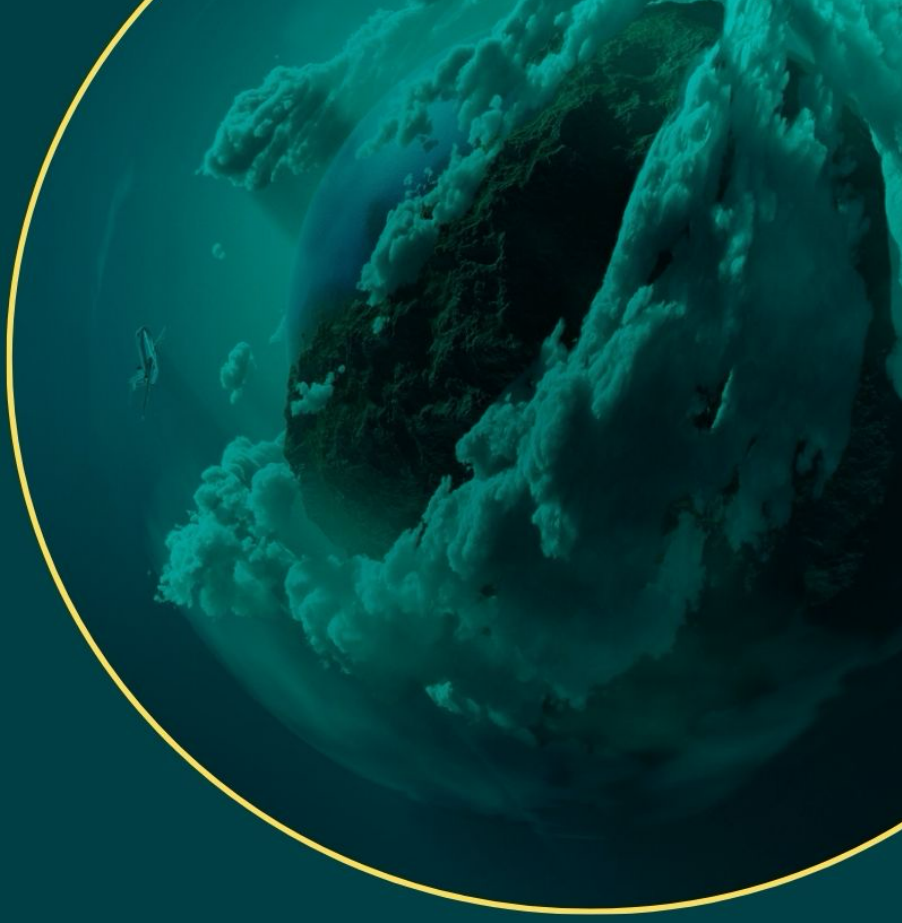
IV 1H-TOXRUN INTERNATIONAL CONGRESS 2025

No Boundaries for Toxicology: One Health, One Society, One Planet

EMPOWERING THE FUTURE GENERATIONS: THE SYNERGY OF SCIENCE, EDUCATION AND SOCIETY

8-9 May 2025 | Porto, Portugal



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TOX RUN
ONE HEALTH TOXICOLOGY RESEARCH UNIT

IV 1H-TOXRUN INTERNATIONAL CONGRESS 2025

NO BOUNDARIES FOR TOXICOLOGY: ONE HEALTH, ONE SOCIETY, ONE PLANET

EMPOWERING THE FUTURE GENERATIONS: THE SYNERGY OF SCIENCE, EDUCATION AND SOCIETY

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08-09 MAY, 2025

Porto, Portugal

EDITORIAL

EDITORIAL

The future of toxicology research in the era of artificial intelligence: the vision of 1H-TOXRUN Hub

