XIII JORNADAS DE GENÉTICA
E BIOTECNOLOGIA
III JORNADAS IBÉRICAS
DE GENÉTICA Y BIOTECNOLOGÍA

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XIII Genetics and Biotechnology Conference/ III Genetics and Biotechnology Iberian Conference

The Genetics and Biotechnology Conference (JGB) of the University of Tras-os-Montes and Alto Douro (UTAD) is an annual scientific event organized jointly by the Nucleus of Students of Genetics and Biotechnology (ADNGB) of UTAD and the Direction of the Course of Genetics and Biotechnology in collaboration with the teaching staff of the Department of Genetics and Biotechnology (DGB). As a result of the scientific-pedagogical partnership established between professors of DGB (UTAD) and of Faculty of Biological and Environmental Sciences of the University of León (UL), Spain, it was considered important to repeat the shared organization of this event between professors and students of the UTAD and UL designating it as XIII Genetics and Biotechnology Conference / III Genetics and Biotechnology Iberian Conference (XIII JGB / III JIGB). The main objective of the XIII JGB /III JIGB is to update knowledge in the area of Genetics and Biotechnology. To this end, the focus of this event is the conferences given by renowned national and international scientists and the thematic workshops that will constitute more practical sessions. The XIII JGB /III JIGB will also focus on interaction, exchange of experiences and scientific debates between Portuguese and Spanish students and professors. The best oral and posters presentations will be awarded. The target audience is Portuguese and Spanish students, researchers and university professors from the scientific areas of Biological Sciences and Biotechnology as well as High School teachers from the Biology area. A wide variety of topics will be discussed, in the different areas of Genetics and Biotechnology, such as Plant, Animal, Human, Microbial, Evolutionary, Cancer, Forensic, Ethics, Entrepreneurship, among others.

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Professor María Luz Centeno

Students and members of ABLE:
Patricia de la Madrid Salmerón & Marina Pérez Gutiérrez

PROGRAM

Wednesday 14th April

PT	ES	
16:00	15:00	Workshop **Irene Oliveira, Professor** *Bioinformatics and applications to Life Sciences*

The remaining workshops will be held on April 21, 2021

Thursday 15th April

PT	ES	
09:00	10:00	**Opening Session**
09:15	10:15	Conference **Antonio Fernández Medarde, PhD** *Biotechnological applications of secondary metabolites form marine microorganisms*
10:15	11:15	Conference **Alfredo Corell, Professor** *Inmunidad: cuando las pinzas "laser" de tender la ropa neutralizan al CoronaVader*
11:15	12:15	**Coffee break**
11:30	12:30	**Oral Communications – Session 1** Carnicero-Mayo Y. - *Assessment of microbial diversity in traditional sourdoughs made from organic farming or conventional farming wheat flour*

Guío J. - *Induction of lin genes in Anabaena sp. PCC7120: laying the foundations for the development of a lindane biosensor*

Carvalho IV.. - *Filamentous fungi screening in small ruminants*

14:00 15:00 Conference

Dezso David, PhD

Deciphering the Pathogenesis of Genomic Disorders by Whole Genome Sequencing

15:00 16:00 Conference

Miguel Castanho, Professor

From Zika virus to SARS-CoV-2. Developing drugs to fight viruses in the brain

16:00 17:00 **Coffee break + Musical Moment**

16:30 17:30 **Poster - Session 1**

17:00 18:00 **Oral Communications- Session 2**

Rodrigues R. – *Perirenal Adipose Tissue and Clear Cell Renal Cell Carcinoma: "The triad: macrophage-cancer cell-adipocyte".*

Lucas D. – *Comparing the FA-SAT ncRNA profile in different human cell lines: Is the expression of FA-SAT (dys)regulated in the cell cycle of HeLa cells?*

Gonçalves M. – *Mutation-adapted U1 snRNA as a therapeutic strategy for Mucopolysaccharidosis IIIC: in vitro and in vivo studies.*

Silva A.I. - *Genetic polymorphisms in DNA repair genes and possible influence on DNA damage and repair activity in a population submitted to physical exercise.*

18:00 19:00 **Round Table with Former Students**

Exclusive event for former and current students of 1st, 2nd and 3rd cycles in the field of Genetics and Biotechnology.

Alumni:

Doutora Eliana Margarida Barros (investigadora no Queen's University Belfast, UK)

Doutor Francisco Morinha (CEO e investigador do Morinha Lab - Laboratório de Biodiversidade e Genética Molecular, Vila Real)

Doutora Cátia Pinto (Secretária Executiva do Laboratório Colaborativo para a Inovação Digital na Agricultura, Torres Vedras)

Doutora Sandra Pereira (investigadora, CITAB-UTAD)

Dr. Diogo Coelho (estudante de doutoramento, Univ. Lisboa)

Dr. João Lage (Técnico da Fitolivos)

Dra. Juliana Silva (Investigadora no CeNTI - Centre for Nanotechnology and Smart Material)

Friday 16th April

PT	ES	
09:00	10:00	Conference **Antonio Molina, Professor** ***From the lab to the field: the key value of innovation for agricultural sustainability***
10:00	11:00	Conference **Fernando González Candelas, Professor** ***Genomic epidemiology and evolution of SARS-CoV-2***
11:00	12:00	**Coffee break**

11:30 12:30 **Poster - Session 2**

12:00 13:00 **Oral Communications – Session 3**

Jorge N. – *Kinetics and mechanisms of acid orange 7 dye removal from aqueous solution using Dactylis glomerata L. seeds powder as adsorbent.*

Santos M. – *Study of fruit quality and gene expression under pre-harvest application of calcium and seaweed as mitigation strategy of sweet cherry cracking.*

Monteiro E. – *Biofungicides elicit defense responses in 'Touriga Franca' against fungal diseases.*

14:00 15:00 Conference

Agostinho Antunes, Professor

Unravelling life diversification with Genomics and Bioinformatics: Biotechnology Relevance

15:00 16:00 Conference

Raquel Godinho, Professor

Hybridization and conservation genomics of populations at extreme environments

16:00 17:00 **Coffee break**

16:30 17:30 **Oral Communications – Session 4**

Castro-Ribeiro C. – *The use of Ganoderma lucidum to prevent obesity – effects on the genetic damage index.*

Ribeiro M. – *Chronic life-cycle studies of the priority pharmaceutical Metformin with Danio rerio: molecular and biochemical assessment.*

Barros S. – *Metformin induces growth and reproductive changes in zebrafish (Danio rerio) after full life-cycle exposure to environmentally relevant concentrations.*

Costa-Pinho L.. - *Green-synthesized magnesium hydroxide nanoparticles influence the osteoblastic and osteoclastic differentiation: an in vitro co-culture approach*

17:30 18:30 **Award Ceremony and Closing Session**

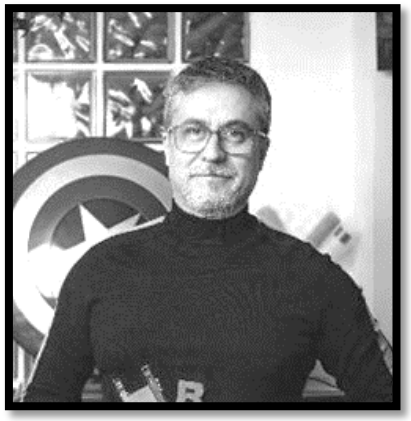
SPEAKERS

Antonio Fernández-Medarde, PhD

Antonio Medarde obtained his PhD at the Anderson Cancer Center of the University of Medicine of Texa (Hostoun), where he also did post-doc work in the Departments of Cell Biology and Molecular Pathology.

Later, in Boston, he worked as a researcher on the Department of Tumor Immunology at the Dana-Farber Cancer Institute and in the Department of Surgical Research at Children's Hospital, both associated with Harvard Medical School.

In 2001, started working in Spain, at the Research University of the Hospital de León. Joined Biomar Microbial Technologies León in February 2002, where he is Executive Director and Chief Executive Officer.



Alfredo Corell, Professor

Alfredo Corell (Madrid, 1963) is a Spanish immunologist, university professor and scientific disseminator. He is member of the Spanish Society of Immunology and winner of the II Educa Abanca Awards, "Best University Lecturer in Spain in 2018".

He obtained the Degree and the PhD in Biology at the Universidad Complutense of Madrid, specializing in Immunology and doing his doctoral thesis at the Hospital 12 de Octubre in Madrid. He carried out a postdoctoral stay at the Anthony Nolan Foundation in London. For eighteen years, he carried out research and immunological diagnosis in various hospitals such as the Hospital 12 de Octubre in Madrid.

His work in immunogenetic and histocompatibility areas includes the discovery of a pseudogene on human chromosome 6, within the HLA system: HLA-DRB6. He has more than sixty scientific publications in high-impact journals, including the prestigious New England Journal of Medicine, Lancet, Immunology Today y Mucosal Immunology.

Since 1999, he has been Immunology assistant professor, and Professor from 2020, at the University of Valladolid. He was director of the Permanent Training and Teaching Innovation Area at the University of Valladolid until July 2020, when he was appointed as Vice-rector for Teaching Innovation and Digital Transformation of said university.

He is also the coordinator of the Immunomedia teaching innovation project. A digital dissemination platform in which professors from six Spanish and two foreign universities participate, which has been recognized both nationally and internationally.

In his role as a scientific disseminator, he frequently collaborates with the informative television program La Sexta Noche, presented by the journalist Iñaki López, as well as with the program Horizonte: Informe Covid, created by Iker Jiménez; in La Hora de La 1 (RTVE), presented by Mónica López, in Está Pasando (Telemadrid), presented by Inés Ballester, and in Cuatro al día (CuatroTv), presented by Joaquín Prats. In 2016 and 2018 he gave TEDxValladolid and TEDxUdG talks on educational innovation and scientific dissemination.

Dezso David, PhD

Researcher at the Department of Human Genetics at the National Institute of Health Doutor Ricardo Jorge (INSA). He has several decades of experience in elucidating Mendelian diseases' molecular basis and, more recently, of genomic diseases caused by cytogenomically visible or cryptic variants.

To identify disease-causing genes and genomic elements and their pathogenic mechanisms, in collaboration with Harvard Medical School and its collaborators, he applied an innovative approach to genomic sequencing. This new approach proposes a genomic medicine flow chart to be adopted in clinical practice.

Miguel Castanho, Professor

Group leader of the Physical Biochemistry Unit of Pharmaceuticals of the João Lobo Antunes Institute of Molecular Medicine, Miguel Castanho is also a full professor and director of the Department of Biochemistry at the Faculty of Medicine of the University of Lisbon (UL) since 2007. With a Degree in Biochemistry from the Faculty of UL Sciences in 1990, completed a PhD in Molecular Biophysics at the Chemical Engineering Department of the Instituto Superior Técnico (IST).

Between 2016 and 2017, Miguel Castanho was vice-president of the Portuguese Foundation for Science and Technology. From 2013 to 2016, he directed the M2B-PhD - Medical Biochemistry and Biophysics doctoral programs, which combine UL, IST, the University of Coimbra and the University of Porto. Between 2011 and 2016, he was vice-director of the Faculty of Medicine of UL and, between 2009 and 2012, he chaired the Portuguese Biochemistry Society.

Among other distinctions, Miguel Castanho received the Leonidas Zervas award, given by the European Peptide Society.

Antonio Molina, Professor

Antonio Molina is a Professor of Biochemistry and Molecular Biology at the Department of Biotechnology and Plant Biology of the Universidad Politécnica de Madrid (UPM). Dr. Molina completed his doctoral thesis at the UPM under the supervision of Dr. Francisco García-Olmedo and did a postdoctoral at the Agricultural Biotechnology Research Unit (ABRU) Syngenta (NC, USA) under the supervision of Dr. John Ryals.

Since February 2016 he is Director of the Centro de Biotecnología y Genómica de Plantas (CBGP), a joint research centre between the UPM and the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA). The CBGP has been awarded as Center of Excellence "Severo Ochoa" by the Spanish Research Agency. He is also Co-Director of the recently created Centre of Excellence for Plant-Environment Interactions between the CBGP (UPM-INIA) and the Institute of Genetics and Developmental Biology (IGDB) of Beijing and the Shanghai Plant Stress Centre (PSC), two research centres of the Chinese Academy of Sciences. The research activity of the group of Dr. Molina at the CBGP focuses on the study of Plant Immunity and its applications to crop protection. Dr. Molina has published more than 70 scientific articles being a recognised expert in the area of plant innate immunity, resistance to necrothrophíc fungi and the function of plant cell wall in immunity. He has developed an intense activity of innovation and technology transfer in collaboration with national and international companies, that resulted in several Patents exclusively licensed to companies and in the commercialization of an agrobiological product. Dr. Molina is the co-founder of PlantResponse Biotech SL, a UPM spin-off enterprise that has raised more than 12 millions of euros of Venture Capitals.

He is currently Chairman of its Scientific Advisory Board. The company received in 2016 the EuropaBio award to the best innovative European SME in Green Biotechnology, and has been recognised by the Spanish Association of Biotech Enterprises (ASEBIO) as one the Spanish success biotech companies of the last 10 years. Dr. Molina is also co-founder of the UPM start-up FAIR Data Systems.

Fernando González Candelas, Professor

Fernando González Candelas is a Full Professor in the Department of Genetics at the University of Valencia, Spain

He is also a senior researcher at the Foundation for the Promotion of Sanitary and Biomedical Research in the Valencian Community (FISABIO) and at CIBERESP (Center for Biomedical Research in Epidemiology and Public Health) funded by the Spanish Government.

His areas of specialization and interest are: Molecular Epidemiology, Evolution and Population Viability of RNA Viruses and Bacteria; Molecular evolution and phylogenetic reconstruction based on biological sequences; Genomics: annotation and evolutionary analysis of bacterial genomes. Phylogenomics; Microbial forensic analysis, determining sources of outbreaks and transmission chains in viral and bacterial infections.

His main research interests are in Population Genetics and Evolutionary Genetics, Molecular and Evolutionary Epidemiology, Molecular Systematics and Genomics, Bioinformatics and Conservation Biology. Currently works in the Molecular Evolutionary Epidemiology of different pathogens, mainly RNA viruses, such as SARS-CoV-2, hepatitis C virus (HCV) and human immunodeficiency virus (HIV), and bacteria, such as Legionella pneumophila, Treponema pallidum, Neisseria gonorrhoeae , Pseudomonas aeruginosa and Klebsiella pneumoniae, among others. Recently, he began to analyze the Genomic Epidemiology of antibiotic resistance in Enterobacteriaceae.

The basic approach is to analyze the variability of the nucleotide sequence at different levels, from intra-patient samples to samples worldwide, and from particular genes to complete genomes, depending on the specific objectives of the different projects. Previously, he studied Population and Evolutionary Biology of endemic plants from the Mediterranean, such as the Limonium species (Plumbaginaceae), using a series of genetic markers (RAPDs, AFLPs, microsatellites, isoenzymes) and including the analysis of quantitative characteristics. He published more than 200 peer-reviewed scientific articles and wrote two books.

Agostinho Antunes, Professor

Earned his Ph.D. in Genetics and Evolution (2002) at the University of Porto (Portugal), in collaboration with the INRA (Paris, France) and the Washington University (St. Louis, USA). He undertook Post-Doctoral work at the Laboratory of Genomic Diversity (LGD), National Cancer Institute, National Institute of Health, Frederick, Maryland, USA (2002-2004), and he continued affiliated with LGD as a Visiting Scientist until 2011.

He became a Researcher (Computational Biochemistry) at the REQUIMTE, University of Porto, in 2005, then moved in 2007 to the CIIMAR (Interdisciplinary Centre of Marine and Environmental Research), University of Porto, and in 2013 became the Head of the Evolutionary Genomics and Bioinformatics Group. Since 2011 he has been also Professor at the Department of Biology from the University of Porto and Director of the Environmental Monitoring Centre – CMIA, in Matosinhos Municipality, Porto, Portugal.

His major research interests include understanding the evolutionary significance of genomics in natural adaptation, diversification and speciation, and its conservation relevance, notably by integrating genomics, bioinformatics and biotechnology to describe overall diversity patterns from microorganisms to animals.

Raquel Godinho, Professor

Raquel Godinho has a PhD in Evolutionary Biology from the University of Lisbon since 2004, and is currently a researcher at CIBIO, Research Center for Biodiversity and Genetic Resources from the University of Porto, where she leads the research group EcoGenomics. She is also a Invited Teacher Assistant at the Faculty of Sciences of the University of Porto, and Invited Researcher at Johannesburg University.

She has been the main coordinator of FCT projects and is the author of more 80 articles in scientific journals. She has as main research interests i) population and conservation genetics, ii) the hybridization between wild and domestic species, iii) adaptive processes to environments extremes, and iv) the use of non-invasive genetics applied to monitoring and conservation programs mammals in the Iberian Peninsula and Southern Africa.

She has used medium and large mammals in her research as biological models, namely canids, and is part of the IUCN Group of Canine Specialists. She is also a member of the Editorial Board of Scientific Reports magazine in the area of Ecology and Evolutionary Biology.

CONFERENCES

Biotechnological applications of secondary metabolites form marine microorganisms

Fernández-Medarde A. [1*]

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* a.fernandez@biomarmt.com

Keywords: Natural products, bacteria, fungi, drug development, biopesticides.

Marine environments contain high biodiversity that correlates with great chemical diversity of the secondary metabolites produced by resident bacteria and fungi. Biomar Microbial Technologies has established a large collection of more than 70.000 strains of bacteria and fungi isolated from marine samples. This collection is the source of compounds that are identified for their potential for different applications, from Human health to Cosmetics or Agriculture. Once a specific application is defined as a target for the discovery efforts of the company bioassays are defined and validated, to allow for the identification of active extracts and compounds. The basis for the identification of active compounds are the processes of activity guided fractionation and structural elucidation by Mass spectrometry and NMR techniques. During the presentation we will review three examples of how this approach has been used by the company to develop products with application in different sectors. We will start by a description of the identification of a molecule with strong angiogenesis and kinase inhibitory potential, and a very unique chemical structure produced by a marine fungi. We will follow the steps required to start preclinical evaluation of a compound, including establishment of a Maximum Tolerated Dose and selection of an animal model of the target disease.

For agricultural applications, the collection was screened for herbicidal activities. An extract from a marine actinomycete was selected, and the identification of a potent compound led to the registration of a natural herbicide.

