to psychopharmaceuticals

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During the last decades, a variety of psychopharmaceuticals (PP) were recognized as an important source of environmental contamination and increasing concern emerged regarding their potential ecological impact [1]. These pharmaceuticals targets are highly conserved among vertebrates suggesting that aquatic animals like fish are likely to be affected by environmental exposure even at low concentration [2]. Previous studies of our group showed that in *Dicentrarchus labrax* (*D. labrax*) the ratio of RNA/DNA increased after 1 day of exposure to Fluoxetine at the concentration of 0.5 and 50 µg/L. Peroxidase enzyme activity in the plasma decreased after 1 day at the same PP and concentration.

The aim of this study was to evaluate transcriptional levels of genes involved in *D. labrax* central nervous system in response to waterborne exposure to two PP. Selected antidepressants were: Fluoxetine (0.5µg/L and 50µg/L) and Venlafaxine (0.01µg/L and 1µg/L). The chronic exposures were performed in a flow-through system for 21 days, with a 7 day recovery period to assess the reversibility of effects. The brain of *D. labrax* was collected and preserved in RNALater. Quantitative real-time PCR (qPCR) assays were successfully established for genes encoding for proteins involved in the neurotransmitter system, such as 5-Hydroxytryptamine subtype 3A (5-HT3A) and 3B (5-HT3B), serotonin transporter receptor (*SERT*), monoamine oxidase (*MAO*), vesicular monoamine transporter (*VMAT*) and dopamine 2 (*D2*) and 3 (*D3*). Specific primer pairs were designed based on UCSC Genome Browser of the sea bass genome (<http://seabass.mpipz.mpg.de/cgi-bin/hgGateway>). Currently, the qPCR analyses are ongoing and first results will be presented about transcriptional changes of neurotransmitter genes in *D. labrax*. This study aims to establish a potential link between environmental exposure of PP, and physiological and neurological changes in fish through the expression of the genes of their neurotransmitters.

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## **Urban Agriculture and Sustainability of cities: project “horta à porta” in Porto**

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The sustainability of cities has been the target of reflections, due the need to articulate policies aimed at maximizing the social, economic and mainly environmental benefits. Thus, municipalities and other entities have been contributing with practices involving the three referred dimensions of sustainable development. Lipor as Intermunicipal Waste Management Service of Greater Porto, along with other partnerships, reinforces the agricultural activity through the design of biological gardens in urban ecosystem. This practice is seen as a potential strategy, due to its contribution to the increase of urban green spaces, in addition to other beneficial multi-functionalities. This study aims to assess / demonstrate that this Community Project, called "horta à porta", contributes effectively to sustainable urban development and consequent improvements in quality of life for inhabitants of Porto. This conclusion will be supported by the analysis of the 60 surveys intended to citizens covered by the project “horta à porta” and by the interview with the responsible of the same project. The bulk of the study consists of bibliographical research, for greater depth in different issues addressed. Finally, in a more general view, we seek to contribute to helping the several entities in this area, so that a reflection on the extension of such projects can be made, in response to the success and the number of candidates on the waiting list of the same.

When 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.

# Veterinary antibiotics amoxicillin and doxycycline in aqueous environmental samples

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The discovery of antibiotics is considered one of the most significant scientific achievements of the 20<sup>th</sup> century. Although they are used for over 60 years to improve both human and animal health, only recently the existence of antibiotics in the environment has received particular notice [1].

Around the world, antibiotics are massively used in animal food production, accounting for up to 50% of total sales. Moreover, it is estimated that antibiotic consumption in livestock worldwide could rise 67% by 2030. Since animal husbandry excrete 75% to 90% of administered antibiotics, these pharmaceuticals reach the environment by the application of manure to agriculture soils, or by pasture-reared animals excreting directly on the land, followed by run-off or leaching to water. The complex vicious cycle of biotransformation and bioaccumulation of antibiotics in the environment contribute to the emergence and global spread of antibiotic resistance bacteria [2].

In this context, studies about occurrence, fate and effects of veterinary antibiotics in the environment has increased during the last decade. However, further researches on veterinary antibiotics as emerging environmental micropollutants is required. Additionally, the development and validation of new analytical techniques is crucial to obtain accurate data on the concentrations of these compounds in the environmental. In this study, solid-phase extraction (SPE) and high-performance liquid chromatography-diode array detection (HPLC-DAD) analytical procedure for concentration and quantification amoxicillin and doxycycline in aqueous samples was developed, optimized and validated. The proposed methodology can be applied to the simultaneous determination of the target antibiotics, which are the two antibiotics most commercialized in Portugal for veterinary practice, in different water samples. Enabling the fast screening and effective determination of both compounds in the aqueous environment, the presented procedure might be important to understand their dynamic in the ecosystems.

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# Neutrophil elastase gene polymorphisms in the inflammatory response of end-stage renal disease patients

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End-stage renal disease (ESRD) is a growing public health problem with an increasing worldwide prevalence. ESRD patients present a high mortality rate that far exceeds the mortality rate for the non-ESRD population and inflammation is a common feature in these patients under hemodialysis (HD). However, the mechanisms/factors triggering the inflammatory process are still poorly clarified. Our group demonstrated recently that HD procedure induces neutrophil activation and elastase release, which might have an important role in amplifying the inflammatory process in these patients. Neutrophil elastase (NE) is a powerful serine protease, synthesized by neutrophils and stored in their azurophilic granules, has potent catalytic activity against a broad array of extracellular matrix substrates, and is the only protein able to cleave insoluble elastin. The NE expression levels are known to be affected by polymorphisms in the promoter region of the neutrophil elastase (*ELANE*) gene. Until now, there are five polymorphisms identified in the six repetitive tandem motifs of the *ELANE* promoter region: c.-903T/G, c.-741G/A, c.-832G/T, c.-789C/T, and 52 bp extra, between the fourth and the fifth repeats.

Considering the implications of high inflammation/oxidative stress status in morbidity and mortality of ESRD patients, the aim of our work was to identify and evaluate the impact of the polymorphisms identified in *ELANE* promoter region in a group of ESRD patients, and its contribution/association with the inflammatory process and disease prognostic.

To accomplish these objectives, we performed a cross-sectional study with 123 ESRD patients on regular hemodialysis. Blood samples were obtained to determine biochemical and hematological parameters, markers of iron metabolism and inflammation. The promoter region of the *ELANE* gene was screened for all patients using PCR-direct sequencing. Elastase quantification was performed by ELISA.

We found three of the previously described polymorphisms: c.-903T/G, c.-741G/A, and the extra 52 bp between the fourth and the fifth repeats. Additionally, we have found two new polymorphisms that haven't been described yet: c.-801G/A, and an extra block, of fourth and fifth repeats, between the fifth and the sixth repeats. Also, patients homozygous for c.-741G/A polymorphism showed a significant decrease in elastase/neutrophil ratio.

The c.-741G/A polymorphism seems to be associated to a decrease of elastase production in patients with ESRD. We intend to quantify the neutrophil elastase in more patients and to evaluate its impact on the inflammatory process of ESRD patients.

## New steroidal aromatase inhibitors: aromatase-dependent anti-proliferative effects and apoptosis in MCF-7aro breast cancer cells

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Hormone-dependent (ER<sup>+</sup>) breast cancers require estrogen for their growth. One of the therapeutic approaches includes the use of aromatase inhibitors (AIs), which by inhibiting the enzyme aromatase block the conversion of androgens to estrogens. However, the development of AIs-acquired resistance and the occurrence of bone loss are two main drawbacks, highlighting the importance for searching for new and potent AIs [1]. Based on the interaction of androstenedione with the enzyme active site, a range of new steroidal compounds have been designed, synthesized and studied for their anti-aromatase activity, by our group, in order to find new and potent AIs [2]. Three steroidal compounds, 4 $\beta$ ,5 $\beta$ -epoxyandrost-17-one (**43**), 6 $\alpha$ -methyl-5 $\alpha$ -androst-3-en-17-one (**46**) and 6 $\alpha$ -methyl-3 $\alpha$ ,4 $\alpha$ -epoxy-5 $\alpha$ -androst-17-one (**47**) were obtained from chemical modifications in A- and B-ring of androstenedione, the natural substrate of aromatase. In this work, their biological effects were studied in an ER<sup>+</sup> aromatase-overexpressing human breast cancer cell line (MCF-7aro), in a non-cancerous cell line (HFF-1) and in an AI-resistant cancer cell line (LTEDaro). Our results demonstrate that the new AIs decrease MCF-7aro cell viability in a dose- and time-dependent manner, being the anti-proliferative effects aromatase-dependent. Moreover, it was observed morphological changes in MCF-7aro cells, such as membrane blebbing and chromatin condensation, typical features of apoptosis, which was further confirmed by a significant activation of caspase-7. In addition, these AIs prove to be non-toxic for HFF-1 cells and have the ability to sensitize LTEDaro cells. Compound **47** was the most potent and promising AI. These results provide new insights on the most favorable structural modifications in androstenedione structure in order to design new and potent AIs with lower side effects.

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# Optimization and characterization of methotrexate-loaded polymeric nanoparticles: a Box-Behnken design approach

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Methotrexate (MTX) is an antiproliferative and immunosuppressive agent, with great results in the cancer and inflammatory diseases treatment. However, MTX exhibits high cytotoxicity that limits its application. For that reason, the incorporation of MTX in delivery systems, as polymeric nanoparticles of poly(lactic-co-glycolic acid) (PLGA) can deliver the drug to the target, overtaking the limitations connected to the toxic effect. Hence, the first step of this study was the optimization of some parameters for the development of polymeric nanoparticles loaded with MTX following the main criteria: average particle size between 100-200 nm, low polydispersity and drug encapsulation efficiency higher than 75%.