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ABSTRACT

Toxicology, the great science of understanding the toxic effects of chemical substances on living organisms, is increasingly being integrated within the One Health framework – the holistic approach that recognizes the interconnectedness of human, animal, and ecosystem health [1,2]. The One Health concept advocates interdisciplinary collaboration among veterinarians, physicians, environmental scientists, and other professionals to address complex health challenges that transcend species and ecological boundaries. This vision gives legitimacy and uniqueness to 1H-TOXRUN, the research Hub for One Health Toxicology. Integrating toxicology into the One Health paradigm encourages collaboration across disciplines to investigate the mechanisms, sources, and impacts of toxic exposures. This includes studying xenobiotics' cellular and molecular mechanisms, assessing environmental contamination, and evaluating the health outcomes in both animals and humans. Ecotoxicology, as a subfield, emphasizes the effects of pollutants on ecosystems, including plants, animals, and microbial communities, further reinforcing the need for a unified approach [3]. Adopting a One Health perspective in toxicology leads to more comprehensive risk assessments that account for the complexities of real-world exposure scenarios. Therefore, policies and interventions must be designed to address the interconnected nature of these risks, promoting sustainable practices and preventive measures at the ecosystem level.

However, the world is changing, and the massive availability of artificial intelligence (AI) will shape the future of toxicology as a mother science, just as it is already transforming forensic science, as previously highlighted [4]. Integrating AI into toxicology is poised to redefine the discipline, transitioning it from relying on empirical observation to a data-driven, predictive science. By leveraging machine learning, deep neural networks, and generative AI, toxicology will enhance chemical risk assessment, reduce animal testing, and enable personalized toxicity predictions [5]. Indeed, AI models, such as quantitative structure-activity relationship (QSAR) frameworks and read-across-based structure-activity relationships (RASAR), now achieve high accuracy in predicting chemical toxicity, outperforming traditional animal test reproducibility [6]. For example, deep learning algorithms analyze chemical structures, biological activity, and omics data to forecast mutagenicity, streamlining drug development and reducing reliance on in vivo experiments [6]. Tools like eToxPred prioritize compounds for testing, minimizing costs and ethical concerns [7]. In addition, AI can decipher complex molecular pathways by integrating transcriptomic, proteomic, and metabolomic datasets [8]. Moreover, neural networks process high-content imaging from high-throughput screens to identify biomarkers for hepatotoxicity, nephrotoxicity, cardiotoxicity, and neurotoxicity, linking genetic susceptibility to chemical exposure [9]. This capability enables precision toxicology, where AI tailors risk assessments using individual genetic, microbiome, and exposure profiles [10]. AI platforms are also already simulating biological systems to predict human responses. For instance, virtual organ models replicate chemical interactions, enabling dose-response extrapolations and antidote efficacy testing. These systems support probabilistic risk assessments, quantifying uncertainties in exposure scenarios. Toxicology education is also a target of reshaping with AI, enabling personalized learning, and bridging gaps between theoretical knowledge and real-world applications [11]. This transformation, driven by AI's capacity, makes it possible to analyze complex datasets, simulate experiments, and predict toxicological outcomes, offering educators and students unprecedented tools to enhance understanding and engagement. AI-driven toxicology education will offer safer virtual labs for students to conduct impractical experiments in physical settings. Therefore, by embedding AI tools into curricula, educators prepare students to innovate in chemical safety, drug development, and public health crises, ensuring the next generation of toxicologists excels in both computational and experimental realms. But future directions are even more disruptive and revolutionary. Indeed, quantum computing will make it possible to solve complex systems, such as toxicology models, including multi-organ interactions, beyond classical computational limits, and cloud-based platforms will democratize access to predictive tools, empowering low-resource regions in chemical safety management. Moreover, quantum annealing optimizes the prioritization of 10,000+ chemicals for regulatory review, focusing resources on high-risk compounds [12].