Finally, we will comment on how our collection of extracts was screened looking for potential cosmetic products. A marine extract with activity against Propionibacterium acnes was fractionated until a potent peptide for the treatment of acne was identified, and brought to the market.

Immunity: when laser clothes pegs neutralise the coronavirus.

Corell A. [1*]

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Keywords: Immunology, science dissemination, mass media.

I have been doing immunology dissemination on the streets for 7 years, with the help of medical and nursing students at the University of Valladolid. But this dissemination was part of the learning process and was one of the strategic lines of action of the University project Immunomedia. In a complementary way with street activities and with the support of the students, we have been working on the dissemination of immunology in different social media.

However, the personal trajectory in the dissemination of immunology in the different media (TV, radio, newspapers) of the country, emerged during the first days of the strict lockdown decreed during the month of March due to the Covid-19 pandemic. After participating in several television programmes, I realised that the public did not fully understand the complex concepts of the immune response. So when I was asked what neutralising antibodies meant, and since we could not leave the house because we were confined, I used a small minion, an aluminium water bottle, and a clothes pin. With these 3 simple elements, the concept of neutralising a virus by an antibody in just 1 minute was explained without any confusion.

I received live congratulations from the show's host, and many comments on social media and on my private communication channels. So I understood... that this was the way to get people to understand complex aspects of physiology, through simple, everyday elements, or through metaphors that were visually simple and appealing.

I went into the star-wars universe, which is very well known by different age groups, and has managed to gain the loyalty of both children and grandparents, and I have not hesitated to use other elements such as music or costumes. I have always opted for maximum rigour within simplicity. Based on the comments and the impact on social networks, it seems that we have managed to bring an immunologist into many Spanish homes.

Deciphering the Pathogenesis of Genomic Disorders by Whole Genome Sequencing.

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Keywords: Genomic disorders, Structural variants, Balanced chromosomal abnormalities, Whole genome sequencing, ANKRD11 - KBG syndrome, TAD, Precision Genomic Medicine.

Balanced and unbalanced chromosomal or genomic structural variants (SVs) have long been recognized as a major cause of genomic disorders (GDs). These are clinically undiagnosed, isolated or syndromic pathologies, often manifested as developmental and neurodevelopmental anomalies, but can also overlap with known syndromes or monogenic disorders. Approaches used for detection of SVS, such as balanced chromosomal abnormalities (BCA) evolved significantly from classical and molecular cytogenomic technologies, such as FISH and microarrays, to whole genome sequencing (WGS) with high physical coverage and low sequence depth. The spectrum of disease-associated SVs, at DNA sequence-level resolution, and the emerging pathogenic mechanisms will be presented. Like classical haploinsufficiency due to point mutations, disruption of the coding regions or genomic elements controlling quantitative expression of a dosage-sensitive gene will lead to a haploinsufficient phenotype. As such, among others, disruption of ANKRD11 and WNT3 by the t(16;17)(q24;q21.3)dn breakpoints, resulting in autosomal dominant KBG syndrome will be presented. Certain BCA can yield clinical phenotypes that are similar to microdeletion syndromes caused by hemizygosity of a major causal gene locus. Position effect, due to disruption of the genome architecture of topologically associated domains (TADs), a chromatin unit that define regulatory landscape, by SV breakpoints is a major pathogenic mechanism of GDs. There is no direct correlation between complexity of rearrangements and severity of clinical phenotypes. Such genomic approach, that allows personalized medical genetics care, has necessarily to be accompanied by deep phenotyping. The emerging picture from these data highlights the extent to which the human genome can be affected by SVs, its tremendous plasticity, and the intricacy of pathogenic mechanisms leading to such disorders. The presented approach and workflow represents a major step towards Precision Genomic Medicine approach.

Acknowledgments: This research was supported by Fundação para a Ciência e a Tecnologia Research Grants PTDC/SAU-GMG/118140/2010 and HMSP-ICT/0016/2013.

From Zika virus to SARS-CoV-2. Developing drugs to fight viruses in the brain.

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Keywords: Drug, virus, peptide, conjugate, brain, NOVIRUSES2BRAIN.

Targeting drugs to the brain is a very hard task. The brain endothelium, named the brain-blood barrier (BBB), is very impermeable. While this property protects the brain from toxic effects of exogenous molecules and microorganisms, it is a serious limitation to chemotherapies in the brain. Drugs aimed to inactivate viruses in the brain, for instance, have to overcome the BBB in addition to being effective and non-toxic. These conjugation of properties (BBB translocation, activity, low toxicity) is difficult to achieve. The rationale behind a drug development strategy to inactivate viruses such as zika and SARS-CoV-2 in the brain will be presented.

From the lab to the field: the key value of innovation for agriculture sustainability.

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Keywords: no keywords selected

Understanding plant nutrition and response/adaptation to environmental interactions/changes is mandatory to develop novel technological innovations for sustainable agriculture. The Universities and public research institution will have a capital contribution in the development of new technologies for sustainable crop production, but to achieve this goal novel models of cooperation with enterprises and new open innovation schemes would be required, particularly in Europe. The experience of CBGP (UPM-INIA) in this innovation/tech transfer process will be presented and some models of public-private partnerships will be introduced that can contributing to close the gap between the excellent research in academia/public research centres and the technological needs of agriculture enterprises and market. One example of these technological needs is the development of agrobiological products that have a clear potential to reduce the use of chemical pesticides and fertilizers in agriculture. Among these potential agrobiologicals are some plant/fungal cell wall-derived carbohydrates that trigger crops disease resistance responses. Some results obtained with this technology in experimental greenhouse and using different plant-pathogen interaction systems will be presented and the potential of using this technology in sustainable agricultural production discussed.

Genomic epidemiology and evolution of SARS-CoV-2

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Keywords: High-throughput sequencing, variants, lineages, founder events, selection, population structure, super-spreading events.

The rapid expansion of SARS-CoV-2 has profoundly altered the life of a large majority of humankind. One of the most useful tools to tackle this problem is obtaining the complete sequence of the virus genome and compare it with those of other viruses. Viruses evolve at a very fast rate, in which relevant changes occur at epidemiological time-scales. This provides an opportunity for integrating evolutionary and epidemiological dynamics under a single framework, known as phylodynamics. This approach has provided information about the origins of the virus and the processes that have governed its spread and dynamics since the end of 2019. In Spain, we have established a multidisciplinary consortium integrated by research groups and laboratories from diverse fields such as clinical microbiology, sequencing, bioinformatics, epidemiology, etc. Our goal is to obtain and analyse complete genome sequences of about 20000 viruses obtained in our country. By comparing these sequences with others from all over the world, we have observed a large number of independent introductions (>500) of the virus in the initial stages of the pandemic. Most introductions arrived from other European countries but only a few of them originated the vast majority of transmissions within Spain, mainly through superspreading events. One of these seeded a specific lineage (known as SEC8) that is genetically different from those that spread initially in other European countries and it disappeared almost completely after the lockdown. Another lineage emerged a few weeks later and it was initially detected among agricultural workers at the beginning of the summer season. It was associated to the virus spread during the second wave of the pandemics. International tracking of this variant revealed that it spread from Spain to other countries, arriving at very high frequencies in some of them.
Genomic surveillance of this virus is revealing its tremendous power and potential as an instrumental tool in Epidemiology and Public Health.

Unravelling life diversification with Genomics and Bioinformatics: Biotechnology Relevance.

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Keywords: Genetics, genomics, bioinformatics, biotechnology, animals, microorganisms.

Deciphering genetic disease and health, species evolution and the diversification of phenotypic traits, can be largely advanced with whole genome sequencing projects. Here, recent results from our group retrieved from comparative genomic/proteomic and bioinformatic analyses of varied animal and microorganism species will insightfully illustrate cases of adaptive successes to thrive into diverse ecological conditions. The findings pinpoint unique molecular products of critical relevance in species evolution, diversification and conservation, but also highlight genomic novelties with relevance in biomedical research and biotechnology.

Hybridization and conservation genomics of populations at extreme environments.

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Keywords: conservation genetics, desert foxes, extreme environments, Iberian wolf, non- invasive sampling

Genomic studies addressing relevant questions in the field of Mammalian Hybridization and Conservation have now become widespread, including the analysis of genetic drift, the evaluation of inbreeding and hybridization and their consequences, or the investigation of adaptive variation, among many others. However, the application of genomic data as a tool for understanding hybridization and adaptation to extreme natural settings is highly relevant for conservation and have so far received much less attention. Conservation genomic practices in mammals, especially medium and large species with elusive behaviours, is also much challenging and largely limited to the availability of samples as these species are difficult to capture. One of the major revolutions of conservation genetics was the possibility of using non-invasive sampling, allowing natural populations to be investigated in unprecedented detail without the manipulation or disturbance of individuals in the wild, but these samples are still difficult to use in genomic approaches. In this talk, I will illustrate in two levels the application of genetic tools in mammal's conservation by i) an analysis of genomic data to investigate time, demography and hybridization of mammals living at extreme environmental settings, and ii) the use of non-invasive samples in the characterization of population parameters and its application in conservation.

ORAL COMMUNICATIONS

01

Perirenal Adipose Tissue and Clear Cell Renal Cell Carcinoma: "The triad: macrophage-cancer cell-adipocyte".

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Keywords: Renal Cell Carcinoma, Clear Cell Renal Cell Carcinoma, Obesity Paradox, Triad: Macrophage, ccRCC cell and adipocyte, Tumour Microenvironment Interplay.

Renal Cell Carcinoma (RCC) is considered one of the most common malignancies of the urinary tract and the deadliest urological cancer. Importantly, obesity is one of the recognized risk factors associated with RCC, nevertheless, a number of epidemiological studies report that obesity can also contribute to better disease outcome in certain scenarios. This phenomenon is designated as the obesity paradox. However, the role of adipose tissue in RCC development remains unclear.

An important piece of evidence from "in vivo" xenograft models, shows that adipose tissue stem-like cells can be recruited to the tumour region indicating the existence of a crosstalk between the tumour itself and outlying adipocytes. This, once again, supports the importance of further dissecting the mechanisms underlying the interplay between renal tumour cells and adipocytes.

One of RCC subtypes is Clear Cell RCC (ccRCC), which arises from renal tubular epithelial cells in the renal parenchyma and commonly presents aberrations, such as the loss of short arm of chromosome 3, and VHL tumour suppressor gene inactivation, by epigenetic or genetic events. The morphological hallmark of ccRCC is the clear cytoplasm, with lipid and glycogen accumulation in the cells that induce important metabolic alterations during the course of the disease. Furthermore, ccRCC is considered an immunogenic tumour because it is characterized by the accumulation of pro-inflammatory cytokines and immune cells infiltration, among which, macrophages. These phagocytes are reported to potentially have a relevant role in ccRCC development and progression.

In line with this, the present work aims at characterizing the interplay between the triad: adipocytes, ccRCC cells and macrophages. For that, macrophages and tumour cells profile will be assessed, and the molecules involved in this communication scrutinized. Finally, key players of the triad communication will be validated in human ccRCC tissue samples from surgical resections.

02

Kinetics and mechanisms of acid orange 7 dye removal from aqueous solution using Dactylis glomerata L. seeds powder as adsorbent

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Keywords: Acid orange 7, *Dactylis glomerata* L., Bio-adsorption, Pseudo-first order kinetic, SIPS isotherm, FTIR, SEM.

Dactylis glomerata L. (DG) is considered an invasion plant, with high costs for its removal. For this work, DG seeds were crushed into powder, to be used as a bio-adsorbent for the treatment of a wastewater polluted with acid orange 7 (AO7). To our knowledge, DG was never used for bio-adsorption of AO7, therefore its efficiency is still unknown. Therefore, the aim of this work was (1) characterize the bio-adsorbent DG by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and Brunauer, Emmet, Teller (BET), (2) optimize bio-adsorption process, (3) evaluate the kinetic rate of AO7 removal, (4) study the mechanism of bio-adsorption.

An initial analysis to DG by FTIR showed an absorption band at 3390.86 cm-1 linked to the presence of proteins, fatty acids, carbohydrates and lignin. SEM images and BET showed the DG materials exhibit a heterogeneous and relatively porous morphology. It was optimized the pH, DG dosage, temperature and AO7 concentration and under the best operational conditions pH = 3.0, [AO7] = 50 mg/L, [DG] = 3.0 g/L, T = 298 K, V = 100 mL, agitation = 350 rpm, t = 30 min it was obtained an AO7 removal of 99.2%. The bio-adsorption process of AO7 by DG was observed to follow a non-linear pseudo-first order kinetic model (r2 = 0.998) with a kinetic rate of k1 = 0.405 min-1, and the adsorption isotherm was best fitted by SIPS model (r2 = 0.998), with a n = 1.05, confirming the homogeneity of the DG adsorbent. The UV-visible spectra analysis showed that an adsorption/desorption mechanism occurred and FTIR analysis showed the disappearance of AO7 characteristic absorption bands, indicating the destruction of the N=N bonds of AO7. In conclusion, Dactylis glomerata L. seeds powder is an efficient, economic and safe product for textile dye wastewater treatment.

03

The use of Ganoderma lucidum to prevent obesity – effects on the genetic damage index.

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Keywords: C57Bl/6J mice; Wester Diet; Animal model of obesity; Ganoderma lucidum; Liver.

Obesity is an emerging health problem worldwide and can be influenced by the regular consumption of natural bioactive compounds. Mushrooms, such as Ganoderma lucidum (GL) include a wide variety of biomolecules with medicinal properties. The aim of this study was to evaluate the effect of a hydroethanolic extract of GL in the liver of an animal model of obesity. All ethical issues were followed (approval nº 8776). Forty-eight male mice (C57BL/6J) were acquired and divided into 5 groups: Group (G)-1-Western Diet 0.2% Cholesterol (WD); G-2-Western Control; G-3-WD+0.7%g/kg of GL; G-4-WD+1.4%g/kg of GL; G-5 WD+2.8%g/kg of GL. At the end of 13 weeks, animals were sacrificed, and the liver collected for genotoxicity assessment and histopathological studies. The chemical composition of the extract was profiled by HPLC-DAD-ESI/MS: ganoderic acid H and p-hydroxybenzoic acid were the main triterpenic and phenolic acids found in the extract, respectively. All animals showed weight gain. The relative masses of the liver ranged between 0.038-0.047g. Histologically, the most frequent morphological changes were microvesicular (83%) and macrovesicular fatty change (steatosis). The Genetic Damage Index (GDI) mean of G-1 was significantly higher than all other experimental groups and G-5 the group with the lowest GDI. The percentage of weight gain was higher in the groups that didn't have GL supplementation, which suggests its anti-obesity properties. The animals developed steatosis, which was not modified by GL supplementation during the period studied, but the relative masses of the liver, lower in the groups supplemented with GL, suggest an ongoing process of restoring to normal hepatocyte's phenotype in these groups. GL dietary supplementation can abrogate genetic damage caused by hyperlipidemia.

04

Comparing the FA-SAT ncRNA profile in different human cell lines: Is the expression of FA-SAT (dys)regulated in the cell cycle of HeLa cells?

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Keywords: *FA-SAT*; non-coding RNA; transcriptional profile; cancer cells; dysregulation.

The emerging interest in studying the non-coding RNAs (ncRNAs) and their functions in the cells have revealed the role of different satellite DNA (satDNA) transcripts in the regulation of several molecular processes.

FA-SAT, the major satDNA of Felis catus, is transcribed in various species (including cat and humans), yielding ncRNAs with known functions in the proliferative and apoptotic pathways. These transcripts were found in the nucleus and the nucleolus of cat non-tumor and tumor cells, presenting distinct distributions throughout the cell cycle: in the normal cells, this ncRNA was only found in G0, G1 and S phases, while in the cancer cells, it was detected in all the cell cycle phases (showing dysregulation of its transcription).

With this work, we aimed, initially, to access and compare the transcriptional profile of this satDNA in different human cell lines (HeLa, A549 and H1299). Regarding the *FA-SAT* RNA (relative) quantity, significant differences were found in the cell lines analyzed, through real-time RT-qPCR. The RNA-FISH experiments enabled us to detect, for the first time, *FA-SAT* transcripts in the nucleus of the HeLa, A549 and H1299 cells, showing discrepancies in their distribution (scattered and/or clustered).

In a second approach, RNA-FISH and immunofluorescence protocols were combined to perform a more detailed characterization of the *FA-SAT* RNA profile in the HeLa cells. These transcripts were, then, additionally located in the nucleolus (and in its periphery) and they were observed in all the phases of the cell cycle (similarly to the reported in cat tumor cells).

The results obtained in this study, combined with a similar further characterization in human non-tumor cells, will allow us to infer the state of (dys)regulation of the *FA-SAT* expression in cancer versus normal cells and it will be crucial to unveil the *FA-SAT* ncRNA contribution to different cellular and molecular outcomes.

O5

Mutation-adapted U1 snRNA as a therapeutic strategy for Mucopolysaccharidosis IIIC: in vitro and in vivo studies.

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Keywords: *HGSNAT*, Mucopolysaccharidosis IIIC, mice C57BL/6, U1snRNA.

A significant number of mutations that change the splicing process and lead to aberrant mRNA production have been identified in Lysosomal Storage Disorders (LSDs). Mucopolysaccharidosis type IIIC (MPS IIIC) is a very rare LSD caused by mutations in the *HGSNAT* gene which encodes an enzyme involved in heparan sulphate degradation. Splicing mutations are one of the most frequent (~20%) genetic defects in MPS IIIC. Around 55% correspond to 5' splice-site (ss) mutations thus constituting a good target for splicing therapeutics. Recently, we have demonstrated that a modified U1snRNA vector designed to improve the definition of the *HGSNAT* exon 2 5'ss can restore splicing impaired by the mutation c.234+1G>A.

Currently, our goal is to evaluate in vivo the therapeutic potential of that modified U1 snRNA by testing it in mice expressing the human splicing defect. For this purpose, two full-length constructs were generated by cloning the wt or the mutated *HGSNAT* splicing-competent cassettes in the pcDNA 3.1 vector. Then, the wt and the mutant constructs were transfected in Hep3B and COS-7 cells. Both minigenes reproduce the healthy control and patient cDNA's splicing pattern. Therefore, they were used to generate C57BL/6 mice expressing the wt (c.241+1G) or mutant (c.234+1A) alleles in the liver. These mice can be used for testing the modified U1 snRNA efficacy in vivo. Thus, wt or mutant minigenes were administrated in mice by hydrodynamic injection following a protocol described by Balestra et al. After 48h, animals were sacrificed, the liver was collected, and the molecular analysis was performed. Preliminary results showed the expression of the mutant construct in the liver of at least one animal. Thus, further tests will be carried out to optimize experimental conditions, by testing other forms of minigenes administration and using other mice strains.