MTX-loaded PLGA nanoparticles were successfully prepared by emulsion-diffusion evaporation technique using polyvinyl alcohol as surfactant. For this combination a 15-run, 3-factor, 3-level Box–Behnken design was employed to optimize the process according amount of surfactant, amount of drug and amount of solvent [1]. The characterization was conducted according to their physico-chemical properties such as: particle size, polydispersity index and encapsulation efficiency. Size and polydispersity index are evaluated by dynamic light scattering. For the encapsulation efficiency the concentration of non-incorporated MTX was determined by absorption spectroscopy.

Utilizing gold nanoparticles [2], it will allow to externally guided the delivery of methotrexate. So, gold nanoparticles were synthesised following the Burst method [3], for further incorporation in the optimised MTX-loaded PLGA and characterised by transmission electron microscopy.

The optimized nanoparticles will be explored for the application in the therapy of inflammatory diseases.

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# Paclitaxel-loaded nanostructured lipid carriers – An iterative optimization

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Paclitaxel is one of the most effective broad-spectrum chemotherapeutic agent in the treatment of cancers. However, its clinical application has been limited due to its poor water solubility. Moreover, paclitaxel causes several side effects due to a wide biodistribution and has undesired pharmacokinetics. Therefore, there is a need of an effective incorporation of paclitaxel in a drug delivery system that can enhance its solubility, permeability and stability and promote a sustained, controlled and targeted delivery, increasing thereby the therapeutic effects and reducing the dosages and side effects.

The objective behind this study was to apply an iterative approach in the optimization of nanostructured lipid carriers' varied characteristics and assess its potential as Paclitaxel vehicles and therefore as anti-neoplastic drug delivery systems. Lipophilicity assays determined Gelucire<sup>®</sup> 43/01, Compritol<sup>®</sup> 888 ATO and Miglyol<sup>®</sup> 812 as the best lipid blend, among a wide variety of tested lipids, for Paclitaxel dissolution. From a starting composition the formulation's physicochemical properties, such as mean particle size, zeta potential and polydispersity index, were evaluated. The composition was iteratively changed as molar ratios between the lipids and the surfactant (Tween<sup>®</sup> 80) are concerned. Each and its composition was for the next one. After optimization, a formulation with good entrapment efficiency, mean diameter of 245.5±1.0nm, polydispersity index of 0.113±0.008 and zeta potential of -29.23±0.47 was achieved. This optimized Paclitaxel-loaded nanostructured lipid carrier formulation already seems a potential candidate as a cheap and safer parenteral anti-neoplastic agent.

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## Preparation and characterization of polymeric particles containing resveratrol

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Resveratrol (RES) is a polyphenolic compound found in grapes and red wines used as an active constituent in pharmaceutical and cosmetic preparations. A rapid isomerization occurs after ultraviolet irradiation exposure which impairs its effectiveness [1]. The aim of this study was to encapsulate RES into polymeric particles to be incorporated in topical formulations, as a strategy to increase its photostability. Polymeric microparticles with RES and empty particles were produced using a piezodriven spray generation apparatus. RES was dissolved in ethanol: water solution (20:80) until saturation and mixed with Eudragit® RS 30D (0.5%). Laser diffractometry (LD) was performed in order to analyze the particle size. The study of the degree of crystallinity was performed by Differential Scanning Calorimetry (DSC). Resveratrol assay was performed with a reverse phase HPLC method. Elucidation of the sedimentation of the particles was carried out by measuring the particle size after preparation and after 24 h rest. For the *in vitro* RES release study, 5 ml samples (0.07 mg/ml, phosphate buffer pH 5.6 solution) were placed into plastic tubes and incubated at 32°C with constant stirring. At regular intervals, a tube was withdrawn and the sample was filtered (0.45 µm) before being analyzed using a spectrophotometric method. Cell viability was analyzed using MTT reduction assay. The viable cells with active metabolism convert MTT into a purple colored formazan product with an absorbance maximum near 570 nm. HaCaT cells (10<sup>4</sup> cells/well, 96 wells microplates) were incubated for 24 h in DMEM supplemented with 10% fetal bovine serum containing the empty and RES-loaded polymeric particles (0.01, 0.05 and 0.1 %). Afterwards, cells were washed with HBSS and MTT (0.5 mg/mL) was added. After 2 h incubation, dimethyl sulfoxide was added and absorbance was measured at 570 nm.

The particles showed a homogenous size distribution with a Dv 50 of 5.36±0.01 µm and drug loading of 4.78%. The analysis of the thermograms of the microparticles showed no detectable peaks for RES. Release studies demonstrate that after 1 hour 80% of the RES was released from microparticles. The results obtained in the cytotoxicity assay were inconclusive which could be related with the sedimentation of the microparticles in the cell medium. When analyzing the sedimentation process, we found that after 24 h a residue was composed by particles with a Dv 50 of 6.03±0.05 µm while the particles on the supernatant presented a Dv 50 of 1.31± 0.02 µm. Other cytotoxicity assays should be performed, for instance using reconstructed epidermis, in order to avoid the interference of sedimentation. Considering the fast release in aqueous medium, the use of these particles in lipophilic or powder formulations is recommended. The effect on RES photostability and skin penetration should be further assessed to establish the interest of these particles for topical application.

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# Production of Solid Lipid Nanoparticles and Nanostructured Lipid Carriers containing antitumoral drug (saquinavir)

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The blood-brain barrier (BBB) is a limiting barrier to the passage of a large number of drugs, particularly antitumoral drugs. This is mainly due to the tight junctions between the endothelial cells lining the blood vessels of the brain. Inevitably, this barrier makes these drugs ineffective on the treatment of brain tumors, since they can't diffuse freely across this barrier [1]. Glioblastoma (GBM) is the most common and most malignant form of primary brain tumors, with survival rate relatively short (9-12 months) [2]. There have been several attempts to overcome the BBB and nanotechnology emerges as leading solution [3]. By developing colloidal delivery systems such as Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs), new boundaries have been opened to improve the delivery of drugs. These nanoparticles are supposed to be non-toxic, biodegradable, with high loading capacity of lipophilic drugs and increased bioavailability in target cells [4].

In this Master's thesis project we developed and optimized SLNs and NLCs incorporating saquinavir (SLN-SQV and NLC-SQV). SQV is a lipophilic and poorly water soluble HIV protease inhibitor, also used as a potent antitumoral agent efficient against numerous tumor cell lines *in vitro* and *in vivo* [5]. The nanoparticles were produced by High-Pressure Homogenization and High-Shear Homogenization techniques with different lipid (5%, 10%), surfactant (1%, 2%, 3%) and drug (0.5%, 1%) concentrations and physico and chemically characterized in order to assess which ones are the most efficient and that best meet the desired requirements. The stability, encapsulation efficiency (HPLC) and thermodynamic behavior (DSC) of SLN and NLC were also tested. The results of the two techniques were generally similar, but slightly better for nanoparticles with lipid 5%, polysorbate 2% and drug 0.5%. The SLN-SQV while having a zeta potential higher than the NLC-SQV, had lower encapsulation efficiency. The drug was solubilized in the lipid both in SLN-SQV and the NLC-SQV, because there weren't any drug fusion events as showed by the DSC technique.

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# Risk of metabolic complications and association with cooking skills and Mediterranean food pattern in a Portuguese economically disadvantaged population

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Actually, approximately 18.7% of the Portuguese population is at poverty risk. Socioeconomic factors have been reported to influence lifestyle and nutritional status. The present study is part of a project which is being developed by a Portuguese Social Solidarity Private Institution. The aim of this study is to verify the association between the risk of metabolic complications and two factors, cooking skills (CS) and adherence to the Mediterranean Food Pattern (MFP).

The population consisted of 99 low income householders, 82 females, between the ages of 21 and 66. A questionnaire containing several sections was applied (socioeconomic data, physical activity, smoking habits and presence of diseases, 24 hours dietary recall, adherence to MFP<sup>[1]</sup> and level of CS<sup>[2]</sup>). Height, weight and waist circumference were measured according to international procedures. The waist-to-height ratio<sup>[3]</sup> (WHtR) was used to assess the risk of metabolic complications.

This population is characterized mostly by individuals with only 5 to 6 years of education. It was found 1.2% of underweight, 30.2% of normal weight, 26.7% of overweight and 41.9% of obesity. Using the WHtR, 82.4% of the population showed risk of developing metabolic complications. Adherence to the MFP showed a mean value of 5.63 ( $\pm 1.87$ ), in a scale varying from 0 to 14 points. For CS, a mean of 4.74 ( $\pm 1.02$ ) was obtained in a scale varying from 1 to 6 points. Correlation between adherence to MFP and CS was low and not significant ( $\rho=1.160$ ;  $p=0.165$ ). Mann-Whitney test using the variables WHtR and MFP or CS showed non-significant results ( $p=0.335$ ;  $p=0.651$ , respectively).

It was observed a trend for overweight and obesity and a high risk of developing metabolic complications. The adherence to MFP was low and not related with CS. No significant association between metabolic risk and MFP or CS was found.

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# Role of RNA-Binding Proteins in Microglia Activation/Deactivation

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Microglia (MG), the resident macrophages of the Central Nervous System (CNS), play important roles in monitoring synapses, phagocytosis, and controlling neuroinflammation [1]. Neurodegenerative disease and ageing research are recently being centred on MG-driven inflammation, since heightened neuroinflammation is a main feature of pathologically affected tissues, such as in Alzheimer's disease [2].

Under normal physiological conditions, MG acquire a 'resting' ramified morphology allowing a constant surveying of the neural microenvironment. As a consequence of brain injury, MG change their morphology to an ameboid-like, 'activated' form [3]. Such morphological switch is associated with fast changes in gene expression and function. It is now clear that activation of microglia, like macrophages, is heterogeneous and can include pro- (M1) and anti- (M2) inflammatory signatures, which are driven by the local environment that can provide M1 and M2 polarizing cues simultaneously [4]. After injury repair, microglia "deactivates", reverting to a ramified resting morphology [3]. MG activation and deactivation require a coordinated gene expression program in part regulated by RNA-Binding Proteins (RBPs). These molecules regulate every aspect of RNA metabolism, including RNA translation, and disruption of RBP function in the brain can lead to neuronal disease.