The IV 1H-TOXRUN International Congress, held in Porto, Portugal, on May 8–9, 2025, promises to be a dynamic and enriching event for professionals across toxicology, biomedical, and environmental sciences, exploring the new expected world for toxicology. Under the visionary theme “EMPOWERING THE FUTURE GENERATIONS: THE SYNERGY OF SCIENCE, EDUCATION, AND

SOCIETY", this congress is set to foster interdisciplinary debate, knowledge exchange, and collaborative reflection within the One Health framework. Four carefully curated sessions anchor the scientific program this year: the i) **Session I: Pioneering the Future – Bridging Science and Entrepreneurship** is the spotlight of the intersection of research and innovation, exploring how scientific advances translate into entrepreneurial ventures and societal benefits. By merging cutting-edge science with commercial agility, academic institutions and startups are translating mechanistic insights into scalable solutions, from AI-driven risk assessment platforms to targeted therapeutics; the ii) **Session II: Shaping the Future of Education – Innovation, Impact and Digital Transformation** focus on transformative educational strategies, digital tools, and the impact of innovation on the training of tomorrow's scientists and health professionals. Integrating digital innovation into toxicology education is driving a paradigm shift, equipping future scientists with skills to address global chemical safety challenges through data-driven, ethical, and interdisciplinary approaches; the iii) **Session III: Global Challenges on Migration and Health** addresses the pressing health implications of global migration, emphasizing the need for integrated, cross-sectoral responses to emerging public health challenges. Global migration is one of the defining challenges of the 21st century, with more people than ever crossing borders due to conflict, economic hardship, environmental change, and opportunity. This movement brings significant health implications for both migrants and host populations [13], and toxicology plays a critical but often underappreciated role in this context. While infectious diseases often receive the most attention, non-communicable diseases (NCDs) and environmental exposures, including toxicological hazards, are major contributors to morbidity and mortality in these populations [14]. Moreover, continued use of traditional medicines, cosmetics, or imported foods may involve substances banned or regulated in the host country, leading to unintentional poisonings or adverse drug interactions; and the iv) **Session IV: Innovation for Sustainability and Environmental Transformation** is intertwined with advances in toxicology, particularly through the emergence of "green toxicology". This discipline reshapes how chemicals and materials are designed, produced, and regulated, prioritizing human and environmental health alongside economic and functional considerations. In other words, green toxicology offers a proactive, preventive approach to chemical safety [15]. Unlike traditional toxicology, which often assesses risks after products are already used, green toxicology integrates toxicological principles early in the design and development. This strategy enables identifying and mitigating potential hazards before chemicals reach the market, minimizing adverse impacts across global supply chains, and supporting the broader goals of sustainability and environmental stewardship [16]. Participants explore the cutting-edge research and initiatives aimed at environmental sustainability, resilience, and mitigating health risks from environmental pressures.

Altogether, the congress underscores the importance of safety, health risk prevention, and promoting resilient communities and circular economy models. By integrating cross-cutting issues aligned with the Sustainable Development Goals, the event aims to advance a unified approach to optimizing people, animals, and environmental health. All authors with relevant work in the aforementioned areas are presenting their oral and poster presentations, both representing fundamental components of scientific congresses, each playing a distinct and complementary role in the dissemination of research, fostering collaboration, and advancing scientific dialogue, and contributing to the success and vibrancy of the 1H-TOXRUN International Congress brand of credibility.

Cordial greetings
Ricardo Jorge Dinis-Oliveira

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IV 1H-TOXRUN INTERNATIONAL CONGRESS 2025

08-09 MAY, 2025

Porto, Portugal

SCIENTIFIC PROGRAMME

MAY 08

09h00 **Opening Session** | **Ricardo Jorge Dinis-Oliveira, Ana R. Freitas** & **Sara Ricardo**, 1H-TOXRUN Management Board

09h30 **Opening Lecture:** Migration in a One Health Context: Integrated Approaches to Global Health Security.| **António Vitorino**, President of the National Council for Migration and Asylum - Portugal

10h15 **Coffee Break**

Session I
Pioneering the Future: Bridging Science & Entrepreneurship

CHAIRS: Eduarda Silva (UCIBIO, 1H-TOXRUN, IUCS-CESPU), Hassan Bousbaa (UNIPRO, IUCS-CESPU), Joana Prata (UCIBIO, 1H_TOXRUN, IUCS-CESPU)