O6

Assessment of microbial diversity in traditional sourdoughs made from organic farming or conventional farming wheat flour.

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Keywords: conventional farming, durum wheat, Kamut® wheat, lactic acid bacteria, organic farming, sourdough, yeasts.

Fermentation with sourdough is one of the most ancient biotechnological processes. Traditional sourdough is generated when a mixture of flour and water is fermented spontaneously by the microorganisms present in the flour and in the environment. Lactic acid bacteria (LAB) and yeasts predominate in the sourdough ecosystem. Aside from other factors, the composition of the sourdough microbiota is believed to be affected by the farming method. The main objective of this study was to characterize the microbiological diversity in two traditional sourdoughs, one made from wholemeal durum wheat flour (*Triticum turgidum* ssp. *durum*), which is a modern cultivar grown by conventional farming; and another made from wholemeal Kamut® brand khorasan wheat flour (*Triticum turgidum* ssp. *turanicum*), an ancient grain grown by organic farming. As far as we are concerned, this is the first study that tackles the microbiological characterization of a Kamut® wheat traditional sourdough. Yeasts were isolated from the sourdoughs using YGC medium and LAB were isolated using MRS medium with pimaricin. Yeasts were identified by sequencing the 26S rDNA D1/D2 domain. BAL were identified by sequencing a 900 bp fragment from the 16S rDNA. In the durum wheat sourdough, the yeasts *Pichia fermentans* and *Kazachstania exigua* and the LAB *Lactobacillus brevis*, *Lactobacillus buchneri* and *Pediococcus parvulus* were identified. In the Kamut® wheat sourdough, the yeasts *P. fermentans, Torulaspora delbrueckii* and *Wickerhamomyces anomalus* and the LAB species *L. brevis* and *P. parvulus* were identified. In conclusion, the durum sourdough showed a higher LAB diversity compared to the Kamut® sourdough. On the contrary, the highest yeast diversity was found in the Kamut® sourdough.

07

Induction of lin genes in Anabaena sp. PCC7120: laying the foundations for the development of a lindane biosensor.

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Keywords: cyanobacteria, lindane, biosensor.

Lindane (γ-hexachlorociclohexane, γ-HCH) is a persistent pesticide that triggers environmental and health problems. Although it is durable and recalcitrant, some microorganisms such as *Sphingomonas paucimobilis* are capable of degrading it. *S. paucimobilis* contains in its genome several enzymes involved in HCH degradation, which are encoded in the catabolic lin genes and are dispersed across the genome. As previous studies showed that the cyanobacterium *Anabaena* sp. PCC7120 was also able to degrade lindane we sought to investigate if this cyanobacterium contained genes involved in lindane degradation.

A comparative genomic study with *S. paucimobilis* identified potential lin genes in its genome, whose expression was analyzed in the presence of γ-HCH and 2,5-DCHQ (a degradation intermediate that acts as an inductor of lin genes expression in other organisms). Our results showed that the expression of one of them increases more than 15 times in the presence of lindane and consequently could be a good candidate for the development of a whole-celll lindane biosensor. The degradative capacity and tolerance of *Anabaena* PCC7120 to α-HCH, β-HCH and δ-HCH isomers were also studied, proving that this cyanobacterium is able to tolerate and possibly degrade other HCH isomers.

O8

Chronic life-cycle studies of the priority pharmaceutical Metformin with Danio rerio: molecular and biochemical assessment.

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Keywords: *Danio rerio*, metformin, chronic effects, mechanisms of action.

More than 451 million people worldwide have diabetes mellitus and the vast majority of whom are affected by type 2 diabetes (T2DM). Metformin (MET) is the first-line pharmaceutical to treat patients with T2DM since it has the ability to reduce hyperglycaemia. However, the total understanding of MET's mechanisms of action remains incomplete.

The main objective of this research is to integrate up-to-date biochemical and molecular methodologies to address the mode(s) of action of MET on the freshwater fish Danio rerio (zebrafish) after a chronic exposure of 9 months. Zebrafish were exposed to three relevant environmental concentrations of MET (361 ng/L, 2166 ng/L and 13000 ng/L). In this study, the underlying toxicity mechanisms of MET were determined by the characterization of biochemical markers (cholesterol and triglycerides of adult zebrafish); and the transcription level of key genes (e.g., *prkaa1, hmgcra, ins, igf, acaca*) of zebrafish larvae and adults.

The results of the biochemical parameters showed a significant decrease of cholesterol levels in 9 mpf zebrafish males and females and a significant decrease of triglyceride levels only in males. The transcription level of igf in 20 dpf zebrafish larvae significantly increased after exposure to MET. Gene expression of *prkaa1, hmgcra* and *ins* of 20 dpf larvae was not significantly altered. In 9 mpf females, *acaca* and *hmgcra* were significantly up regulated.

These findings provide important biochemical and molecular data for risk assessment, given that aquatic organisms are chronically exposed to MET during multiple generations, in the range of the concentrations tested in this study.

O9

Study of fruit quality and gene expression under pre-harvest application of calcium and seaweed as mitigation strategy of sweet cherry cracking.

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Keywords: calcium, cherry cracking, Cv. Burlat, gene expression, *Prunus avium L.*, seaweed.

Actually, cherry cracking is a disorder with strong implication in the quality and profitability of cherry orchards, which highly affects the commercial value of the fruit once the consumers prefer fruits with good appearance, size and flavour. To study and try to reduce this physiological disorder by crop nutrition, calcium and seaweed based biostimulant (both at high and low doses) were applied at foliar level in Cv. Burlat in an orchard located in Resende region. However, it's important not only to solve this problem but also maintain or even increase the fruit quality. So, parameters related to fruit quality and routine analysis were evaluated in the collected fruits as well as the cracking index and the quantification of cuticular waxes. Considering the effects in human health, phenolic, *ortho*-diphenol and flavonoid contents, and the antioxidant activity were also determined. In general, low dose of calcium produced bigger fruits, with higher content of phenolic compounds and antioxidant activity as well as higher cuticular waxes content and lower cracking index. Moreover, it´s known that different gene expression patterns can be correlated with cherry cracking, namely genes involved in cell wall modifications and biosynthesis of cuticular waxes. So, a set of genes involved in these mechanisms was selected and their expression was evaluated by a semiquantitative analysis, using fruits with and without cracks at green/red stage. Observing the preliminary results, appears to exist differences between treatments and fruits with and without cracks. However, these results need to be complemented with a qPCR analysis and more maturations phases.

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O10

Biofungicides elicit defense responses in 'Touriga Franca' against fungal diseases.

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Keywords: Grapevine, Plant fungal diseases, Plant extracts, Defense genes, qRT-PCR.

Grapevine (*Vitis vinifera* L.) is susceptible to many fungi and fungi-like pathogens, that compromise its production and economic profit. The increasing use of chemicals to control diseases, namely through copper-based fungicides, leads to accumulation in the upper soil layers and residues in wines, affecting its quality and ultimately human health. Farm to fork European strategy establishes the need to reduce pesticides and promote alternative plant protection. In this context, it is of utmost importance the implementation of alternative environmental-friendly plant protection strategies, as the use of natural biofungicides.

The aim of this study was to verify the effect of plant-based biofungicides on grapevine, by analyzing the expression of the genes Gluc, PR1 and PR17. In a trial installed in UTAD, with Touriga Franca cultivar, ten foliar applications were made throughout 2019 growing season, with nettle extract, Japanese knotweed extract, conventional fungicide, and water (control). The genes expression was evaluated through quantitative RT-PCR in leaves sampled in veraison and maturation stages.

This analysis revealed that the biofungicides elicit the upregulation of the three genes studied throughout vine growth cycle which may indicate the activation of plant defense mechanisms against pathogens, since Gluc and PR17 genes are related to defense responses and PR1 gene is a marker of systemic disease resistance mechanism. Additional years of field trials and analysis of other genes will allow to better understand plant extracts biofungicides effect on grapevine.

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011

Metformin induces growth and reproductive changes in zebrafish (Danio rerio) after full life-cycle exposure to environmentally relevant concentrations.

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Keywords: *Danio rerio*, Metformin, Histology, NGS, Chronic exposure, Reproduction.

Metformin (MET), an anti-hyperglycemic drug of the class of biguanides, is one of the most prescribed pharmaceuticals worldwide. MET has been reported to suppress the hepatic gluconeogenesis, as well as lipid and cholesterol biosynthesis. It acts by directly inhibiting the mitochondrial complex I of the electron transport chain, which in turn inhibits ATP production and AMP accumulation, and ultimately activates the energy sensing AMP- activated protein kinase (AMPK) pathway. Due to its massive consumption worldwide, large amounts are commonly detected in wastewater treatment plants (WWTPs) and surface waters. Considering its relatively high environmental concentrations in surface waters (as high as 33.6 µg/L in the USA), MET has raised concerns regarding its possible detrimental effects in non-target aquatic species. The present study aimed at evaluating the effects of MET in zebrafish after one full generation of exposure to environmentally relevant concentrations (ng to low µg/L). An integration of multiple key ecological endpoints (survival, growth, reproduction, and embryo development) with histopathological analysis of zebrafish gonads was performed, as well as transcriptomic profile of liver and gonad tissues, in order to gain more insight on the effects of MET exposure in a real case scenario. The results showed that MET was able to increase weight and length of exposed fish at different life stages. Similarly, growth indexes, such as Fulton's condition factor, gonadosomatic and hepatosomatic indexes were significantly higher in exposed animals. Early transcriptomic data revealed altered expression of several genes involved in the reproductive processes. Moreover, the histopathology analysis showed an increase of gonadal abnormalities in MET exposed animals. Overall, the results show that full life-cycle exposure to environmentally relevant concentrations of MET are able to induce several effects at eco-logical level, as well as, in the reproductive machinery of *Danio rerio*.

012

Genetic polymorphisms in DNA repair genes and possible influence on DNA damage and repair activity in a population submitted to physical exercise.

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Keywords: genetic polymorphisms, DNA damage, DNA repair activity, physical exercise.

Regular physical exercise has been associated with increased DNA repair mechanisms, as a result of exercise-induced adaptation, leading to enhanced resistance to oxidative stress. The base excision repair pathway is involved in the repair of oxidative damage, which is generated by reactive oxygen species. However, it has been suggested that the carriers of genetic polymorphisms in DNA repair genes are associated with reduced repair activity. Therefore, the main purpose of this study was to investigate the possible influence of different genetic polymorphisms in DNA repair genes (*hOGG1Ser326Cys, XRCC1Arg399Gln, XRCC1Arg194Trp* and *APE1Asp148Glu* polymorphisms) on DNA damage and repair activity in response to 16 weeks of combined physical exercise training, in 47 healthy Caucasian individuals. Comet assay was carried out in peripheral blood mononuclear cells and enabled the evaluation of oxidative DNA damage (FPG-sensitive sites) and also of DNA repair activity, evaluated by OGG1 activity. DNA was extracted from peripheral blood samples and genetic polymorphisms were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. Regarding differences between pre and post-training, the results showed a significant decrease in FPG-sensitive sites for the wild-type *hOGG1Ser326Cys* ($p<0.001$), *XRCC1Arg399Gln* and *XRCC1Arg194Trp* polymorphisms ($p=0.007$ and $p<0.001$), and also for the mutant *XRCC1Arg399Gln* ($p=0.011$) and *APE1Asp148Glu* ($p=0.001$) polymorphisms. There were no significant changes in DNA repair activity. This preliminary study suggests that physical exercise has different effects in oxidative DNA damage but not in DNA repair, considering the polymorphisms under study.

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013

Green-synthesized magnesium hydroxide nanoparticles influence the osteoblastic and osteoclastic differentiation: an in vitro co-culture approach.

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Keywords: Green synthesis, Mg(OH)2 nanoparticles, osteoblasts, osteoclasts, differentiation, co-culture.

Cases of severe bone loss demand a strategy for bone regeneration to ensure bone metabolism and health. Biomaterials-mediated approaches should ensure biocompatibility, osteogenic potential, and cost-effectiveness. Due to the role of magnesium (Mg) in bone tissue, Mg-based nanoparticles (NPs) are potentially interesting, and a trend for the promotion of bone cell adhesion and differentiation has been suggested. Green-synthesis of NPs reduces cytotoxicity while functionalizing these particles with bioactive relevant molecules. In this work, Mg(OH)2 NPs were synthesized (with MgCl2 as a precursor), and using water (Mg(OH)2), or a Rosehip (RH) extract (Mg(OH)2+RH), and were tested for the bone cells effects in co-cultured osteoblastic and osteoclastic cells. MG-63 osteoblastic cells and THP-1-derived macrophages indirectly co-cultured in a Transwell® insert system were exposed to 10 µg/mL of both Mg(OH)2 NPs and Mg(OH)2+RH NPs for 1 and 6 days, and cell behaviour was characterized for typical osteoblastic and osteoclastic markers, respectively. Additionally, cell cultures treated with osteogenic/osteoclastogenic inducers were used as the positive control of cell differentiation. Results showed that osteoblastic differentiation was induced by both NPs, observed as a three-fold increase in alkaline phosphatase (ALP) activity, compared to that on the positive control, and Mg(OH)2+RH NPs presented a higher effect. For osteoclastogenic behaviour, tartrate-resistant acid phosphatase activity (TRAP) in NPs treated cultures was similar to that measured in the positive control. The same was observed for other phenotypical characteristics like the presence of multinucleated cells and TRAP(+) multinucleated cells. Results indicated a higher induction caused by the green-synthesized NPs in the osteoblastic differentiation when co-cultured with THP-1-derived osteoclastic cells suggesting a great advantage in the use of these NPs in bone regenerative therapies to stimulate bone formation.

014

Filamentous fungi screening in small ruminants.

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Keywords: Fungi; small ruminants; sheep; goats

The aim of this study was to evaluate fungal biodiversity in small ruminants.
In this study, 47 hair samples, 42 (89.4%) from sheep and 5 (10.6%) from goats with origin in 13 small ruminant herds. Regarding gender, 89.4% (n=42) were females and 10.6% (n=5) were males. Concerning age, animals ranging from 1 week old to 7 years old were included. Most animals in the sample were 3 years old (n=15; 31.9%) and 4 years old (n=12; 25.5%). Regarding breed, the sheep were all Churra breed and the goats were Serrana breed. These animals belonged to Carrazeda de Ansiães (21.3%), Carviçais (8.5%), Mogadouro (38.3%), Torre de Moncorvo (8.5%), Vila Flor (12.8%), Vinhais (10.6%). All small ruminants had lesions. Samples were collected 17 (36.2%) on the back, 21 (44.7%) on the face, 8 (17.0%) on the snout, 1 (2.1%) on the chest.
Fungal growth was observed in 91.5% (n=43) of the small ruminants under study. In this study important genera of filamentous fungi were isolated. In 5 (10.6%) samples no identification of the isolated fungi could be made. In small ruminants, the most frequent fungal genera were *Aspergillus* spp. (n=14; 29.8%), *Alternaria* spp. (n=10; 21.3%) and *Cladosporium* (n=9; 19.1%). This was followed by the genera *Penicillium* spp. (n=13; 27.7%) and *Scopulariopsis* spp. (n=8; 17,0%). This work contributes to the knowledge of the fungal biodiversity of livestock, namely in small ruminants.

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POSTERS

P1

The use of histological techniques in the diagnosis of drowning.

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Keywords: drowning, histology, forensic, canine, poisoning, necropsy.

The diagnosis of drowning is described in the literature as one of the most challenging in the area of forensic medicine. In fact, although the macroscopic and histological lesions of drowning have been widely described in the human medical literature, this diagnosis is still a diagnosis of exclusion.

In order to understand the usefulness of histopathological methods in the criminal investigation of suspected drowning, fourteen dead dogs were examined.

Two groups were considered: group A consisted of 7 dogs suspected of drowning; group B comprised 7 control dogs suspected of being poisoned.

Necropsy procedures were performed on the animals in accordance with standard techniques. Representative lung samples were collected for histopathologic examination; fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 microns, and stained with hematoxylin and eosin.

In the animals of group A, the predominant lesions included mild to moderate pulmonary congestion and emphysema. In all cases, the coat was wet and the lungs had moderate to severe edema. Group B animals presented pulmonary congestion and emphysema in the majority of the animals, hemorrhage in three cases, and edema in two cases.

The histological examination performed on lung samples of the animals in group A revealed mild to moderate vasodilatation and rupture of alveolar walls, multifocal, moderate to severe, pale fluid within the alveoli, and in one case intra-alveolar hemorrhages. Similar lesions were observed in the lungs of animals in group B, except for interstitial fibrosis in two cases.

The gross lesions and histological results observed in drowning cases, though characteristic, are inconclusive because they can be detected during necropsies of cadavers who died for other causes such as poising. Additional studies, such as diatomaceous testing, are needed to support the diagnosis of drowning in veterinary forensic medicine.

P2

Coagulation-Flocculation-Decantation with NaCl-Plant Extracts and Microfiltration for Winery Wastewater Treatment.

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Keywords: Winery wastewater, Coagulation-flocculation-decantation, microfiltration, Chelidonium majus L., FTIR, SEM, XRD.

The winery wastewater (WW), is very toxic if released in the environment without treatment. Therefore, the aim of this work was (1) assess, for the first time, the production and application of NaCl plant extracts of Acacia dealbata L., Chelidonium majus L., Daucus carota L., Tanacetum vulgare L. and Vitis vinífera L. in a coagulation-flocculation-decantation process (CFD), (2) test the addition of potassium caseinate, polyvinylpyrrolidone (PVPP), activated sodium bentonite and activated charcoal as flocculants, (3) compare the efficiency of NaCl-plant extracts with aluminium sulfate, (4) perform CFD process to enhance the efficiency of microfiltration process (MF).
Fourier-transform infrared spectroscopy (FTIR) analysis of plants revelled the presence of proteins, fatty acids, carbohydrates and lignin. Scanning electron microscope (SEM) analysis revealed that flocculants exhibit a heterogeneous and relatively porous morphology. XRD patterns of activated sodium bentonite revealed a main composition in montmorillonite, with traces of illite, quartz, feldspar and calcite. Several parameters were optimized in the CFD process (pH, coagulant dosage, agitation, flocculant type, flocculant dosage) and under the best operational conditions it was observed a turbidity removal of 81.6, 96.8, 81.9, 79.6, 81.9 and 98.1%, with a sludge production of 90, 57, 93, 48, 72 and 84 mL/L, respectively. With performance of CFD→MF it was observed a turbidity removal of 97.1, 99.7, 98.2, 99.2, 98.4 and 99.7%, respectively. The filter consumption after CFD→MF was observed to be lower (217, 108, 217, 325, 217 and 108 mg, respectively) in comparison to application of MF to raw WW (1301 mg). A positive correlation between sludge compaction and filter consumption ($y = 2.55x + 12.68$, $r2 = 0.949$) was observed, which indicated that CFD process had a direct effect in microfiltration cost reduction. In conclusion, the combined CFD→MF process with Na-Cl extracts is an economic, efficient and environmentally safe process for WW treatment.