Furthermore, it has been reported that RBPs can regulate the macrophage state of activation, the stability of inflammatory cytokines [5], and we have seen that their expression in MG is modified upon LPS-activation. Preliminary results of the lab identified several putative binding sites for specific RBPs in the 3' Untranslated Region (UTR) of genes associated with the acquisition of the M1 and M2 phenotypes. This analysis led to the possibility that gene translation in the M1 signature is being regulated by a different subset of RBPs from those regulating M2 polarisation. Modulating the expression of such RBPs could in theory regulate the signalling pathway leading to the activation and deactivation of MG.

We initially selected 4 candidates from the previously identified RBPs and we analysed their expression patterns by RT-qPCR in the different MG stages: resting, activation and deactivation. Our work has then focused on the neuro-oncological ventral antigen 1 (Nova1) since it revealed the most interesting pattern. We modulated Nova1 by lentiviral shRNA delivery and found that it can alone modify MG physiology by enhancing and suppressing specific cytokine expression.

These results bring new insights into the modulation of MG inflammation patterns, revealing new potential therapeutic targets for several neuroinflammatory diseases.

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## **Selenium and cardiovascular diseases: evidences from the post-mortem analysis of tissues**

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Cardiovascular diseases (CVD) are a group of disorders of the heart and blood vessels, which are often related to atherosclerosis, a condition that occurs when plaques accumulate on the inner walls of the arteries [1]. Free-radical processes play a central role in the pathogenesis of this process.

It is now well established that selenium (Se), an essential trace element, has unique antioxidant properties through the constitution and regulation of the activity of GPx and several other selenoproteins. Selenium deficiency has been implicated as a risk factor for CVD [2]. However, epidemiological studies have shown controversial results and did not prove unequivocally that a high intake of Se prevents the development of CVD [3].

Based on this background, and in order to look for further evidence of the actual involvement of Se in the pathogenesis of CVD, we performed a study involving the direct analysis of the kidney and aorta tissues. The main objective was to assess the differences in Se levels between CVD patients and healthy subjects. Samples of kidney and aorta (both with and without plaque of atheroma) were collected from individuals submitted to autopsy (n=67; 22–93 years old). After samples microwave-assisted acid digestion in closed vessels, Se was determined by ICP-MS. The results were expressed as µg/g on a dry-weight basis.

When comparing the results for aorta tissue with or without plaque a significantly higher Se level was found in the aorta without plaque (4.71±2.33 µg/g vs. 3.85±2.50 µg/g, p≤0.05). Both in the kidney and aorta, no significant gender- or smoking-related differences were observed.

Individuals with CVD (n=11) showed lower Se levels than healthy subjects in both kidney and aorta tissue: 3.90±2.80 vs. 5.48±5.35 µg/g and 3.59±2.45 vs. 4.46±2.14 µg/g, respectively (p≤0.05), which is in good agreement with current evidence from studies supporting a modest inverse association between Se levels and the risk of CVD [3].

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# Solid Lipid Nanoparticles Encapsulated Antitubercular Drug Isoniazid

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Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* and is still far from being a health concern of the past. In 2014, the World Health Organization (WHO) reported an estimated 9 million new cases and 1.5 million deaths from TB, thus making it the second leading cause of death by infectious diseases in the world. Drug treatment for TB is becoming increasingly difficult due to the long treatment period and patient non-compliance, which frequently leads to the emergence of multi-drug resistant (MDR) strains. The search for new anti-TB drugs is of key importance in this fight, but notwithstanding this search, new drug delivery strategies may also play an imperative role. Isoniazid (INH) is a first-line anti-TB drug in the battle against TB. INH is a Biopharmaceutical Classification System (BCS) class III drug, presenting high solubility and low permeability. One of the major drawbacks in the use of INH for the treatment of TB is the severe toxicity associated. The severe toxic effects induced by INH frequently lead to discontinuation of the therapy. In this context, the present study aims to increase the therapeutic index of INH through the development of a delivery system, using solid lipid nanoparticles (SLN) synthesized by hot ultrasonication method [1]. The developed nanoparticles were characterized regarding their size, zeta potential, encapsulation efficiency (EE) and loading capacity (LC). The results showed that the developed nanoparticles possess a particle size of 152.2 ( $\pm$  1.2) and a polydispersity of 0.219 ( $\pm$  0.004). In addition, the NLC synthesized are stable regarding their high zeta potential of  $-22.68$  ( $\pm$  0.82) mV. The EE obtained was around 15 %, and the LC 1.4%. Therefore, the produced SLN constitute a suitable platform to encapsulate drugs with hydrophilic character, such as INH and may be exploited in the future to effectively fight TB.

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## **Sustained release tablets of thiamine hydrochloride based on glyceryl behenate**

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Sustained release tablets have been a research field with growing interest due to their advantages, such as convenient posology and reduction of side effects. Actually, the main advantages refers to security and efficacy, due to the improved bioavailability of the drug and to the fact that drug concentrations do not oscillate, which reduces the toxicity associated to peaks in the pharmacokinetic profile. One of the most simple and used ways to obtain a sustained release dosage form is the use of matrix systems, in which the drug is homogenously dispersed in a matrix-forming agent, which controls the drug release [1]. The main objective of this work was the development of sustained release tablets using glyceryl behenate as a lipophilic matrix and thiamine hydrochloride as a model drug, and their technological characterization. The formula was constituted by thiamine hydrochloride 2.5% (w/w), glyceryl behenate 53.0% (w/w), lactose 21.0% (w/w), cellulose microcrystalline 36.5% (w/w), talc 4.0% (w/w) and magnesium stearate 1.0% (w/w) [2]. The tablets were obtained by direct compression using a compression machine (Korsch 9048-71, Germany) and evaluated for mass uniformity, hardness (hardness tester Erweka TBH 28, Germany) and friability (friabilator Electrolab EF-1W, India), according to the Portuguese Pharmacopoeia 9.0 [3]. Additionally, to access the drug release through the developed tablets, an *in vitro* dissolution assay was performed in a dissolution apparatus (Sotax model AT7, Switzerland). The dissolution medium was maintained at  $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  and stirred at 100 rpm. Samples (10 mL) were withdrawn at predetermined time intervals, without volume reposition, filtered and assayed by spectrophotometry at 273 nm.

The developed tablets complied with the acceptance criteria established by the Portuguese Pharmacopoeia 9.0, regarding mass uniformity and friability. The main hardness value was 62.9 N, which can be considered acceptable. At the end of 90 minutes, the maximum amount of released drug was 67.9%.

We can conclude that the obtained tablets showed good mechanical resistance, mass uniformity and a sustained release of thiamine hydrochloride.

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## Syndecan 4 role in gastric cancer cell biology

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Syndecans are transmembrane proteoglycans comprising a core protein with covalently attached heparan sulfate chains on their extracellular domain. Syndecans play key roles in cell-cell and cell-extracellular matrix (ECM) interactions and regulate cell adhesion and cell motility, processes that modulate carcinogenesis [1]. There are four members of the syndecan family in mammals, being the syndecan 4 (SDC4) expression ubiquitous. SDC4 and beta1 integrin have been demonstrated to cooperatively act for the cell-ECM adhesion through focal adhesion formation and actin cytoskeleton modulation [2]. Aberrant expression of SDC4 has been reported in breast cancer, melanoma, hepatocellular carcinoma and malignant mesothelioma [3]. In addition, we have shown that, during the gastric carcinogenesis, *H. pylori* infection results in increased expression of SDC4 but its biological implications remain unknown [4].

The aim of this work is to decipher the role of Syndecan-4 in gastric cancer cell behavior and evaluate its putative involvement in gastric tumorigenesis.

The AGS human gastric adenocarcinoma cell line was used to evaluate changes in cell morphology and behavior upon SDC4 siRNA treatment. SDC4 silencing efficiency was monitored by Western Blot and Real Time PCR. SDC4 and beta1 integrin expression and subcellular localization were evaluated by immunofluorescence, and migration assays were performed to evaluate SDC4 silencing impact on cells migration capacity. Immunohistochemistry for SDC4 was performed in human gastric cancer tissue samples.

We observed that SDC4 silencing affects cell morphology with loss of lamellipodia and filopodia formation. In accordance with the previously described SDC4 and beta1 integrin interplay, gastric cells with reduced expression of SDC4 showed alterations in beta1 integrin expression and subcellular localization. Furthermore, these phenotypic alterations had an impact on cell migration capacity.

Finally, tissue immunoprofilling with a SDC4-specific antibody showed that SDC4 expression was upregulated in intestinal-type gastric cancer tissues compared to normal gastric mucosa. Our results suggest that SDC4 may constitute an important regulator of gastric cancer cell behavior by modulating cytoskeleton organization, cell adhesion and migration.

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# Synthesis and structural elucidation of a new carboxyxanthone

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Xanthenes represent a structurally diverse group of compounds with a broad range of biological activities. This important class of compounds may contain a wide variety of structures depending on the substituents, including methyl and carboxylic acid groups [1]. A remarkable example among the large number of natural and synthetic xanthone derivatives is 5,6-dimethylxanthone-4-acetic acid (DMXAA), a simple carboxylated xanthone. DMXAA was originally developed as an anti-tumor agent and was the first of its class to enter Phase III trials [2]. Carboxyxanthenes are not only interesting bioactive compounds but they are also very important as basis to molecular modifications in order to obtain new derivatives, such as chiral derivatives [3].