10h45 From Research to Product: Conquering the Hard Road of Entrepreneurship. | **Cidália Pina-Vaz**, FASTinov

11h15 Career Path or Career Portfolio? A Journey from Academia to Digital Health Innovation. | **Fabíola Costa**, Sword

11h45 Insects in Food: A Sustainable Solution for the Future. | **Vasco Esteves**, Tecmafoods

12h30 **Lunch & Poster Viewing**

Session II
Shaping the Future of Education: Innovation, Impact & Digital Transformation

CHAIRS: Patrícia Antunes (UCIBIO-FCNAUP), Rui Azevedo (UCIBIO; 1H-TOXRUN, IUCS-CESPU), Vítor Seabra (UCIBIO; 1H-TOXRUN, IUCS-CESPU)

14h00 Educational Technologies for Inclusive Pedagogy: Evidence-Based Insights to Improve Teaching Practices and Student Learning Outcomes. | **Fernando Remião**, FFUP

14h30 MicroMundo: Citizen Science and Service-Learning against Antimicrobial Resistance. | **Victor Cid**, Universidad Complutense, Madrid

15h00 From Registration to Teaching: How Vet-OncoNet Contributes to the Training of Veterinary Doctors. | **Kátia Pinello**, ICBAS-UP/ISPUP

15h30 **Coffee Break & Poster Viewing**

16h30 **Presentation of Selected Oral Communications**

OC 01 | The effects of methylparaben exposure on drinking water bacteria virulence and tolerance to antibiotics. **Ana Rita Pereira**, FEUP

OC 02 | Development of new phytochemical-based disinfectant formulations for the control of healthcare-associated bacterial infections. **Mariana De Sousa**, FEUP

OC 04 | MicroMundo@IUCS_CESPU (2022-2024): Uncovering Soil's Hidden Treasures and Its Impact on Science and Antimicrobial Resistance Awareness. **Beatriz Antunes**, UCIBIO, 1H-TOXRUN, IUCS-CESPU

OC 05 | Characterisation of the poisoning profile in the emergency department of Lamego hospital. **André Araújo**, IP Guarda

OC 07 | Alcohol consumption, depression, stress and anxiety in post-pandemic university students: exploratory study. **Beatriz Lopes**, UFP

OC 08 | From resistance to sensitivity in non-small cell lung cancer: newly established multidrug-resistant cancer cells contribute to identifying DNA damaging agents as collateral sensitizer drugs. **Sara Silva**, i3S

18h30 **Closing of the First Day**

MAY 09

Session III
Global Challenges on Migration & Health

CHAIRS: Bruno Peixoto (UCIBIO, 1H-TOXRUN IUCS-CESPU), Joana Barbosa (UCIBIO, 1H-TOXRUN IUCS-CESPU), Luís Fernandes (UCIBIO, 1H-TOXRUN IUCS-CESPU)

09h30 Migration: Public Policies and Food Security. | **Alexandra Bento**, INSA

10h10 Is a Gentrified City a Healthy City? | **Ana Isabel Ribeiro**, ISPUP

10h30 Demography and Migration: Discussing the Connection with an Eye on the Replacement Migration Concept. | **Jorge Malheiros**, IGOT-UL

11h00 **Coffee Break & Poster Viewing**

11h30 **Presentation of Selected Oral Communications**

OC 09 | Development and validation of an HPLC-DAD analytical method for psilocybin and psilocin. **Beatriz Senra**, FMUP

OC 10 | Binding of Synthetic Cannabinoids to Human Serum Albumin: Site Characterization and Recognition Mechanisms. **Ana Rita Santos**, FFUP

OC 11 | Assessing the Herbicidal Potential of *Rhodopirellula rubra* LF2 Extracts: A Promising Approach for Sustainable Weed Control. **Miguel Silva**, FCUP

OC 12 | Citizen science biomonitoring of metal(loid) pollution in honey from Northwest England. **Tony Walker**, Dalhousie University