P3

Bioinformatic approach to define specific probes for Colletotrichum acutatum.

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Keywords: *Colletotrichum acutatum*, probe design, olive, olive oil, food safety..

Olive tree (*Olea europaea L.*) cultivation has expanded over the last decades, accompanying the growing demand for olive oil consumption. This crop is particularly important in the Mediterranean countries, both economically and culturally, as olives and olive oil are a staple in their diets. Portugal represents the seventh largest olive producer country globally and is one of the main suppliers for the European Union. There are several diseases that affect this crop, including anthracnose, that can lead to major yield losses and negatively impact the olive oil quality. Olive anthracnose is caused by species within the Colletotrichum genus, most of them associated with the complexes Colletotrichum acutatum and Colletotrichum gloeosporiodes. Disease severity and incidence varies with environmental conditions, such as humidity and temperature, cultivar susceptibility, and the pathogen's virulence. This study aims to develop a method capable of both detecting Colletotrichum and differentiating between the mentioned complexes. Data mining was performed on single-copy genes well conserved within the genus, using the program Geneious and 28 possible probes were obtained, for the gene Cgl-SLT2, of which 3 were selected for further studies. These sequences will be incubated with nanoparticles in order to obtain a portable, specific and highly sensitive biosensor that is both time and cost-effective. In the future, this methodology could be employed to prevent anthracnose outbreaks in olive orchards, thus preventing the olive oil quality deterioration and protecting consumer's interests.

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P4

Biomedical Big Data: Ethical and Legal Aspects.

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Keywords: Big Data, biosanitary field, patient, privacy, rights, risks.

Data has always been with us, multiplying in recent years with the advent of the Information Age. In this context, Big Data is presented as the most appropriate option for processing and getting information from them. This technology can be particularly useful in one of the areas where Biotechnology plays an important role, the biosanitary field. However, any implementation carries its risks. In this case, where people's most intimate personal data comes into play, danger becomes more apparent. Through a bibliographic review of the existing doctrine, we will be able to know the true impact and risk that are brought with this technology. We will also define some possible solutions and conclude what attitude we shall have towards Big Data.

Acknowledgments: This work is based in my Degree Final Project, which was done under the direction Salvador Tarodo Soria and Marina Morla González, both professors at the Faculty of Law in the Universidad de León. I would like to give an especial thanks to Marina Morla González who's teachings and supervision have made possible the realization of this Poster.

P5

Seed priming with boron in common wheat.

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Keywords: biofortification, boron, germination, mitosis, seed priming, wheat.

With an annual production of 749 million tonnes, wheat is one of the cereals with high economic and nutritional importance worldwide. Common wheat (Triticum aestivum L.; 2n = 6x = 42) represents the second cereal with Portugal's most increased production and cultivation area. The main challenge of wheat producers is to ensure sustainable production regarding climate changes. Biofortification strategies have been applied to common wheat to improve its germination, seedlings emergence, plant resilience, yield and seed quality. Among those strategies, seed priming with micronutrients (nutripriming) is one of the most used. The germination and mitotic cell analyses in roots harvested from seeds pretreated with micronutrient solutions at different concentrations (nutripriming) allowed the determination of the better micronutrient dosage to be used in wheat biofortification. Boron is a micronutrient that should be used with caution because there is a tenuous line between its benefits and toxic effects. With this work, we intend to revise some previous studies related to seed nutripriming in cereals, namely, performed with boric acid (H3BO3) at different concentrations, and to extrapolate about their effects on the germination and mitosis of wheat.

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P6

Novel Biosensor Applied to Non-Small Cell Lung Cancer Detection.

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Keywords: Biosensor, Cancer Diagnosis, Non-Small Cell Lung Cancer, Piezoelectric Biosensor.

Nowadays, lung cancer is the main malignant cancer reported worldwide, being responsible for one third of cancer-related deaths registered annually. Its low survival rate is related to the lack of an early diagnosis, as almost 65% of lung cancer patients are diagnosed in a late stage of the disease. In the last decade, due to advances in the genomics field, specific genes which suffer mutations associated with particular subtypes of lung cancer, like non-small cell lung cancer (NSCLC) have been identified. These advances have led to molecular-based therapies targeting those specific genes, increasing the survival rate of some patients. Some of the genes that are relatively well studied and are commonly mutated in NSCLC patients, which can be used as biomarkers, include epidermal growth factor receptor (EGFR) and the transforming EML4-ALK fusion gene. The need of fast, reliable and early detection means applied to NSCLC has led to the development of highly sensitive devices able to detect cancer-associated mutations. Such devices, known as biosensors, are a promising alternative to more conventional detection methods and can potentially alter the way cancer is diagnosed and treated. This project will focus on the potential development of a biosensor, namely a quartz crystal microbalance (QCM), applied to the detection of NSCLC. The detection, as is the case of most DNA biosensors, is based on the hybridization between the NSCLC specific DNA probe and the sample DNA (containing specific mutations associated with NSCLC).

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P7

Nanoparticle-based biosensor: a potential alternative for COVID-19 detection.

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Keywords: biosensor, COVID-19, SARS-CoV-2, diagnosis.

The current COVID-19 outbreak, caused by the SARS-CoV-2 virus, has resulted in more than 120 million of infected individuals and nearly 3 million deaths worldwide. Although vaccination is already underway in several countries, it is still essential to maintain high levels of testing. The development and optimization of fast, sensitive, and cost-effective detection methods is very important to mitigate any possible outbreaks by identifying and isolating the infected individuals. Techniques such as Real-Time Polymerase Chain Reaction (RT-PCR) and serology tests have been widely implemented. However, problems like low specificity and sensitivity, high cost, excessive waiting time and lack of equipment and qualified technicians still need to be addressed to assure all-inclusive reliable accessibility. A potential solution to overcome the current limitations is the development of a biosensor, which is a device that combines a biological component to detect an analyte, and a physicochemical component to generate a measurable signal. This would provide a fast, portable, highly sensitive and large-scale diagnostic tool for COVID-19. The biosensor presented is nanoparticle-based and relies on the identification of SARS-CoV-2 RNA through probe hybridization. The reaction will be detected by a change in fluorescence and the process takes less than 20 minutes from sample collection to result; it is low-cost and does not require specialized personnel. This biosensor is an easily adaptable device that allows the recognition of new SARS-CoV-2 variants by changing or adding a new probe. This is a major advantage due to the virus high mutation rate, which is one of the emerging challenges regarding the current pandemic.

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P8

How herbicides affect nucleolar activity?

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Keywords: Cytotoxicity, herbicides, nucleolus, nucleolar changes, stress sensor.

The nucleolus is a multifunctional nuclear domain that plays noncanonical roles in crucial cellular processes, including stress response. Regardless of the plant species, the nucleolus acts as a stress sensor, responding with changes in morphology, number and area, giving evidence about the plant's tolerance or susceptibility.

Cytotoxicity caused by different stressful agents may lead to mitotic index reduction, alterations in the cell cycle, chromosomal and nucleolar anomalies.

Nucleolar activity is one of the most sensitive cytotoxicity parameters. Plants exposed to pollutants like herbicides or their active substances showed nucleolar changes. Such studies have been done in model species like Allium cepa. However, the development of similar studies in cereals would be desirable regarding the abundant use of herbicides in their cultivation and their putative role in phytoremediation. The presence of herbicides or their active substances in the soils will result in an irregular cell division, nucleolar activity. It will affect the protein synthesis and plant development of the target crop and non-target species.

This work will focus on reviewing the current knowledge about the impact of herbicides and/or their active substances on plant species' nucleolar activity.

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P9

Dermatophytes occurrence in herding dogs from the Northeast region in Portugal.

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Keywords: Dermatophytes; Fungi; herding dogs.

The aim of this work was to screen dermatophytes in the fur of herding dogs from herds in the Northeast region. The samples were collected by the Mackenzie technique and sent to the Medical Microbiology Laboratory. Samples were inoculated in Dermathophyte Test Medium® and microscopic identification was performed using dichotomous keys and the Lactophenol with Cotton Blue technique.
In this study, 24 hair samples from herding dogs were analyzed. Regarding gender, 66.7% (n=16) were male and 33.3% (n=8) were female. Regarding age, half of the animals were 2 to 5 years old (n=12), 41.7% (n=10) were up to 1-year-old, and 8.3% (n=2) were 6 or older. Regarding breed, most animals were Podengo (41.7%) followed by Transmontano Cattle Dog (33.2%). Most animals belonged to Freixo de Espada à Cinta (29.2%), Torre de Moncorvo (20.8%) and Vila Flor (12.5%). Regarding lesions, 79.1% (n=19) had no lesions and 20.9% (n=5) had lesions, one of which was an atopic dermatitis type lesion. As for sampling sites, 17 samples were collected on the back, 3 on the face, 3 on the lesion areas, 1 on the chest. Dermatophytes of the genus Microsporum were isolated in 3 samples. Dermatophytes were isolated in samples from 2 male and 1 female. One animal was a Transmontano Cattle Dog breed and 2 were unbred. Two animals were 2 years old and one animal was 7 years old. One of the positive animals had lesions.
The occurrence of dermatophytosis in this study was 12.5%. This study allowed increasing the knowledge about dermatophytosis in shepherd dogs of the Northeast region in Portugal.

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P10

Fungal Biodiversity in the Beds, Collars and Blankets of Shepherd Dogs from Terra Quente Transmontana.

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Keywords: Fungi, fomite; shepherd dogs.

In this study, 21 samples from sheepdog objects were analyzed in order to evaluate the fungal biodiversity in these objects.

The Mackenzie technique was used to collect the samples. Samples were sent to the Medical Microbiology Laboratory of the University of Trás-os-Montes e Alto Douro, where cultures were performed in Dermatophyte Test Medium® (DTM®).

Concerning about the dogs to whom the objects belonged, 11 (52.4%) were females and 10 (47.6%) were males, aged between 2 months and 13 years. As for breed, 16 (76.2%) were unspecified, 2 (9.5%) were German Shepherd, 1 (4.8%) was Podengo, 1 (4.8%) was Transmontano Cattle Dog, 1 (4.8%) was Yorkshire. None had lesions. These animals belonged to the Terra quente Transmontana, namely, the municipalities of Alfândega da Fé (n=7; 33.2%), Macedo de Cavaleiros (n=2; 9.5%), Vila Flor (n=9; 42.9%), and Freixo de Espada à Cinta (n=2; 9.5%), Mirandela (n=2; 9.5%). Most of these animals (n=13; 61.9%) were companion animals and 4 lived with livestock (n=8; 38.1%).

The objects where samples were collected were bedding (n=3; 14.3%), collars (n=16; 76.2%) and blankets (n=2; 9.5%).

Fungal growth was observed in 85.7% (n=18) of the objects under study. Several filamentous fungi were isolated in this study. No dermatophytes were isolated. In 5 (23.8%) samples no identification of the isolated fungi could be made.

In these samples, the fungal genera isolated were *Alternaria spp*. (n=7; 33.3%), *Penicillium spp*. (n=3; 14.3%), *Cladosporium spp*. (n=3; 14.3%), *Aspergillus spp*. (n=2; 9.5%), *Chrysosporium spp*. (n=1; 4.8%), *Fusarium spp*. (n=1; 4.8%), *Mucor spp*. (n=1; 4.8%). The isolated fungal genera were opportunistic, however, all have infective potential in human and veterinary medicine. This study contributes to the knowledge of fungal biodiversity in dogs and their environment.

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P11

Talaromyces marneffei isolation on a horse blankect in Miranda Do Douro region – the importance of a One Health approach.

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Keywords: Fungi; pathogenic; horse.

Talaromyces marneffei is a dimorphic fungus that is endemic in many parts of Asia and causes a fatal fungal infection in immunosuppressed individuals. Till now, the only known natural reservoirs are Rhizomys (bamboo rats), Cannomys spp. and humans.

Objects from horses were screened for fungal microbiota associated with fomites. Samples were collected by the Mackenzie technique and analyzed using routine mycological methods. Samples were inoculated on Potato Dextrose Agar and Sabouraud Dextrose agar and incubated at 25 ºC and 37 ºC during 3 to 7 days. The growth of a colony of Talaromyces marneffei was observed in samples from a horse blanket. The case documented in this paper is the first case of isolation of T. marneffei in a horse's fomite. As this is considered an opportunistic infection, it is important to note that T. marneffei was not isolated from the horse to which the blanket belonged; however, the horse died of unknown causes one week after the collection of the material.

These results demonstrate the presence of the pathogen in Portugal, further research is needed to understand the role of animal-borne fomites in public health through a One Health approach.

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P12

Cytogenomic response of almond tree to two biostimulant and two boron-based fertiliser treatments.

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Keywords: Biostimulants, chromosomes, fertilisers, leaf mitosis, molecular markers, *Prunus dulcis*.

Extreme environmental factors affect the productivity and quality of nuts like almond (*Prunus dulcis*). Biostimulants and fertilisers can mitigate the negative impacts of extreme climate conditions as seen in other species. Seaweed extract (AN) and free amino acids(AA) (biostimulants), and two boron-based fertilisers (boron ethanolamine - BE and solid boron - BS) were applied to almond trees of the cultivar 'Vairo' growing in an orchard at the NE of Portugal. Biological processes depend on cell division regularity and genomic stability. Cytogenomic analyses were used to select the best treatment for cv. 'Vairo', in comparison with control (untreated trees). Young leaves, sampled in June, July and August of 2019, were used for Cytogenetics and molecular analyses. Treatment AA increased significantly ($p<0.05$) the mitotic index (MI) relative to the control in all sampling dates and showed the lowest percentage of dividing cells with anomalies (%DCA) (30.58%) in August. Treatments BE and BS showed the highest mean numbers of telophase, suggesting mitosis progression. Genomic template stability (GTS) was inferred by comparing molecular patterns produced by five marker systems in treated versus control plants. Newly and/or disappeared bands (polymorphisms) relative to the control patterns suggested genomic instability. The highest GTS values were found in trees treated with BE, AA and BS, and in August. Overall, these treatments showed higher cytogenomic stability and seemed to be suitable for cv. 'Vairo' growing at the NE of Portugal.

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P13

Expression of genes related to cell wall modifications under foliar application of potassium and magnesium in sweet cherry – a semiquantitative analysis.

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Keywords: cherry cracking, Cv. Burlat, gene expression, magnesium, potassium, *Prunus avium L.* .

The *Prunus avium* L. species, most commonly known as cherry tree, in Portugal is mainly found in North and Centre, being a predominant tree in regions with temperate climate. The fruits of this tree, known as sweet cherry, have a particular interest in Portugal and other parts of the world due to its chromatic, aromatic and nutritional attributes, and its effects in human health.

Cherry cracking is considerate a severe problem since it affects, with a high incidence, many cultivars of cherries. A cracking rate of 20-25% is enough to consider an unviable harvest once the cracked fruits lose its value as fresh fruit and can only be sell to processing industry. Several treatments have been tested with the purpose to know which is more effective in cracking reduction.

The main goal of this work is to analyse the expression of genes that may be associated to the cracking of the sweet cherry, such as genes associated to cell wall modifications, through a semiquantitative analysis, to better understand how the application of different concentrations of potassium and magnesium in the green/red and red maturation phases affects the gene expression in *Prunus avium* L. fruit. For this, fruits at two maturation stages were collected, RNA was extracted and then cDNA was synthetized. The semiquantitative analysis of the selected genes involved in cell wall modifications revealed differences among treatments and maturation phases. To validate the results, a housekeeping gene was used as control, which maintained their expression to all treatments and maturation stages.

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P14

Analysis of the XPDAsp312Asn polymorphism in a human population sample.

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Keywords: XPD gene; Nucleotide excision repair; Polymorphisms; PCR.

The *XPD* gene, located on the 19q chromosome arm, encodes a protein essential for multiple cellular signalling pathways. The XPD protein acts as a helicase and is part of a protein complex known as TFIIH, having a crucial activity in the nucleotide excision repair (NER). This mechanism recognizes the damage in the DNA molecule, cut the affected fragment and synthesize the new complementary DNA chain. Genetic variations like polymorphisms difficult the efficiency of the DNA repair proteins, leading to genetic instability. Many studies have shown that the *XPDAsp312Asn* polymorphism is related to a reduction in repair capacity increasing the risk for several diseases.

The main objective of this study is to genotype the XPD gene, more specifically the *XPDAsp312Asn* polymorphism in a human population sample. DNA samples extracted from blood of 22 individuals were used for the detection of alleles A and G of the polymorphism by PCR. Two different PCR reactions, each of them using a specific pair of primers for one allele amplification (bands with an expected size of 150bp), were done. Homozygous were detected when the amplification occurred only in one reaction (Asp/Asp or Asn/Asn genotypes) and heterozygous samples (Asp/Asn) were identified when bands appeared using both pairs of primers.

Based on the results we can conclude that most of the individuals are wild-type (GG – *Asp312Asp* ≈ 59%) homozygous, about 18% are heterozygous (GA – *Asp312Asn*) and approximately 23% are mutant homozygous (AA – *Asn312Asn*).

Therefore, this genetic analysis based on this technique allowed the detection and genotyping of the *XPDAsp312Asn* polymorphism and also to characterize the population under the study for this polymorphism.

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P15

Detection of the RAD18Arg302Gln polymorphism in a human population sample.

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Keywords: *RAD18* gene; multiplex PCR; polymorphism; DNA repair; *RAD18Arg302Gln*.