In this work, we described the total synthesis of a new carboxyxanthone by a multi-step pathway. The synthetic strategy involves an Ullmann reaction between an aryl bromide with a phenol possessing two methyl substituents, and using picolinic acid, CuI in DMSO/K<sub>3</sub>PO<sub>4</sub> at 80°C, leading to the formation of a diaryl ether intermediate [4]. The structure elucidation of the synthesized carboxyxanthone and its intermediates was established by spectroscopic methods (IR, <sup>1</sup>H and <sup>13</sup>C NMR).

The new carboxyxanthone will be evaluated for growth inhibitory activity on human tumor cell lines and for anti-inflammatory activity by inhibition of enzymes involved in the inflammatory cascade. Additionally, it will be used as suitable functionalized chemical substrate to obtain new chiral derivatives of xanthenes.

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# Synthesis of a xanthonic compound with antitumor activity and development of proliposomes as its delivery system

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Liposomes are vesicular systems, composed of phospholipids that mimic biological membranes [1], with a range of size between nanometers and micrometers [2]. These systems have been widely explored for the delivery of drugs [2]. However, liposomes present stability problems, limiting their storage for a long period [3]. Therefore, there is the necessity to develop strategies to overcome these problems. Preparation of proliposomes is a promising and cost-effective strategy to formulate liposome dispersions. Proliposomes are dry, free-flowing granular products composed of drug and phospholipids which, on hydration, convert into vesicular structures [4]. Proliposomes have potential to be exploited in different routes of administration.

The aim of this study is to synthesize a xanthonic compound (LEM2) with antitumor activity, namely in some glioma cell lines, and to develop proliposomal formulations to encapsulate and deliver LEM2. Proliposomes were prepared with egg phosphatidylcholine (EPC) and cholesterol (CHOL) as the lipid part and mannitol as the carrier material, using two different methods: film deposition on carrier and spray drying. Proliposomal formulations were studied in varying ratios of EPC and CHOL and in varying ratios of lipid part and mannitol using film deposition on carrier. The preferred formulation was used to prepare proliposomes using spray drying and to compare the products from both methods. In this communication the synthesis, structural elucidation of LEM2, and properties of developed formulations such as size, zeta potential and physical state of proliposomes will be presented.

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## Synthesis of alkylated derivatives of 3,7-dihydroxyflavone with potential antitumor activity

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Flavonoids 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]. In particular, 3,7-dihydroxyflavone has been shown to inhibit the growth of human tumor cell lines, being this effect at least in part associated to caspase-dependent apoptosis [2]. Recently, as result of the search for new antitumor agents by our group, four 3,7-dihydroxyflavone derivatives have been identified as potent inhibitors of human tumor cell lines [3]. Additionally, one of these derivatives, 3-hydroxy-7-geranyloxyflavone, has revealed to be a caspase-7 activator [4]. These studies support the hypothesis that the alkylation of the flavonol scaffold could represent a promising strategy to obtain anticancer compounds that act by activating caspases. In the present work, the synthesis of seven 3,7-dihydroxyflavone alkylated derivatives has been achieved by the reaction of 3,7-dihydroxyflavone with alkyl halides in alkaline medium under microwave irradiation. The synthesized compounds were characterized by NMR techniques (<sup>1</sup>H NMR, <sup>13</sup>C NMR, HSQC and HMBC). In the future all synthesized compounds will be evaluated for their ability to modulate caspases-3, 6, and 7 activity using yeast cell based assays.

**Acknowledgments:** This research was developed under the Project PEst-OE/SAU/UI4040/2014 and partially supported by the European Regional Development Fund (ERDF) through the COMPETE - Operational Competitiveness Programme and national funds through FCT - Foundation for Science and Technology, under the project PEst-C/MAR/LA0015/2013 and REQUIMTE-Pest-C/EQB/LA0006/2013.

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## Synthesis of alkylated derivatives of chrysin with caspase modulatory activity

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Flavonoids represent a very interesting class of naturally occurring compounds regarding not only the impact and health benefits but also because of their wide range of biological/pharmacological properties, being the antitumor activity one of the most studied. The antitumor activity of flavonoids is associated with, at least in part, to their ability to induce apoptosis by affecting the expression or activity of a wide variety of molecules involved in apoptosis pathways, namely the caspase family proteins [1]. Recently, as result of the search for new caspase modulators by our group two prenylated flavonoids have been identified as caspase-7 activators [2].

Inspired by the potential of these flavonoids as caspase modulators, we have synthesized seven alkylated derivatives using chrysin, a natural flavone, as the starting material. The synthetic approach was based on the reaction with alkyl halides 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). In the future all synthesized compounds will be evaluated for their ability to modulate caspases-3, 6, and 7 activity using yeast cell based assays.

**Acknowledgments:** This research was developed under the Project PEst-OE/SAU/UI4040/2014 and partially supported by the European Regional Development Fund (ERDF) through the COMPETE - Operational Competitiveness Programme and national funds through FCT - Foundation for Science and Technology, under the project PEst-C/MAR/LA0015/2013 and REQUIMTE-Pest-C/EQB/LA0006/2013.

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# Synthesis of promising bioactive chiral derivatives of xanthones with two stereogenic centres

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Xanthones are polyphenolic compounds that are widely produced by higher plants, fungi, and lichens as secondary metabolites [1]. In the literature, there are many examples of natural chiral derivatives of xanthones (CDXs), but scarce from synthetic origin [2,3]. However, synthetic CDXs have demonstrated to inhibit the growth of three human tumor cell lines, with high enantioselectivity [2]. Interestingly, it was found that the CDXs bearing aryl groups in the chiral moiety were the most active compounds in all human tumor cell lines tested. In fact, the most active compound was synthesized by coupling the xanthonic building block 6-methoxy-9-oxo-9*H*-xanthene-2-carboxylic acid with the enantiomerically pure amino alcohol (1*R*,2*S*)-(-)-2-amino-1,2-diphenylethanol. Moreover, good enantioselectivity between this (*R,S*)-CDX and its antipode, i.e. (*S,R*)-enantiomer on all the human tumor cell lines were also observed.

In this communication we report the synthesis and characterization of two new CDXs which are the diastereomers of the CDXs referred above; i.e. with the (*S,S*)- and (*R,R*)-configurations. The structures of the synthesized compounds were elucidated by spectroscopic methods (IR, <sup>1</sup>H and <sup>13</sup>C NMR).

The evaluation of growth inhibitory effect on the different tumor cell lines of the synthesized CDXs will be performed in order to complete the study of the influence of the stereochemistry on this activity.

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# Synthesis of Xanthone Derivatives with Potential Dual Mode of Action for Alzheimer's Disease

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Alzheimer's disease (AD) is the most prevalent form of dementia. It is known that the malfunctions of different, but interconnected, biochemical complex pathways are related to the pathogenesis of AD. Inhibition of acetylcholinesterase (AChE) is one of the most accepted therapy strategies for AD. Consequently, several AChE inhibitors have been approved for commercial use. Nevertheless, due to the lack selectivity of AChE inhibitor drugs, AD-patients suffer from side-effects, suggesting that there is a considerable need for strategies for development of new AChE inhibitors [1]. The multifactorial nature of AD requires new therapeutic strategies. Among the multipotent approaches, the association between cholinesterase inhibition and antioxidant activity has been considered as an attractive strategy [1]. Considering that concept, this work aims to obtain new AChE inhibitors with antioxidant activity based on the xanthonic scaffold. Here in, we describe the synthesis of the 1,3,8 – trihydroxyxanthone (X1) and its methoxy derivative (X2) [2].

1,3,8 – trihydroxyxanthone (X1) was synthesized by two methodologies: Eaton's reaction and Grover, Shah and Shah's reaction (modified method for microwave). The synthetic approach used for the synthesis of X2 involves the reaction of X1 with methyl iodide in the presence of K<sub>2</sub>CO<sub>3</sub> in anhydrous acetone at reflux (60°C). The synthesized compounds were purified by column chromatography and their structure elucidation was established by NMR techniques.

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# Synthesis, Development and Characterization of Isoniazid Solid Lipid Nanoparticles

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Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. TB ranks as the second leading cause of death from a single infectious agent, after the human immunodeficiency virus (HIV). The World Health Organization (WHO) estimates that 9 million people fell ill with TB in 2013, resulting in 1.5 million of deaths. Indeed, TB becomes a serious health problem and deserves all focus on their combat. The identification of novel anti-TB drugs, the modification of existing drugs, and the development of new delivery systems are urgently needed to shorten TB chemotherapy. For more than 60 years, isoniazid (INH) has been a front-line drug in the battle against TB. INH is a Biopharmaceutical Classification System (BCS) class III drug (high solubility and low permeability). One of the major drawbacks in the use of INH for the treatment of TB is its severe toxicity. The toxic effects caused by INH frequently lead to discontinuation of the therapy and the non-compliance. In this context, the present study aims to develop a delivery system of INH using solid lipid nanoparticles (SLN) synthesized by double emulsion method to reduce the toxicity of this drug and increase its efficacy [1]. The developed particles were characterized regarding their size, zeta potential, encapsulation efficiency (EE) and loading capacity (LC). The results showed that the produced SLN present a hydrodynamic diameter of 314.0 ( $\pm 2,6$ ) nm, associated with a low polydispersity of 0.124 ( $\pm 0.014$ ). The synthesized SLN can be considered stable regarding their high potential charge, namely - 23.1 ( $\pm 0.5$ ) mV. The EE obtained was approximately 26%, and the LC 3.9%. These results demonstrate that SLN represent a suitable strategy for the encapsulation of this hydrophilic drug and can be used in the future for the treatment of TB.