OC 03 | Identification of Linezolid-Resistant *Enterococcus* spp. in Calves: findings from Portuguese High-yielding Dairy Farms. **Alícia Ribeiro**, IUCS-CESPU

OC 06 | Unveiling the Toxicological Risks of Synthetic Cannabinoid Smoking: A GC-MS-based Investigation. **Filipa Pita**, FFUP

13h00 **Lunch & Poster Viewing**

Session IV
Innovation for Sustainability & Environmental Transformation

CHAIRS: Cláudia Ribeiro (UCIBIO, 1H-TOXRUN, IUCS-CESPU), Daniel Barbosa (UCIBIO, 1H-TOXRUN, IUCS-CESPU), João Carrola (CITAB, UTAD)

14h30 GreenLab Portugal: Promoting Sustainable Practices in Portuguese Research Institutions. | **Joana Magalhães**, Greenlabs / i3S-UP

15h00 Pioneering Change: The Role of Training in Environmental and Sustainable Transitions. | **Ana Ramos**, INEGI

15h30 Human Sustainability: The Engine of Organizational Performance. | **Rita Carvalho de Matos**, Happiness Business School

16h00 **Innovative Talks**

Building Digital Transformation in Health. | **Sara Marques**, SPMS

Innovating Wastewater Management: The Power of Digital Twin Solutions. | **Virgínia Carvalho**, AquaInSilico

MyVet and MyDoc – A Laboratory in Your Phone. | **Teresa Barroso**, INESCTEC-CRIIS

New Solutions from Microalgae for Health and Agriculture. | **Ralph Urbatzka**, Fykia Biotech

Round Table Discussion

Closing Session

17h00 **Awards Presentation & Closing Ceremony** | **Ricardo Jorge Dinis-Oliveira, Alexandra Teixeira, Áurea Carvalho, Carla Batista Pinto**, UCIBIO, 1H-TOXRUN, IUCS-CESPU

17h30 **Closing cocktail**

IV 1H-TOXRUN INTERNATIONAL CONGRESS 2025

08-09 MAY, 2025

Porto, Portugal

INVITED SPEAKERS

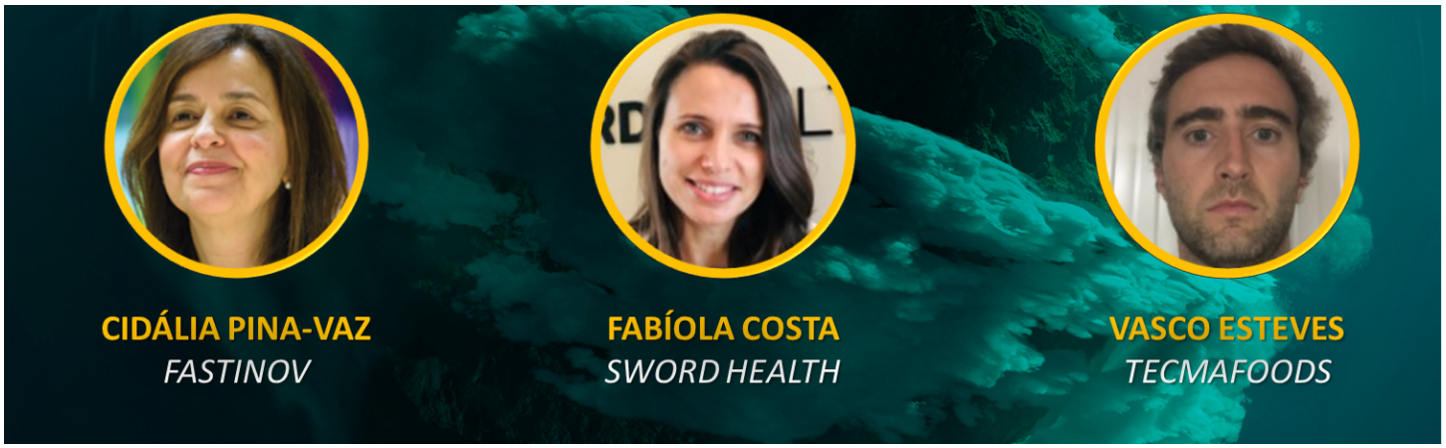
OPENING LECTURE

OL Migration in a One Health Context: Integrated Approaches to Global Health Security.