The human *RAD18* gene, located on the short arm of chromosome 3 comprises 14 exons and codes for a protein of 484 amino acid residues. Functionally, it plays an important role on the ubiquitination process, because codifies an E3 ubiquitin ligase that binds to the DNA molecule with a ring-finger domain. This protein has a major role in the maintenance of genomic stability integrating a protein complex involved in the post-replication repair systems, interacting with the *RAD6* protein. Genetic variations like single nucleotide polymorphisms (SNPs) are frequent in the human population, such as the *RAD18Arg302Gln* polymorphism which results by a transition between a guanine and an adenine, which leads to the changes of an amino acid by another.

The main goal of this study frequency of the *RAD18Arg302Gln* in a population of 21 individuals. The gene was amplified, and the polymorphism was detected by multiplex PCR using two specific groups of primers, one for each allele, which allowed to detect the corresponding bands with the expected size of 146pb to Gln allele, 106pb to Arg allele, and also a band of 206bp as amplification control.

The results demonstrated that the majority of the individuals are heterozygous (Arg/Gln≈81%) while the remaining individuals are wild type (Arg/Arg≈19%) homozygous. This genetic analysis allowed to identify alleles of the *RAD18Arg302Gln* polymorphism and also to characterize the population under the study to this polymorphism.

This genetic analysis allowed to identify alleles of the *RAD18Arg302Gln* polymorphism and also to characterize the population under the study to this polymorphism.

Acknowledgments: This work was supported by National Funds by FCT (Portuguese Foundation for Science and Technology), under the project CITAB-UIDB/04033/2020 and the program VerãoComCiência summer course "Curso Prático-Aplicação de modelos biológicos em Estudos de ecotoxicologia".

P16

Biological roles of tensins.

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Keywords: tensins, focal adhesion, extracellular matrix, mechanosensors, cancer.

Tensins are focal adhesion proteins, whose functions are linked to the transduction of biochemical signals from the extracellular matrix to the cytoskeleton, so they participate in multiple physiological and pathological processes. The aim of this review is to summarize the current state of knowledge about the biological roles of tensins.

Tensins family is constituted by four members: tensin-1 (TNS1), tensin-2 (TNS2), tensin-3 (TNS3) and tensin-4 (TNS4/cten). The four tensins are conserved in mammals and are involved in a variety of physiological processes such as cell proliferation, attachment, migration and cellular mechanical sensing. These functions are due to the presence of multiple domains that mediate the connection between the extracellular matrix and the cytoplasmic tails of β-integrins, as well as participating in the transduction of biochemical signals.

Tensins play important roles in the maintenance of the normal cell activity but they can be involved in multiple human and animal diseases, such as cancer. There are many studies reporting their involvement in melanoma, colorectal, breast, gastric, liver, thymoma, kidney, lung, pancreatic and prostate cancer. The expression of the four tensins is different among cancers and they can act as either as oncogenes or tumour suppressors, depending on their expression level. They regulate the Rho GTPase family, and their activity plays critical roles in cell migration and cytoskeletal dynamics. Tensins also regulate other signalling proteins and pathways, such as EGFR, PI3K/Akt, FAK and c-Met. The c-Met and EGFR have been reported to be overexpressed in many tumours, and an enlightenment of their correlation with tensins may be important to develop efficient anti-cancer therapies and biomarkers.

A deeper understanding of the biological roles of tensins can be extremely valuable not only for future investigations but also for clinical applications.

P17

New roles for FUR (Ferric Uptake Regulator) proteins in Anabaena sp. PCC7120 beyond transcriptional regulation: exploring FurA interaction with photosynthetic electron carrier proteins.

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Keywords: FurA, cyanobacteria, photosynthesis, flavodoxin, ferredoxin.

Photosynthesis is one of the main cellular processes in cyanobacteria. In this process electrons extracted from water by photosystem II (PSII) are transferred through several electron carriers to photosystem I (PSI) and ultimately to ferredoxin to produce NADPH via FNR. Previous studies from our group showed that the Ferric Uptake Regulator FurA regulates several genes involved in photosynthesis and revealed that its overexpression has a strong effect on the photosynthetic activity. It has also been shown that FurA can interact with small molecules (heme or 2-oxoglutarate), affecting its DNA binding activity. Consequently, we sought to investigate the interaction of FurA with soluble photosynthetic electron carriers, namely plastocyanin, ferredoxin, flavodoxin and ferredoxin:NADP+ reductase (FNR).

Cross-linking assays and bacterial two hybrid assays (BATCH) showed that FurA interacts only with ferredoxin and flavodoxin both *in vitro* and *in vivo*. This interaction does not appear to affect FurA DNA binding activity or to have a redox implication, since none of these proteins interfere FurA DNA interaction or are able to reduce FurA. Conversely, the observed interaction seems to modulate the transport of electrons from FNR to flavodoxin and ferredoxin, as seen by in vitro enzymatic assays.

P18

Assembly and Annotation of the Lentil Transcriptome.

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Keywords: Bioinformatics, RNA-seq, transcriptome, de novo assembly, Trinity.

Lentil (*Lens culinaris medik*) is a traditional pulse in the Mediterranean area grown mainly for human consumption. In the context of pursuing a healthy diet, lentils are highly appreciated because their high content in proteins and fibre. Lentil has a large genome of about 4 Gb, which together with extensive repeated regions makes it a complex genome to analyse. Here, we assembled the transcriptome of unstressed lentil cultivar Alpo and used it to gain knowledge on the genes present. RNA-seq was performed with 100pb long Illumina paired-end reads. Low-quality raw sequencing reads were cleaned with Trimmomatic, checking the quality of the reads with Fastqc before and after cleaning. High-quality filtered reads were used for a de novo transcriptome assembly with Trinity. The quality of the assembly was characterized in different ways. First, using BUSCO to explore full-length reconstructed transcripts and completeness according to a conserved ortholog set, 425 BUSCO groups were searched of which 89,2 % were complete (76,5 % single and 12,7 % duplicate), 9,2 % were fragmented and 1,6 % missing. Second, studying the transcript representation using three different analyses: i) examining the read representation in the transcriptome to assess the percentage of reads that are present in the assembly. We found 98,44 % overall alignment rate; ii) computing the N50 transcript contig length, which gave a value of 1,934; and lastly iii) evaluating the representation of protein-coding genes using the NCBI plant protein database, finding that 60,5 % of proteins were represented by nearly full-length transcripts with >80 % alignment coverage. This last step also provided us with the annotation of the transcripts. Trinity was also set to output supertranscripts, which ideally would correspond to the consensus gene once all alternative splicing transcripts are considered. Gene Ontology and Cytoscape were used for gene ontology analysis and visualization.

P19

Transposable elements dynamics in the human genome.

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Keywords: Genome dynamics; Transposable elements; Retrotransposons; Neuromuscular disease; Spinal Muscular Atrophy.

Transposable elements (TEs) are interspersed repetitive DNA sequences with the ability to mobilize in the genome accounting for approximately 45% of the human genome. Despite being highly abundant, only in the recent years their role in the genome started to be gradually recognized. Initially thought as genomic "parasites", the development of advanced tools recognized TEs as important players in genomic evolution, genome organization and gene regulation. TEs are now seen as important sources of new regulatory sequences, capable of altering host genes' sequences and expression, and are also important for genome evolution and diversification. However, their presence in the genome also comes at a price. TEs activity may cause several problems to normal gene expression, genome organization, stability, and integrity. TEs transposition is the main mechanism associated with TE-induced diseases. Fixed TE insertions are also capable of causing problems since TE insertion in gene introns are capable of altering splicing patterns and promoting chromosomal rearrangements that ultimately can lead to disease. Spinal Muscular Atrophy (SMA) is an autosomal recessive neuromuscular disease mainly caused by deletion of the Survival Motor Neuron 1 gene (*SMN1*), gene conversion of *SMN1* into Survival Motor Neuron 2 gene (*SMN2*) or missense mutations in *SMN1*. *SMN1* sequence is highly enriched in transposable elements, with multiple Alu elements and Long Interspersed Nuclear Elements 1 (L1) being inserted in key gene regions.

A comprehensive analysis, using a combined approach based on *in silico* analysis and molecular cytogenetics, of TEs in *SMN1 locus* was made in order to perceive TE dynamics in *SMN1*, understand their role in the gene regulation and try to establish a link between these elements and SMA onset.

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P20

Gene-stealing plants: parasitic plants and horizontal gene transfer (HGT).

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Keywords: *Cuscuta*; functional HGT; haustorium; *Lophophytum mirabile*; parasitism; *Phelipanche aegyptiaca*; plasmodesmata.

Parasitism is a special form of nutrition and a way of life of approximately 4750 plant species. Plant parasitism is made possible by a unique organ: the haustorium, which can penetrate host tissues to connect with xylem, phloem, or both vascular tissues. Plant parasitism has arisen in twelve independent events throughout evolution, and can be of different types, with holoparasitism being the most studied form. Horizontal gene transfer (HGT) is a very frequent event in microorganisms, but less common in plants. Nevertheless, parasite-host interaction in plants reveals a high concentration of HGT events due to the intimate contact between both parts. HGT can be mitochondrial (most frequent), nuclear or plastidial. In addition, an HGT event can be functional or nonfunctional; in the first case, the accepted genetic material has a known function in the receptor. The mechanisms of HGT are diverse, and direct DNA capture, fusion of mitochondria or plastids, RNA intermediates or transposons have been proposed. Plasmodesmata connections between parasitic plant and host cells facilitate these mechanisms. Here we have selected three interesting examples of HGT in parasitic plants: 1) *Lophophytum mirabile*: its mtDNA is mostly formed by foreign genes that functionally replace the native ones; 2) *Phelipanche aegyptiaca* imports host defense genes via HGT: these genes are functional in parasite tissues, and their functions could be defense against host pathogens, attenuation of the host immune system, or defense in the zone of infection; 3) Cuscuta spp.: most of the genes imported by HGT are actively transcribed, and convergent retention of certain genes has been detected with parasitic Orobanchaceae plants. In conclusion: HGT events could have a major influence on the evolution and adaptation of parasitic plants, but more efforts would be necessary to shed light on this interesting phenomenon.

P21

Cross-species models of human bladder cancer: molecular targets.

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Keywords: Urothelial cancer, Animal models, biomarkers, bovine, canine.

Bladder cancer is the most common malignant tumour affecting the urinary system, with rising incidence and mortality. Human bladder cancer, as opposed to other tumours, has increased in recent decades. Urothelial cancer is the ninth most common cancer worldwide and the most common cancer affecting the urinary tract. It has become more prevalent in developed countries. Regarding therapies, no significant progress has been made in recent decades and, despite continued efforts to improve treatment options, there is still no cure therapy available for high-grade or metastatic tumours. Animal models thus offer a unique opportunity to study cancer in humans, allowing to understand carcinogenesis, progression and dissemination of the tumour. Moreover, preclinical development of new treatments requires an animal model that accurately simulates disease in humans. They can be useful for the identification of diagnostic and prognostic biomarkers for disease progression and preclinical identification and validation of therapeutic targets/candidates.

Here we review animal models of bladder cancer namely dogs and cows. Interestingly the bovine urinary bladder occurs in association with bovine enzootic hematuria, linked to the ingestion of Pteridium aquilinum and bovine papillomavirus infection. We also highlight the potential for interspecies comparative genomic studies of bladder cancer to identify key molecular events that cause this disease.

Canine and human bladder cancer are epidemiologically, histologically and molecular comparable, which promotes the dog as an invaluable animal model. In the case of cows, further studies are necessary to understand its potential as a model to study bladder cancer.

P22

Can the pre-harvest application of different nutrients affect the expression of genes related to cell wall modifications in sweet cherry?

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Keywords: cherry cracking, crop nutrition, Cv. Burlat, gene expression, *Prunus avium L.*.

Sweet cherry production has been increased in Portugal, being the Resende region one of the main productive regions of cherry in our country due to its excellent edaphoclimatic conditions. Although sweet cherry is considered one of the most popular spring-summer fruits and with a high commercial value, cherry cracking highly affects the orchards profitability which devalues the fruit price. In this follow up, two assays have been developed in Resende region with the application of different nutrients at foliar level, one with the application of potassium and magnesium and other with the application of calcium and seaweed based biostimulant, trying to decrease or to avoid the cherry cracking.

The expression of genes related to cell wall modifications has been correlated with susceptibility to cracking, so, this study intended to understand how these genes are influenced by the applied compounds. For this, fruits of *Prunus avium* L. cv. Burlat were collected at green/red stage once is the phase when these genes are most expressed since the fruit is growing. Using fruits from all treatments, total RNA was extracted from fruit exocarp and then cDNA was synthetized. The expression of genes related to cell wall modifications was studied by a semiquantitative analysis and a housekeeping gene was used as a control. The expression of the analysed genes appears to have differences among the different treatments while the housekeeping gene maintained their expression. However, these results will be complemented by a qPCR analysis, which will allow to complement the preliminary results.

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P23

Pectin role in Psudomonas syringae defence processes.

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Keywords: RNAseq, bean, Pseudomonas, cell-wall, pectin, Pectin-Methyl-Esterases, Wall-Associated-Kinases.

Plants have developed a sophisticated immune system against bacteria attackers such as *Pseudomonas syringae pv phaseolicola* (Pph). In this system, plant cell wall (CW) becomes the first physical barrier of plant cells, which recognizes Pph. Plants can remodel the CW architecture to limit the proliferation of the pathogen.
To study infection processes, the compatible interaction between common bean cultivar Riñon and Pph has become an interesting model for studying susceptibility. In our lab, physiological analyses at early stages of infection showed that Riñon bean perceives the Pph through PvWAK1, a protein involved in "pectin state sensing, although some components of the subsequence response pathway, which participate in resistant varieties, were not observed. Interestingly, Riñon variety could be elicitated with INA, a structural homologue of salicylic acid, which produced changes in the extractability, molecular weight and epitope changing of non-cellulosic CW components, mainly pectins. All these changes resulted in a more enzymatically digested resistance CW. These findings suggested that pectins play a crucial role in this pathosystem, not only in detection but also in resistance. In order to deepening into this hypothesis, RNAseq analyses were performed at early stages after Pph infection. After differential gene expression and functional analyses, some genes involved in pectin metabolism were clearly miss-regulated during the Pph infection. Among them, there were some pectin methylesterases genes, which determine homogalacturonan (HG) structure by modifying its degree of methylesterification, feature that determines if the HG is degraded or influencing the CW biomechanical properties. Taking into account all these results, the importance of pectin in perception and CW remodelling against Pph infection in bean has been revealed, which gives new steps into further research in pectin related genes and their role in defence processes.

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P24

Ferrets as an animal model for the understanding of cystic fibrosis.

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Keywords: CFTR, cystic fibrosis, ferret, gene targeting.

The aim of this essay has been to assess the viability of ferrets (*Mustela putorius furo*) as an animal model which imitates the etiology and the development of Cystic fibrosis (CF) in humans. *CFTR* is a gene which encodes an epithelial chloride channel and that is defective in CF. To get *CFTR*-knockout ferrets, researchers have designed a transfection protocol based on recombinant adeno-associated virus-mediated gene targeting in fibroblasts, followed by Somatic Cell Nuclear Transfer (SCNT). After obtaining litters of *CFTR-/-* ferrets, the histopathological progression of the disease has been studied -with and without treatments- in gut, lungs, pancreas, liver and vas deferens and a comparison with the human disease has been established. In addition, a gut-corrected transgenic *CFTR*-knockout ferret model has been developed, achieving the *CFTR* gene expression exclusively in the intestine and not in other organs. In pancreas, lungs and vas deferens tissues, pathogenesis is fairly similar to the human one. Nevertheless, intestinal alterations and malnutrition are more severe in ferrets. To counteract that, a treatment based on Golytely, several antibiotics, UDCA, Omeprazole and pancreatic enzymes has reported great results as it enabled *CFTR*-knockout ferrets to live longer. In short, ferrets are a greatly useful animal model for the study of CF and they can make a valuable contribution to the future research of this disease, of which there are still plenty of unclear issues.

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P25

Evaluation of genistein-induced bone and cartilage malformations in zebrafish larvae.

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Keywords: Alcian blue; Alizarin red; zebrafish; genistein; cartilage; bone.

Genistein, an isoflavone from soy origin is present in many hormone substitution pills used for menopausal symptoms relief. It can be found in wastewaters and may act as an endocrine disruptor in non-target aquatic organisms. The disfunction of endocrine systems can result in adverse outcomes, including at early life stages alterations in growth of skeletal structures. Aiming to identify possible alterations on bone and cartilage development, zebrafish embryos were exposed to three genistein concentrations until 6 days postfertilization (dpf), after which they were fixed in formaldehyde and stained with Alizarin red and Alcian blue stain, bleached, cleared and visualized in a glycerol solution.
Morphometric parameters were used to assess genistein effects on cranial bone and cartilage development. Zebrafish larvae exposed to 20ng/L presented, compared to control, increased ceratohyal cartilage and palatoquadrate hyosymplectic cartilages (PC) lengths, and cranial distance between the most anterior Meckel and the lateral fins and between the ceratohyal cartilages joint and lateral fins. Additionally, zebrafish larvae exposed to 200ng/L of genistein, showed PC length, Cranial distance and CH distance decreases compared to control, while the Meckel length increased. The angle parameters evaluated presented no significant changes.
The current work extends the knowledge on toxicological profile of genistein during zebrafish development. It is concluded that genistein causes stage-exposure dependent developmental anomalies being more pronounced when low concentrations of genistein are used at early stages of development. Furthermore, this work shows that this endocrine disruptor may induce potential non-wanted effects in zebrafish studies.

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P26

The effect of ozone on the fight against multi-resistant bacteria.

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Keywords: antimicrobial resistance, antibiotics, ozone.

Antimicrobial resistance is a major human and animal health problem as it threatens the effective prevention and treatment of a growing range of infections caused by various bacteria. This is because most bacteria have developed resistance to antibiotics, so new strategies and therapies need to be implemented. Ozone may be a good alternative to antibiotics since it has a non-specific action, and it has been shown to be active against all microbiological forms. Thus, our aim was to investigate the effect of different concentrations of ozone on the growth of different multidrug-resistant bacterial strains. Six bacterial strains (*Listeria monocytogenes, Eschericia coli, Klebsiella pneumoniae, Enterococcus faecium, Enterococcus faecalis* and *Bacillus cereus*) were seeded onto BHI agar plates and incubated at 37º C for 24h. Then, each strain was diluted in Mueller-Hinton broth to a final concentration of 0.5 of McFarland scale and 200 µL was added to wells of 4 microplates. Each microplate was placed inside an appropriate bag and an ozone gas stream was introduced into the bag. Three difference concentrations of ozone were used in each bag. Then, the microplates were incubated at 37ºC for 24h. Then, the absorbance of each microplate was read at 600 nm. Ozone at minimum concentration did not have any effect on bacterial growth. However, at the maximum concentration, ozone inhibited the growth of all bacteria with the exception of B. cereus. Our results show a promising use of ozone to treat infections caused by these bacteria.