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## The cardiotoxicity of mitoxantrone: A comparative *in vitro* study between mitoxantrone and its naphthoquinoxaline metabolite

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Mitoxantrone (MTX) has been used in several types of cancers and also in multiple sclerosis though its use implies a high incidence of cardiotoxicity. Still, the MTX cardiotoxic mechanisms are poorly known. Some anticancer drug metabolites have been proved to be, at least, partially responsible for their cardiotoxicity. Regarding MTX, toxicological data concerning its metabolites are scarce. Therefore, this work aimed synthesizing the MTX-naphthoquinoxaline metabolite (NAPHT) and to compare the cytotoxicity of the metabolite with the parent drug in 7-day H9c2 differentiated cells at pharmacological relevant concentrations (0.01-5  $\mu$ M). MTX was more toxic in equivalent concentrations in all cytotoxicity tests performed [MTT reduction, neutral red (NR) uptake, and lactate deshydrogenase release assay] and incubation times tested (24 and 48h). NR was the most sensitive test for the MTX cytotoxicity. Ethidium bromide/acridine orange and Hoescht stainings showed no significant apoptotic signs in both MTX and NAPHT. However, a marked cytoplasmatic damage was seen in MTX-incubated cells at 24h. 3-Methyladenine (an autophagy inhibitor) gave a partial protection against the MTX and NAPHT observed cytotoxicity. MTX and NAPHT caused a significant decrease of mitochondrial membrane potential at 24h. However, ATP levels were affected in a different manner after MTX or NAPHT incubation, revealing that these two compounds may impair cellular pathways in a dissimilar manner: ATP increased and lactate levels decreased after 24h incubation with MTX. For the same incubation time and concentrations, NAPHT did not caused any significant effect. For the best of our knowledge, this was the first broad study on NAPHT cardiotoxicity, indicating a higher toxicity of the parent drug in a reliable cardiac model.

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## The procarcinogenic effect of 17 $\beta$ -estradiol in MCF-7 cells is not related to interference with glucose cellular uptake

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Breast cancer is one of the most frequent cancers in the population, especially in older women, and its rate has been increasing over the years. Among several distinguishing characteristics, cancer cells present an increase in glucose uptake together with an increase in lactate production –the Warburg effect [1]. These characteristics are associated with an increase in glucose facilitative transporters (GLUT) expression and activity. GLUT1 is the main glucose transporter in breast cancer cells [2]. Estrogen is known to be a key hormone in the development and progression of mammary carcinogenesis [3,4]. Previously, our group found that the antiproliferative effect of kaempferol on MCF7 cells was dependent on GLUT1 inhibition [5]. So, we decided to investigate if the effect of 17 $\beta$ -estradiol is dependent on changes in GLUT1 activity.

MCF-7 cells were exposed to 10, 100 and 300 nM 17 $\beta$ -estradiol for 24h, 48h and 72h and then we evaluated cellular viability (through quantification of extracellular LDH activity), cell proliferation rate (by the <sup>3</sup>H-thymidine incorporation assay), culture growth (through the sulforhodamine B assay) and migration capacity (with the injury assay). GLUT1 activity was assessed by measuring uptake of <sup>3</sup>H-deoxy-D-glucose.

We were able to show that 17 $\beta$ -estradiol (48h; 100 nM) induces a 16 $\pm$ 4% increase in culture growth as well as a 24 $\pm$ 7% increase in cell proliferation rate. Additionally, an increase in cell viability (of 36 $\pm$ 7%,) was observed. In contrast, 17 $\beta$ -estradiol (48h; 100 nM) did not significantly affect the migration capacity. The effect of 17 $\beta$ -estradiol upon uptake of <sup>3</sup>H-deoxy-D-glucose was then studied. Uptake of <sup>3</sup>H-deoxy-D-glucose (10 nM; 6 min) was not affected by 17 $\beta$ -estradiol (48h; 100 nM), either in the absence and presence of the glucose transport inhibitors cytochalasin B (50  $\mu$ M) plus phloridzin (500  $\mu$ M). Current studies aim to identify the estrogen receptor isoform involved in the effect of 17 $\beta$ -estradiol and the ability of some dietary polyphenols to revert its procarcinogenic effects.

In conclusion, the positive effect of 17 $\beta$ -estradiol upon proliferation and viability of MCF-7 cells is not dependent on inhibition of glucose cellular uptake.

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## Total synthesis of fluorinated chiral derivatives of xanthenes

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Chiral derivatives of xanthenes (CDXs) from natural origin (higher plants, lichens, and fungi), as well as from synthetic origin were found to be promising compounds [1]. Among them there are, for example, an interesting halogenated chiral xanthone isolated from a marine-derived fungus *Chaetomium* sp. with a promising antiprotozoal activity [2], or several synthetic CDXs that revealed growth inhibitory activity on different human tumor cell lines [3].

In this work, we describe the synthesis and characterization of two new fluorinated CDXs. Firstly, the total synthesis of a suitable functionalized xanthonic chemical substrate was carried out by a multi-step pathway, *via* Ullmann reaction with the formation of a diaryl ether intermediate under mild conditions, using picolinic acid as the ligand for copper in DMSO/K<sub>3</sub>PO<sub>4</sub> [4]. Then, the fluorinated CDXs were synthesized by coupling this xanthonic chemical substrate with both enantiomers of a commercially available chiral amine. The structure elucidation of the new CDXs was established by spectroscopic methods (IR, <sup>1</sup>H and <sup>13</sup>C NMR).

The biological activity of the synthesized compounds will be evaluated face to different biological targets in order to discover more bioactive CDXs and verify the potency and stereoselectivity for both enantiomers.

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## Trace elements levels in lung tissue – age and gender differences, and the effect of smoking habits

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According to GLOBOCAN 2012, lung cancer is one of the cancers with highest prevalence and the cancer with highest mortality rate in the world [1]. Risk factors such as smoking habits, occupational and environmental exposures, and personal history of respiratory diseases are, based on numerous studies, directly related to the development of the pathology [2]. Other important factors also associated with lung cancer are gender, age, ethnicity, family history, genetic susceptibility and dietary habits [2]. The association of trace elements to lung cancer has proved increasingly evident. Trace elements are naturally present in the environment and human exposure can occur mainly through ingestion (water, food) and the inhaled air. Many trace elements, in particular several metals, may cause cellular damage and the development of cancer [3].

In order to better understand the role of trace elements in the development of lung cancer, we performed a comprehensive analysis of lung tissue. Dried samples (fragments of ca. 1 cm<sup>3</sup> obtained during autopsy exams from right lung upper lobe) were solubilized through a closed-vessel microwave-assisted acid digestion procedure and the obtained solutions were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) for a broad panel of trace elements.

The main goals were: 1) to define the reference (“normal”) values for trace elements in lung tissue; 2) to study if there was any tendency for an age-related increase/decrease, and 3) to seek for differences between smokers and non-smokers individuals and to determine the impact that smoking has on trace elements levels in lung tissue.

In the general population (n=79), the levels of Al, Sr and Se in the lung tissue tended to increase with age, in contrast with Cd, which showed a tendency to decrease. For the other elements studied (Mn, Fe, Ba, Cr, Ni, Cu, Zn, As, Pb) no significant changes with age were observed. The differences in the Al, Mn, Cd and Pb levels between men (n=48) and women (n=31) were statistically significant (P<0.05). Comparing the group of smokers (n=24) with non-smokers (n=36), statistically significant differences we observed (higher values in smokers) for Al, Cd and Pb (P<0.05).

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# Unravelling the role of ATP on neuromuscular transmission in an experimental model of autoimmune *Myasthenia gravis* (EAMG)

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Adenosine triphosphate (ATP) is co-released together with acetylcholine (ACh) upon electrical stimulation of motor nerve terminals [1]. Released ATP modulates neuromuscular transmission either by directly activating P2 purinoceptors (P2R) [2] or indirectly through the activation of P1 receptors after being metabolized into adenosine (ADO), via ecto-nucleotidases [3]. Adenosine derived from the extracellular catabolism of ATP activates preferentially excitatory A<sub>2A</sub> receptors at stimulated motor nerve terminals [3,4]. Extracellular ATP and P2R also play an important role in the initiation of immune responses [5]. ATP can, thus, assume an important role in autoimmune neuromuscular diseases, like *Myasthenia Gravis* (MG), which is characterized by both skeletal muscle weakness and fatigability on repetitive use due to an immune attack towards muscle nicotinic acetylcholine receptor (nAChR). Here, we measured the ATP levels and its neuromodulatory actions in isolated phrenic nerve-hemidiaphragm preparations from control and experimental autoimmune MG (EAMG) animals.

EAMG was induced in *Wistar* rats through immunization with R97-116 peptide, a immunogenic sequence of the  $\alpha$  subunit of the rat nicotinic AChR [6]. Control animals received the CFA emulsion without the peptide. Animals from the Naïve group were not submitted to treatment. Phrenic nerve hemidiaphragm preparations were isolated and the release of [<sup>3</sup>H]ACh and ATP was evoked by phrenic nerve stimulation with 5 Hz-trains (750 pulses of 0.04 ms duration). ATP and [<sup>3</sup>H]ACh content was measured by the luciferin-luciferase bioluminescence assay and by liquid scintillation spectrometry, respectively.

No differences ( $P > 0.05$ ) were detected in the amount of evoked [<sup>3</sup>H]ACh release in Control (165±36 cpm,  $n=30$ ) and EAMG (151±19 cpm,  $n=30$ ) animals. Under the same experimental conditions, electrically-evoked ATP release was 20±10 pM/mg ( $n=6$ ) and 60±29 pM/mg ( $n=5$ ) in Control and EAMG rats, respectively. Exogenously applied ATP exerts a biphasic effect on evoked [<sup>3</sup>H]ACh release in Naïve preparations; ATP increased the neurotransmitter outflow at concentrations below 100  $\mu$ M (1  $\mu$ M: +56±7%,  $n=5$  and 30  $\mu$ M: +19±5%,  $n=5$ ), whereas it decreased evoked [<sup>3</sup>H]ACh release by 39±9% ( $n=7$ ) when applied in a concentration of 100  $\mu$ M 15 min before stimulation. The inhibitory effect of ATP on evoked [<sup>3</sup>H]ACh release was mimicked by its stable analogue,  $\beta\gamma$ -imidoATP (100  $\mu$ M, -21±11%,  $n=3$ ). The neuromodulatory effect of ATP seems to be, at least partially, mediated by its catabolism into ADO. This was concluded because the effects of the nucleotide were abrogated in the presence of adenosine deaminase (ADA, 0.5 U/mL), the enzyme that inactivates ADO into inosine. Interestingly, pre-treatment with ADA (0.5 U/mL) transformed the facilitatory action of ATP (1  $\mu$ M, +56±7%,  $n=5$ ) into a significant ( $P < 0.05$ ) inhibitory effect (-32±18%,  $n=4$ ).