António Vitorino [1]

[1] President of the National Council for Migration and Asylum - Portugal

SESSION I - Pioneering the Future: Bridging Science & Entrepreneurship



IL01 From Research to Product: Conquering the Hard Road of Entrepreneurship

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ABSTRACT

Background: As a medical doctor and researcher, daily clinical challenges have always been a source of inspiration. One old problem was the delay in antimicrobial susceptibility testing (AST) results from clinical laboratories. In severe infections, biological samples are collected and sent for analysis, but traditional methods require 2–3 days to identify the pathogen and determine effective treatment options. In the meantime, empirical and often blind therapy must be initiated, although the risk of treatment failure increases significantly - by 4% in mortality per hour of delay. Due to rising antimicrobial resistance (AMR), initial therapies can fail in up to 40% of cases, with serious consequences for patient outcomes and public health. **Objective:** To develop a disruptive solution that delivers AST results within 2 hours using flow cytometry—a technology widely used in other laboratory fields but not yet applied to clinical microbiology. **Methods:** To bring this innovation to market, we secured multiple patents, founded a spin-off company from the University of Porto - FASTinov, applied for grants, attracted investors, and carried out extensive internal and external validations. Several FASTinov kits were developed for Gram-negative and Gram-positive bacteria, designed for use with pure colonies, positive blood cultures, and more recently, directly from urine samples. These kits incorporate antibiotic panels, fluorescent probes, and a multiparametric algorithm powered by artificial intelligence to classify bacterial strains as susceptible, resistant, or intermediate within two hours. **Results:** FASTinov kits are now CE-IVD certified and have begun penetrating the clinical diagnostics market. However, challenges remain, particularly in regulatory compliance and the need for automation - requiring full-time dedication to ensure continued progress and implementation. **Conclusions:** This journey transformed a clinician and researcher into an entrepreneur - not by choice, but by necessity. Without this personal commitment, advancing such a transformative solution would not have been possible.

Keywords: Rapid antimicrobial susceptibility tests, sepsis, flow cytometry, FASTinov

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References
www.fastinov.com

IL02 Career path or Career portfolio? A Journey from Academia to Digital Health Innovation

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ABSTRACT

Background: In a world where professional trajectories are increasingly fluid, traditional career paths are being reshaped by individual passions, multidisciplinary skill sets, and emerging opportunities[1]. For many scientists, the journey beyond academia can feel uncertain or undefined. Yet, with the right tools, every experience—professional or personal—can contribute to a unique and fulfilling career portfolio. **Objective:** To share a personal journey from academic research to digital health innovation, reflecting on how a flexible, skills-based approach can unlock diverse career possibilities beyond the academic script. **Methods:** Drawing on over two decades of experience in research (from fundamental research, to translational research, pre-clinical, clinical trials and real world evidence), leadership in funded innovation projects, and a more recent transition to the digital health industry, this lecture offers a narrative perspective on career development. Through storytelling and reflection, it highlights how building and combining skills—scientific, entrepreneurial, creative—can open new venues in both expected and unexpected settings. **Results:** The transition from academia to industry was not a departure, but a continuation of a purpose-driven path. This evolution illustrates how skills developed through research, teaching, mentorship, and even personal interests, can be powerful drivers of career transformation. **Conclusions:** Career journeys are often only coherent in hindsight. There is no universal script—

but rather, a mosaic of experiences that, when linked thoughtfully, create a compelling and impactful career portfolio. By embracing experimentation, leaning into curiosity, and valuing both professional and personal growth, we can empower ourselves—and the next generation—to redefine success in science and beyond.