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P27

Detection of fecal contamination in ornamental animal feed.

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Keywords: Fecal contamination, ornamental animals, feed, *Enterococcus faecalis*, *E. coli*.

The presence of fecal microbial contamination on animal feeds can constitute an indicator of health risk for animals exposed to this feed. For this reason, the aim of this study was to microbial quality survey, detecting the presence of *Enterococcus feacalis* and *Escherichia coli* as indicators of fecal contamination, on ornamental animal feeds. Fifty-seven feed samples of birds, reptiles, fish and mammals were collected from pet shops during 2020-2021. Each sample was pre-incubated in 5 mL of BHI at 37°C for 12 hours. Then, 10 µL of BHI solution were subcultured on Slanetz-Bartley and EMB Levine agar plates, specific media to each bacterium respectively, and incubated at 37°C for 24 hours. The identification of isolates was confirmed by standard biochemical tests. From analyzed feed samples, twenty-eight samples were positive for the presence of *E. faecalis*, in which fifteen were from bird feed, four from reptiles and nine from the fish feed. Additionally, *E. coli* was detected in one bird sample negative to *E. faecalis*. These results demonstrated the existents of fecal contamination on feed consumed by ornamental animals, in particular *E. faecalis*. Future studies are necessary to evaluate the possible impacts for animal and public health, due to the ingestion of these microorganisms by them and their close contact with humans.

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P28

Diversity of Aeromonas species isolated from surface waters: Occurrence of antibiotic resistance to β-lactamase.

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Keywords: *Aeromonas spp.*; Antimicrobial resistance; Anthropogenic pressures; One health concept.

The extensive use of water and anthropogenic activities associated with the inappropriate use of antibiotics and their overuse throughout history are one of the causes for the high incidence of antimicrobial-resistant genes (ARGs) and bacteria (ARBs) isolated from aquatic ecosystems. According to the World Health Organization (WHO) ARGs and ARBs is considered as a major public health concern. *Aeromonas* spp. are ubiquitous bacteria, primarily recovered from aquatic ecosystems. They have been isolated from wastewater, natural water such as rivers, lakes and estuaries, aquacultures, urban drinking water, and in association with numerous autochthonous aquatic organisms in these environments. This study aimed to evaluate antimicrobial resistance among riverine *Aeromonas* spp., taken as representative of the autochthonous microbiota, to assess the level of antibacterial resistance in water, and the potential risk that it represents. Water samples were collected from the hydrographic basins of Tua river, Portugal. Samples were filtered through a cellulose nitrate pore membrane filter. The filters were incubated at 37°C for 24 h in Glutamate Starch Phenol red (GSP) agar. *Aeromonas* isolates were identified by API 20 NE. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method. A broad range of antibiotics covering the β-lactams family were used to determine the resistance of isolates. The results indicate a greater incidence of multiple antibiotic resistance *Aeromonas* isolates, as follows: AML-amoxicillin (93.33%); AMC-amoxicillin/clavulanic acid (73.33%); TIC-ticarcillin (83.33%); TIM-ticarcillin/clavulanic acid (56.67%); PRL-piperacillin (40.00%); TZP-piperacillin/tazobactam (40.00%); ATM-aztreonam (33.33%); IPM-imipenem (43.33%); KF-cephalothin (70.00%); CTX-cefotaxime (40.00%). The high aeromonads β-lactamases resistance suggest that this species can be used as bioindicator organisms for monitoring ARGs in rivers, and should be considered in a “One Health—One World” concept.

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P29

Exploring a link between tumor-associated macrophages and Cox-2 in feline injection site sarcomas.

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Keywords: injection site sarcoma, feline, Cox-2, TAMs, macrophages.

Feline injection site sarcomas (FISS) are malignant tumours that occur in anatomical regions where drugs or vaccines have been previously administered. FISS have high degree of recurrence, and in spite of the existence of several treatments, the rate of therapeutic success is relatively low. The development of therapeutic targeting of inflammatory reactions which promote cancer depends primarily on the definition of the underlying cellular and molecular mechanisms in the tumors. Thus, this work aims to study the possible association between tumour-associated macrophages (TAMS) and the presence of Cox-2 in the feline injection site sarcomas. TAMS and Cox-2 immunoexpression were determined in 58 tumors (classified into three histological grades of malignancy) by the streptavidin-biotin-peroxidase complex methodology. TAMs were categorized in three classes: scarce; moderate, and high. Cox-2 was assessed using a semiquantitative method; the tumour was considered positive when more than 10% of the cells expressed Cox-2. Tumor-associated macrophages were scarce in 29 tumors; moderate in 18 tumors and 11 cases it was considered high. TAMs density was significantly associated with histological grade of malignancy ($p<0,001$). Regarding Cox-2 immunoexpression, 48 (72%) tumors were positive and 28% were negative.The positivity of Cox-2 has been associated with the histological grade of malignacy, being present mainly in tumors with high histological grade.

Comparing TAMS and Cox-2 expression in tumoral cells, they were significantly associated ($p=0,03$). Cox-2 positive tumors had more TAMS than Cox-2 negative tumors. This study demonstrated a close relationship between macrophages and Cox-2 expression in feline injection site sarcomas. This crosstalk is reported in various tumors in humans and animals. By inhibiting the release of prostaglandins from the tumor and by blocking COX activity in immune effector cells, non steroidal inflammatory drugs may also bias the function of immune cells on the way to a more tumoricidal phenotype.

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Covid-19: What led us to lockdown.

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Keywords: Covid-19; Portugal; positive cases; RT-PCR; lockdown.

Since the first outbreak of coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), in the end of 2019, a devastating pandemic has been affecting countries all over the world. Portugal is not an exception and, so far, over than 800 thousand positive cases and more than 16 thousand deaths were confirmed. At UTAD COVID-19 testing center (UTAD Task Force), the molecular detection of SARS-CoV-2 by reverse transcriptase real time PCR (RT-qPCR) is performed on RNA extracts to diagnose COVID-19 from naso/oropharyngeal swab samples from suspicious infection cases. Here, we present the data from a five months testing period, from October 2020 to February 2021, related to patients from four Northern regions: Baixo Tâmega (BTAM), Alto Tâmega e Barroso (ATB), Marão e Douro Norte (MDN) and Tâmega II e Vale do Sousa (TIIVS). The results were in accordance with the pattern observed in Portugal during the same time period, with a worrisome scenario from November to January and a month of February marked by a sharp decline in terms of infected individuals, meaning that the lockdown and other contingency procedures decreed by the government, such as mandatory work from home, were really necessary to limit the spread of the virus and to reduce the number of infections. Furthermore, during the five months period, for the regions considered, the number confirmed of positive cases was higher within female gender, in individuals with ages ranging from 40 to 69 years old and, finally, in individuals from TIIVS region. However, we believe that in order to mitigate further lockdowns, wide testing should be implemented to block possible COVID-19 transmission chains.

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Physiological behavioural and genotoxical effects of psychoactive drugs in aquatic organisms using daphnia as model.

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Keywords: Chiral psychoactive drugs, ecotoxicology, enantioselectivity, pharmaceuticals, daphnia.

Pharmaceuticals are garnering considerable attention as emerging threats to the aquatic environment. In fact, after consumption pharmaceuticals and their active metabolites are excreted by urine and faeces and can contaminate various environmental compartments, including the aquatic systems through direct illegal discharges or sewage system. Among them, psychoactive substances (PAS) contamination are of particular concern as they have the potential to affect physiological functions and ecological behaviours in exposed wildlife critical to reproduction and survival even at low concentrations. For instance, altered phototactic behaviour and reproduction was observed in daphnia exposed at low environmental levels of antidepressants and changes in the swimming activity and aggression alternation was found in fish exposed to sertraline. Even though, the magnitude of the impact is still poorly understood. Besides, the considerable part of PAS are chiral and can occur as enantiomeric mixtures or pure enantiomers depending on their synthesis, metabolism, or biodegradation. Despite the well-established role of enantioselectivity on pharmacokinetics, and pharmacodynamics in biological processes, stereochemistry is generally ignored in environmental studies and there are only a few studies regarding the ecotoxicological effects of chiral PAS. Therefore, enantioselective ecotoxicological studies are crucial for a correct environmental risk assessment and further mitigation measures. Among freshwater organisms, daphnids are ecological relevant organisms of the trophic chain that colonize aquatic habitats. Among daphnids related species, Daphnia magna is a key species to study aquatic environmental toxicity and is a recommended model species for toxicological assessment of chemicals in water and sediment samples due to its high sensitivity to environmental contaminants.

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Serum Protein Cellulose acetate Electrophoresis in hedgehogs (Erinaceus europaeus) a preliminary study.

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Keywords: Hedgehog, Serum protein, Electrophoresis.

The European Hedgehog (*Erinaceus europaeus*) is a widespread wild insectivorous mammal. The Hedgehogs are now found roaming urban areas being more exposed to risks such as environmental pollution, road traffic accidents, and domestic animals' attacks, which predispose them to respiratory and neoplastic diseases. It can be challenging for practicing veterinarians to assess the health status, and to diagnose or monitor specific diseases of rescued hedgehogs, and also to assess whether previously hospitalized animals can be safely released. These problems are in part due to the lack of information about hematologic and biochemical data of normal healthy hedgehogs. The health of each individual is of prime importance and laboratory blood testing, as well as evaluation of their physical condition, is important in their health assessment. Protein concentrations in the blood can be altered by malnutrition and dehydration as well as by disease. Serum protein electrophoresis is a useful tool in the diagnosis and monitoring of a number of diseases. In this study, we evaluated cellulose acetate serum protein electrophoresis on samples from 12 adult hedgehogs. Serum electrophoresis on all samples successfully separated proteins into albumin, α1, α2, β1, β2 and ɣ globulin fractions as in other mammals. Electrophoretic patterns were comparable in all hedgehogs. The results of this preliminary study provide the first data on serum electrophoretic patterns in hedgehogs and may be a useful diagnostic tool in the health assessment of this species in rehabilitation centers.

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Comparison of two tests to detect and quantify HCV RNA: COBAS® AmpliPrep/ COBAS® TaqMan® HCV v.2.0 test vs. Xpert® HCV VL test.

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Keywords: Hepatitis C virus, Viral Load, RT-PCR.

Hepatitis C virus infection (HCV) is a public and individual health problem and affects millions of people worldwide.

At Centro Hospitalar de Trás-os-Montes e Alto Douro, EPE, the current test to detect and quantify HCV RNA is the COBAS® AmpliPrep/ COBAS® TaqMan HCV v.2.0 test (CA/CT), based on real time PCR method. Despite this test present higher sensibility and specificity, is an expensive and long time-consuming test. More recently emerged the Xpert® HCV VL test that present individual cartridges, which allows overcoming the actual problems related with achieve a minimal number of samples described to the COBAS® AmpliPrep/ COBAS® TaqMan HCV v.2.0 test. Furthermore, this test automates the entire test process in one step with more simplicity and facility than COBAS® AmpliPrep/ COBAS® TaqMan HCV v.2.0 test.

This study aimed to evaluate the performance of Xpert ® HCV VL test.

The Xpert® HCV VL test, agreement was found with the CA/CT test in 84% of the samples, of wich 23 were negative and 19 were positive. Considering CA/CT test as gold standard, the sensitivity, specificity, predictive positive value (PPV) and negative predictive value (NPV) of Xpert® HCV VL was found to be 100%, 74.2%, 70.4% and 100%, respectively. The results of both tests showed a strong correlation for the 16 quantifiable samples, $r=0.962$; $p<.001$.

The short run time, random access and the sample loading simplicity of Xpert® HCV VL test suggest that it may play an important role in HCV infection diagnosis and to monitor HCV Viral load. In addition, the performance characteristics must be the best so that the Xpert® HCV VL can replace the current test. In this study, the Xpert® HCV VL test does not have adequate specificity for its validation in determining HCV.

P34

Antigen determination as alternative of HCV RNA quantification- Correlation study in the determination of HCV viremia in CHTMAD patients.

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Keywords: Hepatitis C virus, HCV core Antigen, Viral Load.

Hepatitis C virus infection (HCV) is a global health problem and can result in cirrhosis and end stage liver disease. Currently, HCV diagnosis is based in anti-HCV antibodies detection and HCV RNA detection and quantification by real time PCR, a two-step long consuming process and with a higher associated cost. Antigen core HCV quantification test was developed with the purpose of overcoming these limitations and is presented as a more stable and accessible alternative to the HCV RNA detection and quantification tests.

This study aimed to evaluate the performance of ARCHITECT® HCV Ag test, Abbott Laboratories (HCV-Ag).

At the Centro Hospitalar de Trás-os-Montes e Alto Douro, EPE, 145 samples were analyzed by the COBAS® AmpliPrep/COBAS® TaqMan® HCV v.2.0 test (CA/CT) and by the ARCHITECT® HCV Ag test. Of total samples, 106 samples were from treatment patients. This samples were categorized in three groups: Treatment (T), end of treatment (FT) and patients who achieved a sustained virologic response (RVS).

The HCV-Ag test was presented 88.9% of sensibility and 99.1% of specificity. The correlation analysis was presented a good correlation between the results of both tests ($r=0.890$; $p<.001$). In the evaluation of the HCV-Ag test for different treatment groups was possible to verify a good agreement in the treatment group ($k=0.789$; $p<.001$) and in the end of treatment group ($k=0.638$, $p<.005$) and very good agreement in the SVR group ($k=1$; $p=.001$).

Based on these results, HCV-Ag test can simplify the current diagnosis process and monitor the HCV infection during treatment.

P35

Effect of Bap Concentration on Coffea Robusta L. Micropropagation.

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Keywords: Auxin, benzylaminopurine, *Coffea Canephora*, *in vitro* culture.

The cultivation of plant tissues is a technique that allows the cultivation of plant cells, tissues or organs of the mother plant, in a nutritious medium with optimal conditions of light and temperature. The objective of plant propagation by tissue culture, termed micropropagation, is to obtain a high number of plants similar to plant mother, named clones.

Coffea Canephora (Coffea Robusta) belongs to a genus of the Rubiaceae family, it is a coffee species originally from West Africa, easy to plant when compared to other coffee species, such as Coffea Arabica. The conventional technique of vegetative propagation of coffee requires a lot of time and is expensive: it is in this context that micropropagation of coffee is very advantageous for the production of this plants according to market demands.

This work aimed to observe the effects of plant growth regulators, namely an auxin (IAA) and a cytokinin (BAP), on the *in vitro* growth of Coffea Robusta and its repercussions on micropropagation processes. For this purpose, five base formulations were used in the culture media and the effects of different combinations and concentrations of growth regulators on the response of explants (segments of seeds containing structures of the meristematic type) originating from seedlings obtained and maintained in vitro were verified. In this work, we use the following culture media, for a rapid proliferation and growth of the shoots: MS 0; MS 2 or 4 mg/L BAP; MS 2 mg/L BAP + 0.75 mg/L IAA; MS 4 mg/L BAP + 0.75 mg/L IAA. The results revealed a higher number of leaves and length of shoots in culture medium supplemented with 2 mg / L BAP.

P36

Immunocytochemistry application on cell blocks.

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Keywords: Immunocytochemistry, cell blocks, cytopathology.

The immunocytochemistry (ICC) is a methodology that uses immunoassays to co-locate an epitope of interest in cytological swaps, cytocentrifuged preparations or monolayer preparations. This technique has several applications in cytopathology, such as identification of neoplasms and it is considered an important tool to help in cytological diagnosis. However, it still has some practical limitations.

The technique has been studied in last years but, although it seems to have improved along the time, the results haven not reached yet the desired levels of success. From 1980 to the present day, it was possible to prove that immunocytochemistry was severely conditioned, meaning the interpretation of results is not always easy, obtaining false positives. On the other hand, a useful application of the immunocytochemistry technique has been progressing in time.

Immunocytochemistry has several applications, including cytoblocks, being this characterized as an important complement in the diagnostic process. The use of this technique aims to include cellular/ histological material in paraffin blocks, in order to obtain cytoblocks. This technique is based on diluting the material in 10% Formol, transfer for a rub and centrifuge. The material obtained is then transferred to absorbent paper, placed in a histological cassette, and fixed in a 10% Formol solution, for a few hours/ days. After the procedure, the material was processed, following the steps of dehydration (in growing chains of ethanol), diaphanization (in xylol), impregnation and inclusion in paraffin, microtomy and staining using the Hematoxylin-Eosin (HE) technique. Finally, the slides stained in HE are analyzed under light microscopy.

The application of ICC in cell block is an important complement in the diagnostic process, since it allows the accurate identifications of cellular populations in cavity effusions, allowing a clear differentiation between epithelial, lymphoid, or mesothelial populations.

P37

Evaluation of the expression of biomarkers in individuals with cognitive impairment and its relationship with physical exercise.

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Keywords: cognitive impairment, BDNF expression, exercise, antioxidant system.

Mild cognitive impairment (DCL) is an intermediate state between cognitive changes resulting from, for example, normal cognitive aging and dementia. Individuals with mild cognitive impairment are a high-risk group because they develop dementia at a rate of 10% to 15% per year compared to 1% to 2% per year in the general population. Therefore, it is essential to identify potential protective factors against mild cognitive impairment.
Several studies have shown that physical exercise can decrease the risk of coronary artery disease or type II diabetes and protect against dementia and Alzheimer's disease.
Diagnosis is performed through cognitive tests to assess DCL and later confirmed by quantifying the expression of the Brain-Derived Neurotrophic Factor (BDNF) present in the serum. It is also evaluated the alteration of the expression of some enzymes of the antioxidant system (superoxide dismutase and catalase). Possible changes in the antioxidant capacity in patients with cognitive impairment determine the role of physical exercise in this deficiency's evolution.

P38

Deepening into maize cell wall composition upon Fusarium verticillioides infection: a tool for breeding programs.

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Keywords: Biotic stress, *Fusarium verticillioides*, plant defence, *Zea mays L.*.