These results suggest that ATP modulates neuromuscular transmission through the activation of inhibitory nucleotide-sensitive P2R and excitatory adenosine (A<sub>2A</sub>) receptors. Thus, fine-tuning modulation of ATP release and conversion into ADO, which determines the preferential activation of inhibitory P2 and/or excitatory A<sub>2A</sub> receptors, might be relevant to therapeutically overcome neuromuscular transmission fatigue in myasthenic patients.

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# Phenological modeling of grapevine (*Vitis vinifera* L.). Bioclimatic, genetics and spacio-temporal adaptations

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Premium wine production is limited to regions climatically conducive to potential growing grape-variety with balanced composition and varietal typicity and with opportune well vineyard management. For these intricate fusions of climate, genetics, viticulture and enology, phenological observations have been used by Vintners, in some regions, since the Medium Age.

Phenology is defined as the study of physiologic crop development and the influence of the surrounding environment to the plant, particularly the climate. Vineyard phenological data are essential for making good decision during many phases of grape-growing, such as programming the timing of vineyard operations such as irrigation, pest and disease control, canopy management and harvesting. They also provide a basis for correlative studies with climate and to gauge the behaviour of varieties, for predicting their behaviour in new wine regions and indicator of possible climate change.

This research work proposes to create models to predict the spatio-temporal variability of grapevine phenology response to climate on several grape varieties at different Portuguese Wine Regions as well as their behavior through the different regions.

The approach choose to this work uses an optimization method based on the Simulated Annealing algorithm, that minimize the choice of local optima while searching for the solution for an optimization problem. The Phenology Modelling Platform Version, that allows to parameterize a process-based phenological model, has been used to build several predictive phenological models.

The phenological model will be calibrated and validated with multi-year (+10 years), multi-variety and multi-site data collected in several Portuguese wine regions. As there are records of the same variety in different regions and in different sites within the region, the effect of variety in the models adaptability will be evaluated.

Once the model has been validated, it is possible to use it with the data generated by meteorological satellites coupled Geographic Informatics System platform to map the development of the vineyard over the year.

The preliminary results of this work, points toward the feasibility of the proposed objectives, since the developed models have been able to predict the occurrence of the several phenophases, such as budburst, flowering and *veraison* with error lower than 5 days.

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## Sexual dimorphism of the brown shrimp *Crangon crangon*

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One of the most remarkable phenomena in the evolution of mating systems in living organisms is sequential hermaphroditism, commonly referred to as ‘sex change’ or sex reversal’. As the name suggests, sequential hermaphrodites naturally change their sex during their life: individuals reproduce initially as males and then change to females (protandry) or vice versa (protogyny). Many theoretical models have been proposed, mainly based on sexual allocation theories and the differential advantage of body size on the reproductive success of each sex: sex changers should increase their fitness becoming large males (in protogyny) or large females (in protandry). The brown shrimp *Crangon crangon* L. (Decapoda, Caridea) is an interesting candidate for studies of sex reversal. Its sexual mode of reproduction is still debated, being described as gonochoristic (fixed separate sexes) [1], but also as obligate protandric (male to female sex change) or facultative protandric (some but not all individuals change sex) [2]. This suggests a degree of sexual plasticity and implies that primary females, males and secondary females (resultant from male sex change) coexist in natural populations. Descriptions of the species sexual dimorphism, however, are not validated; moreover, no intermediate morphotypes have been described to date. Therefore, in this work the sexual distinctive features of *C. crangon* were analyzed applying Geometric Morphometric tools in order to objectively describe the sex differences and to depict possible intermediate morphotypes, including the correspondent to secondary females.

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# Spatial localization of arabinogalactan proteins and pectin epitopes in *Quercus suber* L. ovule cells before and after fertilization

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The evergreen Fagacea cork oak (*Quercus suber*) is a dominant monoecious tree species from the Southern Iberian Peninsula forests. *Q. suber* has an extremely important socio-economic, cultural and an environmental value for Portugal. It presents a long progamic phase that provides a comprehensive system for comparative studies in development and sexual reproduction in a non-model plant [1]. Studies on the sexual reproduction of cork oak are essential to understand the molecular mechanisms of fertilization and identify the difficulties associated with the production of acorns destined either for nursery production or for animal food. Cell surface proteoglycans such as arabinogalactan proteins (AGPs) and pectins play important roles in cell growth and development. AGPs and pectins belong to a superfamily of highly glycosylated hydroxyproline-rich glycoproteins found in the entire plant kingdom, in almost all plant organs and cell types from root to flowers [2]. At the subcellular level, AGPs can be found in the cell wall, in the apoplast or anchored to the plasma membrane via a GPI anchor attached to the C-terminal domain of the AGP backbone. In reproductive tissues, the expression of AGPs is associated with the sporophyte–gametophyte transition [3]. Pectins are important cell wall polysaccharides, together with AGPs have been reported to play important roles in plant growth and development. Pectins are mainly composed of homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II).

Immunofluorescent localization of AGP and pectin epitopes in female flowers ovule cells was performed with a set of monoclonal antibodies directed to the carbohydrate moiety of the cell wall polysaccharides, JIM8 and JIM13 recognizing AGPs; and JIM5 and JIM7 recognizing pectins. The antibody binding to pectin HG epitopes for highly methyl-esterified homogalacturonans labeled all cell walls of the female flower tissues. The labeling obtained with anti-AGP antibodies in female flowers ovule cells showed a dynamic distribution making AGPs useful as molecular markers for cork oak ovule development before and after fertilization.

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# Structural irregularities in sagittal otoliths of juvenile flounder *Platichthys flesus* (L.)

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Anomalous otolith formation has been reported in a number of freshwater and marine fish species. The most commonly observed anomaly seems to be the deposition of vaterite instead of aragonite, the most common calcium carbonate polymorph, resulting in different shape and transparency of the otolith.

In this study we report high occurrence rates of anomalous otoliths in juvenile *P. flesus* collected in the river Minho estuary. The most usually observed anomaly appears to be the presence of depressions, projections or holes in different parts of the otolith. Some examples of possible vaterite deposition have also been found but in much less numbers.

A total of 136 pairs of otoliths were randomly selected from juvenile fish (2 - 12.5 cm total length) collected between 2013 and 2015. Otoliths were visually inspected on both distal and proximal sides for the presence of anomalies. Distribution of irregularities within the original samples was examined in relation to site of collection and fish total length. At the site with higher salinity (close to the estuarine mouth), 16 out of 18 otolith pairs were normal while 2 could not be classified. In contrast, at the upstream location, in the tidal freshwater area of the estuary, 43% of the 118 pairs examined were abnormal. In most cases (77%), the otolith pair was affected while a few had only one abnormal otolith, either right or left, although the left presented more extreme abnormalities. The occurrence of anomalies seems to be related with fish size since these were only found in fish smaller than 7.5 cm. Preliminary morphometric analysis indicates that abnormal otoliths have the same proportions (length vs width) as normal ones, but the former seem heavier than the latter, for the same sized fish.

It is presently unclear how these anomalies affect the fish, their growth or their survival. Since fish otoliths are involved in perception of the surrounding environment, modifications in their shape may have negative consequences. Fulton's K condition index, used as an indicator of the fish general condition, did not differ between groups of fish with normal or abnormal otoliths. However, further studies are needed. Also the causes for such anomalies, either genetic or environmental are not known although the fact both otoliths of the same pair are affected might suggest a genetic defect.

Next steps will include crystallography and elemental composition analyses to elucidate whether these otoliths with aberrant structure also differ in composition compared to normal ones. If that is the case, studies using otolith microchemistry to unravel fish life history need to take it into account.

# Study of bacterial communities in sponges by Denaturing Gradient Gel Electrophoresis (DGGE)

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Marine sponges harbour a remarkable array of microorganisms, with the bacteria being the most dominant group within these. We used the technique denaturing gradient gel electrophoresis (DGGE) that allows separation of DNA fragments based on their G + C content [1], to do a comparative study of the diversity of bacterial communities in different species of the *Erylus* sponge.

Sponges were sampled in different locations of the Atlantic Ocean: São Miguel (Açores) – *E. mamillaris*, Madeira – *Erylus* sp and *Sarcotragus* and Berlengas – *E. discophorus*. Portions of sponge tissue were cut and smashed with sterile sea water in order to extract bacteria and subsequently their DNA using the PowerSoil DNA Isolation Kit. To amplify the microbial DNA retrieved from the sponges, polymerase chain reactions (PCR) were prepared with 16S rDNA primers GC-358F (with a GC-clamp at the 5' end) and 907RM. PCR products were run in a 40% to 80% polyacrylamide gradient DGGE gel at 60°C, 100V for 16h. Bands and band types were defined and a presence/absence matrix was formed along with the DNA relative abundance (intensity). Based on the matrix reports the following analyses were done: cluster analysis, non-metric multidimensional scaling (nMDS), similarity percentage analysis (SIMPER) and analysis of similarity (ANOSIM), all based in Bray-Curtis coefficient.

Four gels were made for the comparison of different individuals in the same location and between individuals of different locations. DGGE fingerprinting profiles were similar between different individuals the same species in the same location. The geographic location influenced the microbial community of the sponges as observed by the DGGE profile similarities between the different species in Madeira. Further sequencing of the bands on the DGGE gel could provide more information on the microbial diversity of each community and how they really differ between them.