Keywords: Career in Science; Translational Innovation; Digital Health

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IL03 Insects in Food: A Sustainable Solution for the Future

Vasco Esteves [1]

[1] Tecmafoods

SESSION II - Shaping the Future of Education: Innovation, Impact & Digital Transformation

IL04 Educational Technologies for Inclusive Pedagogy: Evidence-Based Insights to Improve Teaching Practices and Student Learning Outcomes

Fernando Remião [1]

[1] Faculty of Pharmacy, Porto University

IL05 MicroMundo: Citizen Science and Service-Learning against Antimicrobial Resistance

Victor J. Cid[1]

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ABSTRACT

Background: As alerted by WHO and other global or local health agencies, antimicrobial resistance (AMR) poses a global threat to environmental, animal, and human health. Side to side with surveillance and action, research and education stand as basic pillars to collectively tackle the current AMR crisis. The discovery of new antimicrobial drugs or alternative strategies to fight infectious diseases is urgent. Within this frame, we describe here our experience with MicroMundo, a successful international strategy based on principles set up by Jo Handelsman's Tiny Earth initiative in the US, which we have implemented and expanded throughout the Iberian Peninsula [1,2]. **Objectives:** Beyond the isolation of microbial strains showing antibiotic bioactivities, the key objectives of MicroMundo are (i) to create scientific culture in our society on AMR and One Health, and (ii) to inspire vocations in young students towards R&D in Biomedicine. **Methods:** MicroMundo is based on a crowdsourcing research strategy, adapted to Service-Learning pedagogic standards that combine active learning with community service [3]. University students are trained to work in teams, then each team is assigned a Secondary or High School for the academic year. The MicroMundo team is thus in charge of leading younger students into the discovery of antibiotic-producing microorganisms form a soil sample of their choice, thus amplifying the range of search. In addition, MicroMundo teams have the mission of promoting AMR awareness activities directed to the community in which the school is located. **Results:** Along the last eight years, 32 MicroMundo hubs, operating across 31 different Portuguese and Spanish universities, have recruited thousands of teenagers in this quest. Yearly, an average of 500 university students in Spain and Portugal, lead about 3,500 younger students onto the analysis of over 1,500 soil samples, analyzing 30,000 isolates for antibiotic bioactivities. This effort has led to collections of hundreds of antibiotic-producing candidates. But, most importantly, the collective effort boosts wakefulness on One Health-oriented scientific careers in academics in various educational levels. **Conclusions:** MicroMundo is a successful strategy to bring onto society AMR awareness, and to inspire our future researchers into solutions to this current global health challenge.

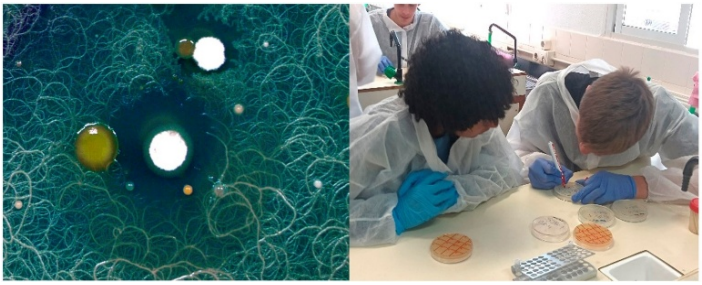

Figure 1. MicroMundo aims to detect antibiotic bioactivities from novel bacterial isolates by exploring soil biodiversity (left, colonies grown on TSA 10% agar; the white colony in the center inhibits the invading *Bacillus mycoides* in the background) by involving secondary and High School students (right).

Keywords: Antimicrobial Resistance, One Health, Citizen Science, Service-Learning, Active Learning.