Maize (*Zea mays L.*) is one of the most important, essential and widespread crops in the world, providing multiple products used for several purposes such as human consumption, animal feeding, or feedstock for second generation biofuels. However, its biomass production may be reduced due to the infection of some pathogens. Maize crop is highly susceptible to *Fusarium verticillioides* fungus, which causes ear rot as well as contamination of the grain with mycotoxins. Plants are able to modify their cell wall (CW) composition to avoid or overcome fungus infection, and these processes play a key role in plant defence mechanisms. From this point of view, it could be interesting to study a putative relationship among CW resistance and CW modifications caused by *F. verticillioides* infection. For this reason, in this study, changes in CW composition upon *F. verticillioides* infection of two maize varieties differing in stalk strength were analysed. This CW characterization was carried out: (1) in a general way by means of Fourier Transform Infrared Spectroscopy; and (2) in a specific way, by analysing the lignocellulosic and non-cellulosic composition, as well as the phenolic profile. In addition, a cell wall enzymatic degradability assay was carried out. Our results point out that differences in stalk strength are not necessarily associated with the particular changes that occur in the CW upon the *F. verticillioides* infection..

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P39

Evaluation of biofilm formation by Staphylococcus pseudintermedius isolated of canine pyoderma.

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Keywords: *Staphylococcus pseudintermedius*, biofilm, Congo Red Agar.

Staphylococcus pseudintermedius is an opportunistic pathogen and the principal agent in the origin of canine pyoderma. This specie is being considering an important biofilm producer, which complicate the treatment of the infection and can further lead to the increase of antibiotic resistance. In this study we aimed to evaluate the biofilm formation of sixty isolates using two methods. Bacterial suspensions of *S. pseudintermedius* was added to the wells of microplates with TSB broth supplemented with NaCl. The plates were incubated at 37ºC for 24h and 48h. For the quantification of biofilm biomass, the Crystal Violet Coloration method was used. The biofilm formation capacity was also evaluated by the Congo Red Agar method. In the first method, after 24 hours, all strains showed ability to form biofilms. The average biofilm biomass of the sixty strains after 24h of incubation was 26,6% which increased to 70,7% after 48 hours. In the Congo Red Agar method only two strains showed a high capacity to form biofilm. Eight strains had a medium capacity, twenty strains had a weak capacity, and thirty strains had no capacity to form biofilm. These two methods showed significant differences in the results. In the first method all strains had the ability to form biofilm, which will complicated the eradication of the pathogen in case of infection. In the Red Congo Agar method most strains did not form biofilm, that is why this method is currently being considering non reliable, because other methods, like genotypic methods, usually prove the opposite.

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P40

Detection and identification of virulence genes of Staphylococcus aureus in a slaughterhouse.

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Keywords: *Staphylococcus aureus*, food safety, meat

Staphylococcus aureus is one of the major pathogens found in meat for human consumption, being the cause of several diseases such as gastroenteritis, thus risking human health and food safety. Its virulence depends on the present of genes like *nuc*, genes encoding enterotoxins, *hla, tsst*, etc. Due to its pathogenicity it is necessary to detect and identify this pathogenic bacterium and identify their virulence genes. So, the aim of this study was to identify the specie *S. aureus* in a set of 16 samples collected from a slaughterhouse as well as to identify the virulence genes and study the prevalence of them in *Staphylococcus aureus*. PCR technique with specific primers followed by agarose gel electrophoresis was the method applied to detect the presence of *S. aureus* in collected samples which was confirmed in all samples by the detection of *nuc* gene. Multiplex PCR allowed the identification of genes encoding enterotoxins (sed, seg, sei) while a single PCR reaction allowed the identification of *hla* and *tsst* genes. Regarding to genes encoding enterotoxins, *sed* gene wasn't found in any samples, *seg* gene was present in 62.5% of samples (10/16) and *sei* gene had a prevalence of 50% (8/16). Concerning to *hla* gene, 75% of samples had this gene (12/16) while *tsst* gene was found in 56.3% of samples (9/16). Therefore, the techniques used in this study allowed to identify the presence of *S. aureus* in our samples and also to analyse their virulence profile by detection of virulence genes.

P41

Enhancer RNAs in Cancer: Regulation, Mechanisms and Therapeutic Potential.

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Keywords: eRNA, enhancer, lncRNAs, cancer, teraphy.

Enhancers have distal cis elements that are part of the genome, with the aim of regulating gene expression, being important in the control of cellular processes, such as proliferation, differentiation and disease progression. Several varieties of human cancers are associated with genetic and epigenetic changes in enhancers. The robust activity of this element is correlated with a greater abundance of RNA polymerase II and enhancer RNAs (eRNAs).

As regulatory elements, system intensifiers play a key role in controlling the expression of target genes, by forming chromatin loops, in order to communicate with the target promoters. After highlighting the function, it was possible to establish a boosting relationship, on the part of the enhancers, with human diseases, including cancer.

The eRNAs have about 500 to 2000 bp and are originated by bidirectional transcription of both strands of DNA. Currently, it is possible to state that eRNAs have an extremely important role in the transcriptional regulation of genes, in various types of human cancer. Based on this premise, studies referring to genetic therapy were made and have proven that some eRNAs may be therapeutic targets in cancer.

The existence of an association between the activation of enhancers and the transcription of eRNAs interferes with the effect they have on each other. It is that eRNAs can promote the formation of a loop between enhancer-promoter. As enhancers, eRNAs have also been studied in relation to their role in carcinogenesis, these studies demonstrate that their functional role, as well as the underlying mechanisms, are not yet fully understood. Complementary studies, especially in vivo, may, in the near future, allow the use of therapy directed to eRNAs, in the intervention of human cancer.

P42

Comet Assay for genotoxicity assessment of psychoactive substances using zebrafish embryos.

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Keywords: psychoactive substances; zebrafish embryo; comet assay; genotoxicity; pollution.

Psychoactive substances (PAS) are increasing as environmental aquatic pollutants. Unchanged drugs and their metabolites reach the environment mainly through wastewater effluents since conventional wastewater treatment plants are not designed to completely remove PAS. The increasing use and abuse of these drugs contribute to their ecotoxicological concern because the impacts on non-target organisms are scarce. Thus, additional investigation is necessary to evaluate ecotoxicological harmful effects of PAS in non-target organisms, like ichthyofauna. Besides, zebrafish (*Danio rerio*) embryo is a very promising model to assess the toxicity of low levels of PAS at different biomarkers, like biochemical, physiological, behavioural, and also genetic damage.
Biological material of the embryos can thus be used to assess genetic damage through the *Single-Cell Gel Electrophoresis* or comet assay. This sensitive and fast technique is a useful tool in geno-ecotoxicological studies to quantify DNA strand breakage, an early biomarker of long-term effects such as mutagenesis and carcinogenesis. After performing the technique, fluorescent "tails" of damaged DNA are observed coupled to an undamaged DNA nucleoid, and the larger the tail (with more DNA) the greater the genetic damage. The use of this technique in zebrafish embryos is important as it allows the assessment of genetic damage following ethical and animal-welfare policies. The data obtained can be important to better understand genotoxic damage (in vivo) under low dose exposures and due to oxidative stress. Translational information can be useful as zebrafish have orthologous genes with humans and hold almost all genes involved in different repair pathways. On the other hand, the wild fish are exposed to complex mixtures of pollutants that need to be studied deeper (cumulative and interactions) for DNA damage.

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P43

Protein profiling and allergenic potential of different almond varieties.

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Keywords: food allergy, almond, almond allergens, nutrition.

Tree nuts are widely considered an important food in a healthy diet however they are one of the most common sources of food allergens. Several almond proteins have been recognized as allergens. Six of them, namely Pru du 3, Pru du 4, Pru du 5, Pru du 6, Pru du 8 and Pru du 10, have been included in WHO-IUIS Allergen Nomenclature Sub-Committee list of allergens. Nevertheless, further studies are needed in relation to the accurate characterization of almond allergens, including their IgE-binding properties. In this work, the protein profile of 27 almond varieties was obtained using 1D-eletrophoresis. The absence/presence of specific proteins was used to establish sample relationships trough dendrograms. Furthermore, the allergenic potential of these varieties was assessed using sera from 4 allergic patients as a source of IgE-antibodies. Results showed that different varieties have small differences regarding their electrophoretic profile, but there were no significant differences for IgE-binding properties. These results seem to indicate a low potential for selecting hypoallergenic almond varieties.

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P44

Cherry cracking: effects of the application of calcium and biostimulants on gene expression.

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Keywords: cherry, calcium, seaweed, expression, genes, cracking.

The species *Prunus avium* L., c.v Burlat, commonly known as cherry tree is originated from Western Asia. The cherry is a fruit that is highly sought by consumers, not only because of its juiciness and flavour, but also due to its nutritional contribution to everyday diet. Occasionally, cherries can be affected by cracks on their surface which makes it lose commercial value, leading to a significant lower profitability. The study was done with the objective of reducing or even avoiding these problems. As such, calcium and seaweed based biostimulant treatments were applied in pre-harvest, with a high dose and a low dose in each of the treatments applied. Cracked and non-cracked cherries were used in order to determine whether the compounds applied in the orchard interfere with the expression of the genes involved in cherry cracking. In terms of methodology, we extracted RNA from cherries exocarp at green/red stage, then the cDNA synthesis was performed and finally a semiquantitative analysis of the gene expression. The analysed genes were *Actin (housekeeping), PaXHT, PaEXP2* and *Paβ-Gal*. According to the treatments applied, actin has a constant expression.The *PaEXP2* gene had a very low expression in the control group (cracked cherries), but a higher expression in the low dose calcium treatment, which led us to assume that the fruit submitted to this treatment would have less cracks. In *PaXHT* we have a similar treatment with CDA with cracked and non-cracked, in CDB cracked and non-cracked and in ADA cracked and non-cracked leading us to believe that the gene has a better expression in this type of treatments. At *Paβ-Gal* we have lighter bands which possibly meant less expression compared to the previous genes, however there was a greater expression in the ADA treatment with cracked and non-cracked cherries.

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P45

Prevalence of methicillin-resistant Staphylococcus aureus (MRSA) isolated from healthy pigs.

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Keywords: Antibiotics, antimicrobial resistance, Staphylococcus aureus, MRSA, swine.

Antibiotics are essential to fight diseases in humans and animals, however their improper and excessive use has been decreasing their efficiency in fighting infections because bacteria manage to adapt and oppose the actions of antibiotics.

Staphylococcus aureus is a Gram-positive bacteria that is capable of adapting and surviving in a wide variety of environments, that is why, a few years after starting to use methicillin against infections caused by this bacteria, the first resistant strain called Methicillin-resistant *S. aureus* (MRSA) appeared.

In this study, we aimed to investigate the prevalence of MRSA in healthy pigs. Forty-six nasal samples were collected from healthy pigs housed in two different pig farms. The samples were seeded onto Chromagar MRSA plates and incubated at 37ºC for 24-48h. One colony with typical MRSA morphology was selected from each plate. From the 46 nasal samples from healthy pigs, 32 (69,6%) MRSA were isolated. Fourteen MRSA were isolated from one pig farm with 24 samples that were collected and 18 MRSA were isolated from 22 samples collected in the second farm. This results allow us to conclude that there is indeed a high prevalence of MRSA in pigs, this being a problem to be addressed due to the negative consequences that it entails.

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P46

Earthworms as a terrestrial model organism to study genotoxicity of chiral pharmaceuticals.

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Keywords: Genotoxicity, Earthworms, Soil, Contamination, pharmaceuticals, Chiral, *Eisenia sp.*.

Presently, due to the increasing soil contamination, it is imperative to assess the toxic and genotoxic effects caused by soil contaminants, namely pharmaceuticals, to prevent the potential risks in ecosystems.

Earthworms are organisms of great importance in terrestrial ecosystems, contributing to soil aeration, degradation of organic matter and soil formation, but they are also important agents in modulating the transfer of inorganic and organic toxicants throughout the food chains. Due to their importance as sentinel organisms to assess soil health, several international guidelines (OCDE and ISO) have been developed to promote the use of earthworms in laboratory and field tests.

Standard ecotoxicological tests, namely with the earthworm species Eisenia andrei/fetida evaluate the effect of chemicals through endpoints such as mortality, growth, reproduction and behaviour, however, the effects at the molecular and cellular level should also be evaluated since these endpoints provide relevant information about the pharmaceutical's mode of action and its potential genotoxic effects.

DNA is the target of genotoxic chemicals and there are several endpoints in genotoxicity tests to assess its damage, such as single- and double-strand breaks, point mutations, deletions, chromosomal aberrations, micronuclei formation, DNA repair and cell-cycle interactions. Methods for detecting genotoxicity include DNA strand break measurements in cells (e.g. comet assay) and cytogenetic assays (micronucleus and chromosomal aberration assays, including the use of fluorescence in situ hybridization and chromosome painting).

Therefore, due to the importance of DNA in maintaining homeostasis and transferring information to offspring, it is important to evaluate the effects of genotoxic chiral compounds on non-target organisms, such as earthworms, for an accurate environmental risk assessment.

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P47

Assessment of the genotype's effect on the micropropagation of the grapevine.

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Keywords: *Vitis vinífera*, genetic diversity, explant.

Vitis vinifera is a plant known worldwide, for its flavor and nutritional values. It is the most cultivated species for the production of wine in the world, with dozens of grape varieties, originating very different wines. The grapevine is a self-pollinating dicot that belongs to the Vitaceae family. It consists of fourteen genera, but most of the edible species belong to the genus Vitis. The different parts of the plant, leaves, buds, stems and seeds are traditionally used as a source of bioactive compounds.

This species has a high economical interest, not just for its production and exportation value, but also for its numerous health benefits, resulting of its high composition in phenolic compound's which work as natural antioxidants. The study of the genetic diversity allows to further the knowledge of this plant and of its behaviour on its mutations that originate the different cultivars.

The micropropagation of selected genotypes can contribute to mitigate the need of vine mother plants and grafts of genetical quality, aiming to improve the cultures. To assess which the best graft or mother plant to use, are considered data relating to explant growth, leaf area, biomass, chlorophyll content and survival rate. Thus, it is possible to choose the plant with the greatest capacity for adaptation and survival in adverse conditions. Comparing the responses of different genotypes for different parameters, it is found that the greater the distance between vines genotypic, more different will the responses be, in relation to the same conditions.

This work aims to show, through the analysis of several articles, the importance of the study of genotypes and evaluate its effect on the micropropagation of the vine.

P48

Development of Biomolecular Tools for Expression and Trafficking Studies of The Human Copper Transporters.

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Keywords: CTR, biomolecular tools, cancer.

Copper (Cu) is an essential metal ion with a crucial role in the biochemistry of every living organism, acting as a cofactor for several enzymes. The cellular Cu uptake occurs through two major copper transporters (CTRs), CTR1 and CTR2. The study of Cu gains more importance when considering its requirement for several cellular phenomena involved in cancer progression, namely cancer cell migration and metastasis.

With this work we intended to develop a set of biomolecular tools for expression and trafficking studies of these transporters. A group of plasmid constructions were designed and constructed following the classical DNA cloning method, harboring *CTR1* or *CTR2*, with GFP or mCherry, at the 5' or 3' ends.

We generated eight plasmids, namely, pCTR1-mCherry, pmCherry-CTR1, pCTR1-AcGFP, pAcGFP-CTR1, pCTR2-AcGFP, pAcGFP-CTR2, pCTR2-mCherry and pmCherry-CTR2. These plasmids will be used for the transfection of different cells lines, including non-proliferative and proliferative glycolysis-dependent cell lines.

The molecular tools developed in this work will be pivotal to characterize the expression and trafficking of fluorescent tagged Copper transporters under distinct metabolic conditions.

P49

Enhancing osteoblastic differentiation through the exposure of MG-63 osteoblastic cells to green-synthesized Mg(OH)2 nanoparticles using MgCl2 as a precursor.

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Keywords: Green synthesis, Mg(OH)2 nanoparticles, rosehip, MG-63 cells, osteoblasts, differentiation.

The use of nanoparticles (NPs) has many advantages to the research and medical fields, namely for bone applications. In the synthesis of NPs, the goal is to produce non-cytotoxic NPs recurring to green-synthesis enrichment with biological properties increasing biocompatibility. Magnesium is one of the most important minerals for bone health due to its abundance in bone and for being a co-factor in more than 300 enzymatic reactions, participating in the activation of alkaline phosphatase, a relevant osteoblastic marker. Osteoblasts, the bone-forming cells, are one of the most important cells for bone turnover. In line with that, this work focused on the exposure of MG-63 osteoblastic cells to green-synthesized Mg(OH)2 NPs synthesized with magnesium chloride (MgCl2) as a precursor. The synthesis was performed in the absence, Mg(OH)2 NPs or the presence of a rosehip (RH) extract, Mg(OH)2+RH. MG-63 osteoblastic cells were exposed to 3 different concentrations (1, 10, and 100 µg/mL) of both Mg(OH)2 NPs and Mg(OH)2+RH NPs for 6 days and cell behaviour was characterized for typical osteoblastic markers. Cell cultures exposed to NPs without RH were used as control for the green-synthesized NPs, cultures treated with osteogenic inducers (ascorbic acid and dexamethasone) as positive control of cell differentiation, and non-treated cell cultures as the basal condition. Results demonstrated that NPs did not negatively affect cell viability and proliferation. When analysing alkaline phosphatase activity and staining, and SPP1 (osteopontin) immunostaining an inductive effect by the Mg(OH)2+RH NPs was observed. The effect of the green-synthesized NPS was inductive when comparing with basal condition and similar to cultures supplemented with the osteogenic inducers, demonstrating an induction of the osteoblastic differentiation into a mature osteoblastic phenotype proposing more studies on the use of these NPs in bone regenerative processes.

P50

Antimicrobial activity of hop and grapevine wastes against Pseudomonas syringae pv. phaseolicola.

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Keywords: Gram-negative, Growth inhibition, Terpenes, Quitinases, Agricultural wastes.

Beans are one of the most important crops in León. However, regular infections caused by *Pseudomonas syringae pv. phaseolicola* (Php) provoke great declines in productivity. That is why the study of ways to avoid these infections is fundamentally important. Apart from the usage of chemical pesticides, in this research we furthermore focus on the antimicrobial activity against Php of two typical cultivars, *Humulus lupulus* (Hop) and *Vitis vinifera* (Grapevine). Through the cultivation of distinct bacteria with the different botanic obtained from agricultural residues which have no other use, we analyze the capacity of the growth inhibitory, obtaining that both species are effective against the upgrowth of the pathogen. At the same time, we carry out a microbial characterization of the principal microorganisms that grow in both crops studied. These results offer us an alternative through the creation of natural pesticides, that can improve previous ones, which cause greater environmental problems.