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## Study of steroid derivatives: aromatase inhibitory activity and biological effects in MCF-7 cells transfected with aromatase

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One of the several therapeutic approaches for estrogen-dependent breast cancer (ER<sup>+</sup>) is the use of Aromatase Inhibitors (AIs). These drugs inhibit the enzyme aromatase reducing the estrogen levels, which are important to promote the growth of this type of cancer. The occurrence of endocrine resistance and bone loss, reinforces the constant search for new AIs, more potent and effective, with lower side effects [1, 2]. New steroid compounds, 7 $\alpha$ -(2,3-epoxypropyl)-androsta-1,4-diene-3,17-dione (**49**) and androsta-4,9(11)-diene-3,17-dione (**52**) with similar structure to androstenedione, the natural substrate of aromatase, were selected for being studied in an ER<sup>+</sup> breast cancer cell line that overexpress aromatase (MCF-7aro cells). The evaluation of their potential as AIs was performed by a radiometric assay, while their effects on cell viability were carried out by MTT assay. The cytotoxicity of these compounds was evaluated by LDH release assay. Morphological alterations in MCF-7aro cells were studied by Giemsa and Hoechst staining. To investigate the effects of the new compounds in non-cancerous cells viability, a fibroblast-type cell line (HFF-1 cells) was also used. The results showed that compound **49** induced approximately 73 % of aromatase inhibition in MCF-7aro cells and its anti-proliferative effects are aromatase-, time- and dose-dependent. In what concerns compound **52**, it presents 94 % of aromatase inhibition in MCF-7aro cells, being its inhibition higher than Formestane, the reference AI used in this study. Nevertheless, the anti-proliferative effects in MCF-7aro are also time- and dose-dependent, but are aromatase-independent. In addition, both compounds demonstrate to be non-toxic to HFF-1 cells. In general, morphological alterations, mainly chromatin condensation, induced by AI **52** in MCF-7aro cells were more pronounced than by **49** and increased with time. These studies allowed biological information of the new steroidal compounds as potent AIs in breast cancer cells, which can contribute to the design of new AIs more potent and specific than the AIs used in clinic.

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## Study of the cell growth inhibitory effect of aqueous extracts from *Tuberaria lignosa* in human tumor cell lines

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*Tuberaria lignosa* (Sweet) Samp. (“alcária”), a plant mostly found in Western regions of the Iberian Peninsula, has antioxidant properties due to its composition in ascorbic acid and phenolic compounds [1]. Given these antioxidant properties, together with the traditional use of the plant to treat several diseases, the aims of this work were to: i) investigate if aqueous extracts from this plant, obtained by infusion and decoction, were inhibitors of cell growth in three human tumor cell lines; ii) study the cellular mechanism of action of the most potent extract.

A screening of tumor cell growth inhibitory potential was performed with the Sulforhodamine B (SRB) assay using three different human tumor cell lines: MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer) and HCT-15 (colorectal adenocarcinoma). Results showed that both aqueous extracts of *T. lignosa* decreased the growth of the cell lines tested and that the *T. lignosa* infusion extract was the most potent one, particularly in the NCI-H460 and HCT-15 cells.

The *T. lignosa* infusion extract was further tested in the NCI-H460 cells. A determination of its effect on cell cycle profile was carried out, by analyzing cellular DNA content by flow cytometry following incubation with propidium iodide. Determination of cellular apoptosis was also performed, with the Annexin V-FICT and propidium iodide assay, and analyzed by flow cytometry.

Preliminary results showed that the selected extract promoted a slight increase in the percentage of cells in the G1 phase of the cell cycle and induced cellular apoptosis.

In conclusion, the *T. lignosa* extract decreased growth of the human tumor cell lines tested and the most potent effect was observed for the *T. lignosa* infusion extract. Future work will confirm if this effect is due mainly to induction of apoptosis.

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# The high adsorption capacity of cork can make it an interesting bedding material for laboratory rodents

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Laboratory rodent microenvironment is a primary consideration for animal welfare and may affect the outcome of experimental studies [1]. The *Guide for the Care and Use of Laboratory Animals* states that rodents should be housed with bedding because it allows foraging, digging and nest building and absorbs urine and feces. Several bedding materials are available for rodents like wood chips, paper products and corncob. Bedding is evaluated for absorbency, biodegradability, toxicity, dust, comfort, cost and experimental results interference [2]. Considering the potential water adsorption and clusters formation capacity of cork [3], we decided to investigate the use of this material as an alternative to corncob. 12 Female Wistar Han rats (aged 10 to 12 weeks) were housed and maintained in accordance with DL n.º 113/2013. Room temperature was  $21.1 \pm 0.9$  °C and room humidity was maintained between  $47\% \pm 2.9\%$ . Rats had *ad libitum* access to autoclaved standard diet and water. Animals were divided into 4 groups: corncob and cork (3 different particles sizes: 1/2 mm, 2/3 mm and 3/4 mm), each containing 1000 mL of bedding. Each group contained 6 rats and was grouped according to different type of bedding materials. All animals were monitored daily in order to evaluate: welfare, individual body weight and water/food intake for 6 days. Preliminary results indicate that cork bedding size 1/2 mm ( $13.11 \pm 3.19\%$ ,  $n=2$ ), 2/3 mm ( $23.70 \pm 4.80\%$ ,  $n=2$ ) and 3/4 mm ( $16.53 \pm 1.49\%$ ,  $n=2$ ) were able to make more clusters (% , day 5) than corncob ( $2.77 \pm 2.03\%$ ,  $n=2$ ). Results indicate that the volume increased 5% in cork 1/2 mm and 30% in corncob bedding, compared to the initial sample of 1000 mL. On the other hand, cork bedding weight increased 34% (size 1/2 mm), 62% (size 2/3 mm) and 59% in 3/4 mm, while corncob bedding variation was lower 24%. Moreover it was interesting to observe that cork bedding 1/2 mm ( $69.00 \pm 8.49\%$ ,  $n=2$ ), was unable to significantly change rats gastrointestinal motility, since total fecal pellets bedding count was similar to corncob ( $71.00 \pm 1.41\%$ ,  $n=2$ ). No evident loss of animal welfare was observed since rats behavior, body weight variation (Corncob:  $10.40 \pm 4.04$ g,  $n=5$  and Cork:  $8.40 \pm 2.88$ g,  $n=5$ ), diet (Corncob:  $18.27 \pm 1.19$ g,  $n=6$  and Cork:  $16.97 \pm 0.85$ g,  $n=6$ ) and water (Corncob:  $28.53 \pm 1.97$ mL,  $n=6$  and Cork:  $27.87 \pm 1.34$ mL,  $n=6$ ) intake had minimal differences between the two groups. Considering that cork bedding showed a higher adsorption capacity than corncob and that animal welfare did not seem to have been compromised, we believe that these comparative studies should continue, in order to evaluate if cork may become an alternative to corncob as a bedding material for laboratory rodents.

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# Unravelling the function and expression pattern of AGP4 and AGP7 in *Arabidopsis thaliana* reproduction

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Arabinogalactan Proteins (AGPs) are hydroxyproline rich glycoproteins that undergo several post-translation modifications. The cDNAs encoding the protein backbones of AGPs show a characteristic domain structure consisting of an N-terminal secretion signal, absent from the mature protein, a central domain rich in proline/hydroxyproline, alanine, serine and threonine, followed by a C-terminal signal sequence for GPI-anchor attachment. Mature AGPs are therefore extensively glycosylated and predicted to be attached by a GPI anchor to the plasma membrane. The GPI anchor can be cleaved by specific phospholipases, releasing the polypeptide into the extracellular matrix in a regulated manner, suggesting that they might play signaling roles. Coimbra *et al.* in 2007 [1] have shown that AGPs can be used as molecular markers for reproductive development, showing that they are present in pistil tissues, especially along the pathway followed by the pollen tube during its guidance to reach the egg cell inside the embryo sac of the female gametophyte. We have been studying the AGPs present in the pistil tissues, in order learn their biological roles along this path. AGP4 and AGP7 were two of the AGPs selected for further analysis and functional characterization based on microarray data available (Geneinvestigator, Arabidopsis eFP Browser, Wuest, S. E. *et al.*, 2010 [2]). In the present work we show some of our results regarding the study of *agp4agp7* double mutant and discuss its possible involvement in multiple pollen tube block.

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# **Budget manipulation and fiscal rules: evidence from the Economic and Monetary Union**

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Economic policies are almost never pursued for purely economic reasons; however politicians find justification for the majority of their actions in economics. Political intentions are thus often the cause for careless budget management and eventually lead to political budget cycles.

This study aims to contribute to the literature on political budget cycles (PBC) by assessing the existence of PBC induced by the manipulation of government budget composition. Moreover, we aim at studying the impact of fiscal rules on the occurrence of this phenomenon. In order to achieve this, our analysis was based on 14 Economic and Monetary Union (EMU) Member Countries and covered the period of 1991-2007. Considering this sample, the chosen estimation method was the Fixed Effects Model. We deliberately excluded the more recent members of the Eurozone (namely Estonia, Latvia, Slovenia and Slovakia) since they represent economic outliers and would otherwise skew the results of the estimation.

The empirical strategy draws on the standard PBC literature and thus relies on several fiscal indicators as dependent variables. The independent variables in the regression include an electoral dummy, the Gross Public Debt-to-GDP (Gross Domestic Product), a Fiscal Rule Index (as calculated by the European Commission), the Real GDP Growth rate and the variation of the Dependency Ratio. The latter refers to the ratio of economically active workers compared to the inactive and was included since countries with higher share of older population typically experience higher public spending (due to particular needs such as social security and health expenditure) which affects their fiscal stance. This fiscal variable mainly affects fiscal policy on the expenditure side since it has an immediate impact on categories such as Social Transfers and Employee Compensation.

A first relevant finding was the fact that there was no evidence of an electoral effect regarding Net Lending, as foreseen by literature. Literature suggests that the absence of budget cycles at this level would be due to the existence of Budget Balance Rules that inhibit the accumulation of high deficits. However, several authors largely agree that the existing fiscal rules have been unsuccessful in preventing these cycles. In this regression, the fiscal rules variable was equally insignificant, suggesting once more the lack of compliance with the existing fiscal rules, or an absence of efficient and preventive fiscal rules. We found no evidence of electoral cycles in budget deficits, but found instead that these cycles occur at disaggregated expenditures and revenue categories. Moreover, evidence showed that these cycles are more predominant in expenditures and in more “visible” categories, namely Social Transfers, Employee Compensation, and Direct Taxes. Even though the fiscal rules indicator does not display significant effects on its own in these categories (except in Social Transfers), it does help mitigate (accentuate) the electoral effect when its value is high (low) enough.

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# **Economic growth, human capital and structural change: an empirical analysis**

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Human capital is identified as one of the main determinants of economic growth and plays an important role in the technological progress of countries. Nevertheless, existing studies have to some extent neglected the importance of human capital on growth via the interaction it can have with a country's industrial specialization. Additionally, the emphasis is mainly placed on supply-side determinants, being demand-side factors quite neglected, particularly the relevance of the processes of structural change. Thus, using a growth model which integrates variables from both the supply side and demand side, we assess the direct and indirect effects of human capital on economic growth, including in the latter the interaction of human capital with the industrial specialization of countries. Based on econometric panel data estimations involving a set of OCDE countries over 1960-2011, we found that the countries' productive specialization dynamics is a crucial factor for economic growth. It is also shown that the interaction between human capital and structural change towards high knowledge-intensive industries impacts on the economic growth. However, the sign of this effect depends on the type of country and length of the period of analysis. Specifically, in the long term and in developed countries, where knowledge-intensive industries already account for a great share of the economy, the impact of the interaction between human capital and structural change is positive. In the case of less developed countries, and considering a shorter time period, the effect of human capital via specialization in high-tech and knowledge-intensive activities emerged as negative.

# Entrepreneurs facing failure

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Entrepreneurship literature has long gravitated around the entrepreneur and his successful projects. Despite the fact that all entrepreneurs want to be successful in their investment, this is not always the case. Whilst failure is lauded as a fundamental learning experience it has been argued that the venture failure can be extremely painful and damaging for the entrepreneur. Business loss may lead to a negative emotional response. Despite these speculations, there is a lack of studies on the subject of failure, except in a primary perspective, of the reasons behind the entrepreneur failure.

This study aims to understand the perceptions that the entrepreneurs possess regarding their failure and the factors that underlie their review. In other words, the study intends to capture the interpretations and evaluations of the entrepreneur about their being unsuccessful or successful.

To meet these goals, we adopted a qualitative methodology of exploratory nature, based on 10 semi-structured interviews of unsuccessful entrepreneurs. The definition of entrepreneurial failure adopted for the purpose of this study is the exit, closing or voluntary sale of the business either for financial reasons (bankruptcy or insolvency), in order to avoid future liabilities, pessimistic prospects or irreconcilable disputes and legal issues (Singh, Corner, & Pavlovich, 2007).

The results show that personal success is not restricted to business growth and profits. Subjective factors are also important indicators, such as personal fulfillment, job satisfaction, professional development and the respect and acceptance of supervisors, peers and colleagues. When the entrepreneur ponders personal success, his/her business failure has a significant weight, giving meaning and also shaping his/her career, personal success and key learning. Experiences with failure may provide an opportunity to learn but can also represent a traumatic experience for the entrepreneur.

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# The importance of incentive schematics in relation to service quality in the promotion of technical efficiency

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The present investigation means to achieve an analysis of International *Benchmarking*, which will assess the importance of incentive schematics in relation to service quality in the promotion of technical efficiency. By using the linear programming methodology DEA, it was possible to measure technical efficiency, including variables related to service quality. The 18 distribution companies analyzed operate exclusively in the European Union and are from Portugal, United Kingdom, Republic of Ireland, Greece and the Czech Republic. This approach is restricted to the evaluation of only technical parameters of distribution regarding high, medium, and low tension. The results demonstrate that the inclusion of quality variables posses apositive effect on technical efficiency indexes. Furthermore, the importance of quality service in the regulatory framework that manages the distribution of electricity was proven.

**Keywords:** Quality, Efficiency, Distribution, Electricity, Regulation.

# Service Convenience on Call Centers: Impacts on Repurchase

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This work focuses on the study of service convenience, which deals with customers' time and effort expenditures to purchase or use a service [1], in a Call Center setting.

Services will add value to consumers by reducing the time and effort they spend [2]. Thus, convenience may represent a distinctive competitive strategy and several studies have reported positive relations between perceived service convenience and consumer satisfaction [3], [4], [5], [6].

Call Centers are fundamental to CRM strategies and customer overall satisfaction with them seems to determine the customer satisfaction with the service itself [6]. It is expected that understanding how Call Centers increase customer convenience, i.e., reduce time and effort, will increase customer satisfaction [3], [4] and loyalty [5].

This research aimed to: (i) relate the concept of service convenience with Call Centers operations; (ii) determine how Call Centers performance may impact on service repurchase; (iii) explore the factors which can influence the relationship between Call Centers performance and service repurchase.

A database of 133 783 customers from a Portuguese telecommunications company and 376 057 contacts were analyzed in what concerns three Call Center performance indicators, representative of customers' time and effort: First Call Resolution, Average Handling Time and Repeated Calls. A chi square test measured the association between variables and Phi and Cramer's V coefficient indicated the strength of association.

Time and effort expenditures on Call Centers were proved to be related to repurchase. Customers at early stages of their relationship with the service, low value customers and those participating on loyalty programs, were the most sensitive to time and effort expenditures on Call Centers.

Understanding the impact of customers' time and effort on customers repurchase behaviour and knowing the customers less likely to wait and expend efforts, managers must seek to improve Call Centers performance, and select who are the customers to answer first. This study is among few empirical studies on service convenience, which assess real customers' behaviour instead of behavioral intentions or perceptions.

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# The influence of Music in Services: Emotions, Satisfaction and Loyalty

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In services, the servicescape (service atmosphere) is a way to create experiences for service's customers (Lovelock, 2001)<sup>[1]</sup> and the music can be manipulated for this purpose (Braga, 2012)<sup>[2]</sup>.

Music has been proved as a popular and necessary resource in a lot of servicescapes, because the sound influences human behavior, stimulating the connection to the memories and the emotions of the customer (Braga, 2012)<sup>[3]</sup>. Music also allows the customer to make inference about the perception of service's value (Baker, Parasuraman, Grewal & Voss, 2002)<sup>[4]</sup> and quality of what is offered by the service (Salazar, Freitas & Lucian, 2008)<sup>[5]</sup>. In turn, customers' emotions (Salazar, Farias & Lucian, 2008)<sup>[6]</sup> and both the perceived value and quality may influence customer's satisfaction and loyalty over the service.

So, this study has as main purpose realize the impact of music on the satisfaction and loyalty in the context of services. Data will be collected from a questionnaire to customers of physiotherapy services, and analyzed through structural equation modelling, testing several consumer behavior variables.

The choice of this study object is supported in the influence and the benefit of the use of music in health care (Sousa, 2013)<sup>[7]</sup>, where physical therapy can be successful with influence of music (Miranda & Godeli, 2002)<sup>[8]</sup>.

This study aims to fill the gap in the literature on physiotherapy services' studies and to increase the studies that show correlation between emotions, satisfaction and loyalty.

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## Analysis of an individual ventral swimming start using a dynamometric station for 3D force measurements

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When swimmers are in contact with the starting block, the force produced its application is critical to guarantee the quality of the start [1]. The dynamometric station developed by our group allows the discrimination of the forces generated by each limb and the block zones where they are applied [2]. We proposed a method for assessing the external forces during the block phase of an individual ventral grab start.

The experimental procedure consists of five repetitions of an individual ventral start (5 min rest) on a dynamometric station with seven force plates [2]. The LabVIEW™ software was used to visualize the curves of vertical, antero-posterior and medial-lateral components of the reaction forces of one competitive swimmer (male, 15 years old, 53 kg and 1.71 m) on the starting block (Figure 1).

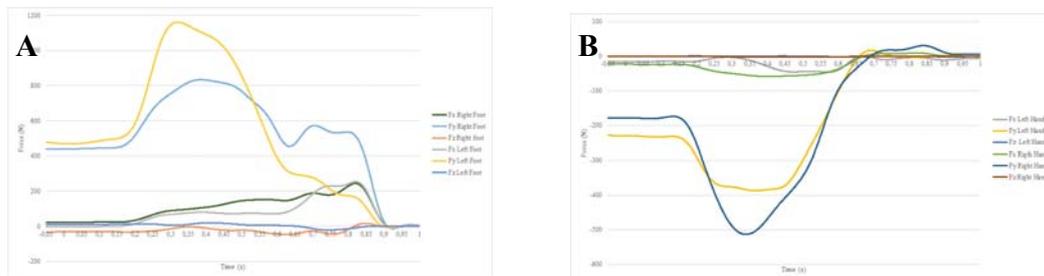


Figure 1 Reaction forces on the starting block produced by lower limbs (A) and upper limbs (B).

Following the mentioned procedure, it was possible to make a detailed analysis of the forces produced by the swimmer on the starting block and parameters like reaction time, total time of contact with the block, horizontal and vertical peak forces and impulse could easily be accessed and interpreted. It is proposed an objective method to evaluate the forces applied by the swimmers on the starting block during swimming starts. This tool can be useful for coaches and swimmers in the sense that provides real-time feedback to correct and improve swimming start technique.

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