Acknowledgments: I would like to thank Alfonso Gonzalo from the Plant Physiology and Biotechnology Department of the University of León for his collaboration and advise during the writing and revision of this work.

P51

Biotechnological strategies to increase the production of artemisinin or its precursors in plants.

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Keywords: *Artemisia annua*, Artemisinic acid, COSTREL, MYB, Nicotiana tabacum, T-DNA binary system.

Malaria is one of the most devastating diseases in human history, affecting millions of people. For example, 219 million cases of malaria were estimated worldwide in 2017, most of them in developing countries. Nowadays, artemisinin-based therapies are the only effective treatment for malaria. Artemisinin is a natural compound produced by glandular trichomes of *Artemisia annua* L., an annual herb native to temperate Asia, but the synthesis in plant is not efficient enough nor economically interesting. As an alternative, it has been developed the drug semi-synthesis from its precursor artemisinic acid, which is produced in engineered yeast. Recently, two biotechnological strategies has been proposed to increase the artemisinin production in plants.

First proposal relies on *AaMYB* gene overexpression in *Artemisia annua* which enhances artemisinin and gibberellin biosynthesis pathway. Besides, this overexpression stimulates the formation of a greater number of glandular trichomes. Transgenic plants were transformed using an *Agrobacterium tumefaciens* strain which contains the *AaMYB* gene in a binary vector system.

The second strategy consists of artemisinin biosynthesis pathway expression in *Nicotiana tabacum*, a high biomass crop. These plants were developed using COSTREL technique that combines two sequential cycles of transformation-plant regeneration. First, genes related with artemisinic acid biosynthesis were introduced into the chloroplast genome. Thereafter, the transplastomic plants were transformed with nuclear genes encoding enzymes to affect flux through the artemisinin pathway.

Both strategies involve an improvement in the artemisinin production, although the most efficient is undoubtedly that carried in *Nicotiana tabacum* as it has been shown in several studies.

Acknowledgments: The authors thank Dra. María Luz Centeno Martín from the Plant Physiology Department of the Universidad de León for her collaboration during the writing and revision of this bibliographic work for the “Plant Biotechnology” subject.

P52

The effect of cannabidiol, a component of cannabis, on the $GABA_A$ receptor in the amygdala of a prenatal infection model of schizophrenia.

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Keywords: Schizophrenia, cannabidiol, GABAA receptor, amygdala, Poly I:C.

Schizophrenia is a complex brain disorder that has positive, negative and cognitive symptoms. The mechanisms underlying the negative symptoms are largely unknown and particularly resistant to current antipsychotics, which also produce an array of side effects. Thus, there is a need for a new schizophrenia treatment, without deleterious side effects. Cannabidiol (CBD) can improve cognition and some of the negative symptoms of schizophrenia; however, the mechanisms underlying these benefits are unknown. Inhibitory GABAergic signalling in the amygdala, a brain region implicated in emotion and that is disrupted in this disorder, may be involved. Therefore, this project aimed to examine alterations in GABAA receptor binding density in the amygdala of a rodent model of schizophrenia and to assess the effect of CBD treatment on this receptor. For this purpose, pregnant rats were administered either a synthetic virus (poly I:C, POLY) to stimulate maternal immune response, which mimics the effects of schizophrenia, or vehicle (saline, CONT). Offspring were treated with CBD or vehicle (VEH) during early adulthood. GABAA receptor binding density in the amygdala was determined in the post-mortem brain tissue using receptor autoradiography methods. Sections containing radioligand binding were quantified (n= 208 coronal sections, in duplicate). Statistical analyses revealed no significant alterations to GABAA receptor binding density in the amygdala in poly I:C offspring (vs CONT), and no effect of CBD treatment (vs VEH). As a conclusion it can be inferred that the GABAA receptor may not be a target of CBD or the poly I:C model of schizophrenia. Therefore, further research is needed to assess how CBD exerts its therapeutic effects.

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P53

Glowing Plants.

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Keywords: Bioluminiscence, *Arabidopsis thaliana*, *Agrobacterium tumefaciens*, glowing plants, biotechnology, light energy.

The aim of our project is the creation of bioluminescent trees using the luciferase gene, found in the genetic code of bioluminescent organisms. By introducing the information needed for codifying Luciferase, an enzyme that accelerates the reduction/oxidation of luciferin molecule through the consumption of ATP, we pretend to be capable of producing bioluminescent plants. The goal we pursue is to try to find alternative ways of producing luminal energy as an alternative pathway of traditional electrical energy for, among others, enlightening streets.

Acknowledgments: We would like to thank all the Universidad Europea of Madrid biotechnology teachers, who instructed us and managed to get us involved into this project, as well as to all the researchers who have done previous work in the field, without whom none of this would have been possible.

P54

Coeliac Disease: Diet Impact In Amylase-Trypsin Inhibitors Microbial Metabolism.

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Keywords: ATIs, coeliac disease, diet, innate immunity, macrophages, microbiota.

Gut microbiota, whose composition can be modified by diet, has been reported as a remarkable environmental factor in development of coeliac disease pathogenesis, due to its capacity of degrade partial-digested gluten products, influencing its immunogenicity. One of these products are amylase-trypsin inhibitors (ATIs), which could tightly stimulate innate immune response mediated by macrophages, through KC inflammatory cytokine. On this basis, the main objective was to evaluate if diet induced changes in gut microbiota, thus affecting ATIs metabolism and immunogenicity on macrophages. Impact of polyamines, one of the main metabolites produced by microbiota and present in food, on macrophages was also analysed. In order to do that, bone marrow-derived macrophages were stimulated with intestinal washes incubated with ATIs, obtained from mice which had been in different dietary treatments and KC production levels were measured. Furthermore, macrophages were incubated during its differentiation process with polyamines and phenotypic changes were analysed by qPCR. Results, showed polyamine incubation confer anti-inflammatory phenotype to macrophages once differenced, and demonstrated the influence of diet in microbiota metabolism and ATIs immunogenicity suggesting an important role of diet in development of coeliac disease.

Acknowledgments: Professors Nicolás Navasa Mayo, Honorina Martínez Blanco and Luis Getino Alonso from Bioquímica Y Biología Molecular department of Universidad de León.

P55

Minigene Assays for the Functional Re-Classification of Splicing-Affecting Missense Variants in Acute Myeloid Leukemia.

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Keywords: AML, splicing, minigene, predictor.

Alterations of the splicing machinery have been extensively studied due to the importance of the targets they affect and their implication in the pathology of Acute Myeloid Leukemia. Nevertheless, the impact that any point mutation can have on the splicing process of a specific gene remains virtually unexplored. Because of this, genetic analysis has mainly been focused on the assessment of pathogenicity based on the effect of a variant onto the protein. In 2016, Papaemmanuil and collaborators performed the largest genomic study on AML published hitherto (1,540 patients) (N Engl J Med 2016;375(9):900-1). However, the effect of the identified variants on splicing was neglected. Therefore, the purpose of this study is the evaluation, through in-silico and in-vitro analysis, of the possible effect that 174 unique point mutations selected among the 5,234 variants identified in this cohort can have over the splicing of the genes they affect. The in silico analysis was carried out using different predictors in order to verify whether these can have an aberrant effect on splicing: i) Human Splicing Finder (CV > 65; Difference CV between WT and mutated sequence >15%) ii) regSNP-intron / regSNP-splicing (Prob> 0.5) and, iii) SpliceAI SpliceAI> 0.2). After the evaluation, it was determined that 14% (n = 22) could be reclassified as "probably pathogenic" due to their putative effect on splicing, with at least 2/3 positive predictors (means for HSF: 80.8, regSNP: 0.57, SpliceAI: 0.45). For the functional validation of these variants, minigene assays were used, with 13 showing no effect on splicing and two variants, in the TET2 and EZH2 genes, altering splicing, causing the skipping of an exon. Thus, we demonstrate that for the accomplishment of an exhaustive molecular study in AML, the effect of a variant on splicing should be considered as complementary to that on the protein.

P56

Aliivibrio fischeri & Eupymna scolopes: A Brilliant Symbiosis.

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Keywords: colonization, tissue maturation, diel rhythm, bacteria, squid, symbiont.

The most common way in which bacteria live with animal cells is by colonization of their epithelial tissues. For this reason, it is important to learn the general rules behind this type of symbiosis. *Euprymna scolopes* is an experimental model for the chronic colonization of epithelial tissues by Gram-negative bacteria, like *Aliivibrio fischeri*. Its study has enabled to understand the mechanisms underlying symbiosis, showing that the specificity of this process can involve both biomechanical and biochemical factors. Moreover, it is clear that symbionts drive host-tissue maturation and that diel rhythms of symbiosis, which involves big changes in gene expression and host-cell ultrastructure, create stability.

Acknowledgments: I would like to thank Dr. Antonio José Laborda Navia, Dean of the Faculty of Biology and Environmental Sciences of the University of León and Professor of the Zoology Department, for his collaboration and supervision during the writing of this work.

P57

Targeting MEK: attempting to overcome cancer resistance to RAS-MAPK therapies.

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Keywords: c RAS-MAPK, MEK, resistance, cancer, CRC, NSCLC.

MAP kinases pathway is a well-known signal transduction pathway activated by several mitogens, resulting in cell proliferation, growth and migration. Nevertheless, mutations in its kinases upregulate this pathway, leading to development of cancer. Among other strategies, MEK (a kinase belonging to MAP kinases pathway) has been identified as a therapeutic target in some types of cancer, including colorectal and lung cancers. Mostly, MEK inhibitors are able to overcome the resistance that tumor cells have acquired to other drugs, but novel strategies look for combining MEK inhibitors with other blockers, obtaining promising results. In this review, we will discuss the current methods of combating cancer resistance, focusing in the central role of MEK.

Acknowledgments: This review has been endorsed by professor Sonia Sánchez Campos, IBIOMED vice principal.

P58

Eosinophils: a new histopathological aggressiveness indicator for canine squamous cell carcinoma?

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Keywords: *squamous, carcinoma, canine, eosinophilia, TATE, eosinophils.*

Squamous cell carcinoma (SCC) is a malignant neoplasm of epithelial cells of humans and animals. In dogs, is the second most common tumour that affect the skin and oral cavity. In humans, tumour associated tissue eosinophilia (TATE) is believed to play an important role in its biological behaviour. However, as far as we know, there are no publications about tumour associated tissue eosinophilia in canine SCCs. So, the objective of this study is to analyse TATE in canine SCC and determine whether there is an association with histopathological characteristics associated with the biological aggressiveness of the tumours.

Congo red coloration was used to evaluated TATE in 49 canine SCC biopsies, 22 of them were cutaneous and 27 oral. TATEs were quantitatively assessed as scarce, moderate, and intense. The eosinophils obtained in SCC stained Congo red were statistically compared with histopathological features.

Our results reveal that the eosinophil infiltration was seen in all the cases, being intense in 20 cases (40,8%), scarce in 16 (32,7%) and moderate in 13 (26,5%) tumours. No statistically significant associations were observed between infiltrate by TATE and ulceration, necrosis and tumour location. But were significant when compare TATE in tumours with and without emboli and in tumours of different histological grades ($p < 0,0001$). Thus, SCCs with embolus and high histological grade generally present few eosinophils.

Through the results obtained, our study revealed that eosinophils are correlated with the aggressiveness in cutaneous and oral SCC and could potentially be used as prognostic factors in this type of cancer.

WORKSHOPS

Bioinformatics and applications to Life Sciences.

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Keywords: Bioinformatics.

Nowadays large amounts of biological data are generated in all sectors of society. As a result of this indeterminable quantities of data, there is a high demand for professionals with multidisciplinary knowledge and skills in areas of data analysis, program writing, knowledge in artificial intelligence, biology and genetics all together, that is demand for professionals in the field of Bioinformatics. This area has great employability both in technology-based companies based on biotechnology and bioinformatics, and in investigation/jobs oriented towards to Industry, Research and Health Services, Genetics and Biotechnology, Environment and Pharmacy.

At UTAD, the Master in Bioinformatics and Applications to Life Sciences had its 1st edition in 2018/2019 with a plan adjusted to the qualification of candidates and with 4 branches in the 2nd year 1st semester. Last editions Applied Computing was open.

In the field of Applied Computing, students may have the opportunity to create their own computer tools and data analysis applications, algorithms and applications based on knowledge of the main Top programming languages, among others C, C ++, R, Matlab, Java, Python, and in the field of Biostatistics based on the R language using Bioconductor packages. In the field of Omics taking Bioinformatics for an analysis of sequences, alignments, annotation of genomes, genetic variations of diseases, gene expression and in Environmental Assessment and Management developing skills in modelling and computational simulation for the evaluation of performance and environmental management systems and GIS.

UTAD accepted the challenge of offering advanced training, which is taking its first steps. We will briefly reveal the first thesis projects in this course showing the multiplicity of careers in Bioinformatics field.

Post mortem examination in understanding the pathogenesis of pandemics.

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Keywords: necropsy, post mortem, pathology, pandemic, sample collection.

Post mortem examination is an essential tool for determining the cause and circumstances of death. It also plays a key role in identifying new infectious diseases, detecting emerging infections and bio-terrorist attacks.

In a pandemic context, post mortem examination is more than a way to determine the cause of death or to rule out other causes of death. Necropsy may reveal secrets that other tests cannot and may even change clinical approaches.

Data obtained in post mortem examination provide new insights into epidemic diseases that include understanding the pathogenesis of the disease; defining the lesions, the transmission mechanism, the individual's immune response. Also contributes to the prediction of the prognosis, and allows evaluating the response to old and new therapies.

In a new infectious disease, necropsy allows monitoring in real-time the evolution of the etiological agent, the disease, and the host immune reaction.

Necropsy also provides an opportunity to collect samples for further examination. However, the value of this examination in the diagnosis depends on the special precautions required for sample collection. An adequate tissue sampling determines the quality of the test results.

To sensitize the participants to the importance of necropsy in the study of epidemic or pandemic diseases, in this workshop, necropsies will be performed in different animal species, with special attention to a basic gross examination and sampling of tissue and fluids for ancillary examinations, such as histopathological, toxicology, chemistry, microbiology and genetic testing.

Abiotic Stress Responses in Fruit Trees: Metabolism and Productivity.

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Keywords: Abiotic stressors, Biostimulants, Climate change, Fruit trees, Mitigation strategies.

The most recent climate projections point to a decrease in water availability, an increase in air temperature, and the occurrence of extreme phenomena, such as excessive rainfall near the harvest periods. Consequently, significant economic losses occur due to a strong reduction of the commercial value of the fruits. Abiotic stresses such as extreme temperatures, drought, salinity, and UV-B radiation are the foremost limiting factors for crop productivity.

Under the current climate changing scenario and also due to the increase of global trade in fruit to meet consumer demand for regular supply of high-quality fruit, it is important to understand the relationship between preharvest treatments with biostimulants and the physiological behaviour of fruit trees. Although no consistent literature is available about the effect of those substances, such as glycine betaine (GB) and Ascophyllum nodosum (AN), on the physiological performance of fruit trees, these compounds might be a new and innovative solution to increase the crop ability to tolerate stressful environments. The accumulation of osmolytes such as GB (quaternary ammonium compound) in cells can stabilize the structures by maintaining the integrity of membranes against the damaging effects of abiotic stresses via osmoregulation or osmoprotection. Seaweed based biostimulants, like AN, are composed of several components, such as plant hormones, proteins, sugars, vitamins, humic substances, and phenolic compounds. Several published reports suggest that biostimulants improve plant productivity by increasing the minerals assimilation and the photosynthetic activity, reducing the transpiration rate and the fruit-cracking incidence.

This workshop will provide an update on recent studies focusing on the physiological responses to changing environmental conditions at different fruit tree levels. Specifically, we will address how we can determine plant stress responses taking advantage of new technologies to link physiology and omics approaches.

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A Hands-on Science Experiments to Promote Sustainable Use of Natural Resources Through Genomics.

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Keywords: Omic Tools; Sustainability; Circular Economy; One health concept.

Climate changes and ongoing globalization are the two major challenges of 21st century food sustainability. It is therefore essential to design innovative market solutions for assessing consumer perception to new products, focusing on by-products and/or green ingredients, with efficient use of natural resources. Sustainable use of natural resources also contributes to food safety, rural development and increasing employment opportunities. According FAO, biodiversity for food are among the earth's most important resources. Crops, farm animals, aquatic organisms, forest trees, micro-organisms and invertebrates – thousands of species and their genetic variability make up the web of biodiversity that the world's food production depends on.

This workshop is a hands-on essential Molecular Genetic workshop aiming to provide an overview of concepts, practical procedures, and tools for overall genomics analysis. Following the One Health concept, this workshop is designed to showcase, formulate questions and points of view, and communicate the latest scientific advances in Molecular Biology, as well as its potential application in the sustainable use of natural resource (e.g., aquaculture) with a holistic perspective, namely i) emerging pathogens, ii) multidrug-resistant species, iii) novel ingredients (green) as alternative sustainable source of protein, and iv) food safety. During the course, the participants will be in touch with current DNA-based molecular techniques (experimental design, DNA and RNA nucleic acids extraction, conventional PCR, real-time qPCR, RT-qPCR), microbiology (antibiotic susceptibility testing), data analyses (Next generation sequencing, in silico analysis).

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Comparative oncology: spontaneous animal models for human cancer research?

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Keywords: Oncology, Cancer, Animal.

Neoplasms, growth de novo, tumors or cancers (also called malignant neoplasms), are neoformations that arise in all pluricellular beings, from the simplest to the most complex, being the result of accumulations of genetic errors and epigenetic changes in cells. Research in oncology in Veterinary Medicine has been following Medicine and is currently considered as a potential model for human oncological research.

With this workshop we intend to show some images and anatomical pieces of cases of animal neoplasms that were received in the Laboratory of Histology and Pathological Anatomy of UTAD, as well as talk about the research related to these adaptive but irreversible lesions.

Silk, an exciting material.

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Keywords: silk, biomacromolecule, biomaterial, thermal behaviour, wettability.

This workshop intends to provide an overview of silk science and highlight the most attractive features of this very attractive biomacromolecule which has a tremendous technological potential in a wide variety of areas, spanning from electronics and optics to biomedicine. The basics of silk processing and characterization will be presented and explored in practical laboratory experiments. Several techniques will be employed to study the morphology, thermal behaviour, surface properties and transparency of regenerated and raw silk fibers.

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The Organizing Committee of the XIII JGB / III JIGB