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IL06 From Registration to Teaching: How Vet-OncoNet Contributes to the Training of Veterinary Doctors

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ABSTRACT

Background: Vet-OncoNet [1] is a national platform in Portugal dedicated to collecting and analyzing data on animal neoplasms, driven by a One Health vision. More than a database, Vet-OncoNet is a collaborative ecosystem connecting veterinary professionals, academic institutions, and laboratories to improve animal health, promote comparative oncology, and drive data-informed decision-making [2-4]. **Objective:** This presentation aims to demonstrate how Vet-OncoNet contributes to the academic training of future veterinary doctors at the Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto. **Methods and Experience:** Vet-OncoNet actively collaborates with undergraduate students enrolled in the Epidemiology course at ICBAS. Students engage in hands-on learning by exploring real-world data, formulating hypotheses, conducting descriptive analyses of selected tumors, and presenting their findings in a final monograph. Through this experience, students strengthen their skills in Excel (including pivot tables and basic analytics), Power BI, and even ArcGIS for geospatial visualization. Moreover, final-year students undertake curricular internships within Vet-OncoNet, allowing them to deepen their understanding of the Evidence-Based Veterinary Medicine (EBVM) cycle—Ask, Acquire, Appraise, Apply, and Act. These students participate in multidisciplinary projects using R-based tools, including RShiny applications, and begin to explore the integration of artificial intelligence solutions in veterinary data analysis. So far 3 PhD collaborations, 2 MSc Thesis, 5 final graduation integrated masters UP, 16 monographs have been produced. At the master's level, students from both Oncology and Public Health programs collaborate with Vet-OncoNet in various research areas. These include the analysis of the national companion animal registry (SIAC) [5,6] and oncology [7] and comparative studies [8] involving both animal and human data. These experiences foster critical thinking, scientific communication, and advanced data analysis skills, preparing students for evidence-based and data-driven veterinary careers. **Conclusions:** This project places veterinary students at the intersection of animal health and the expanding field of data science, equipping them with practical tools and critical thinking skills essential for modern evidence-based practice. Thus, Vet-OncoNet is not just a database—it's a living tool, driven by the One Health vision, connecting science, society, and education.

Keywords: data-driven learning; epidemiology; evidence-based veterinary medicine; One Health

Acknowledgments/Funding

This project received support from ICBAS - Instituto de Ciências Biomédicas Abel Salazar, ICBAS.

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SESSION III - Global Challenges on Migration & Health

IL07 Migration: Public Policies and Food Security

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IL08 Is a Gentrified City a Healthy City?

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ABSTRACT

Background: Gentrification is among the most transformative socio-spatial processes of our times, reshaping places worldwide by reconfiguring their socioeconomic, cultural, and physical landscapes. Gentrification has become increasingly transnational, as local gentrification processes are now linked to global capital flows and the movements of a mobile middle and upper class, including lifestyle migrants, international students, and tourists, primarily from wealthier countries. Gentrification alters living environments and leads to displacement, both of which might have health impacts. Thus, evidence is needed to navigate the public health challenges stemming from gentrification processes. **Objective:** In addition to framing the topic within the broader literature and the Portuguese context, this talk aims to summarize the evidence generated by the HUG project (PTDC/GES-OUT/1662/2020), funded by the Foundation for Science and Technology, which aimed to understand the public health impacts of gentrification in Porto area. **Methods:** We conducted several complementary studies involving participants from EPIPorto, a population-based cohort from Porto (Portugal), and a purposive sample from the community. Both qualitative and quantitative methods were employed, including interviews, Photovoice, and observational studies analyzed using regression models. **Results:** Research conducted within the project revealed that gentrification reshapes urban life, bringing both economic revitalization and a range of negative outcomes such as increased stress, pollution, housing displacement, weakened social ties, and greater health inequalities. Older adults appeared particularly vulnerable, experiencing rising living costs, mobility challenges, social isolation, and mental health decline. Findings also pointed to a growing sense of alienation, loss of community cohesion, and impacts such as depression among older populations. Housing insecurity was associated with loneliness, poorer cognitive function, and a diminished perception of healthy ageing. Displacement further contributed to psychological distress, social disconnection, and worsening living conditions. **Conclusions:** Porto has become a significant site of transnational gentrification, driving up housing costs and profoundly reshaping the city's social and physical landscape. The studies presented here identified a range of health impacts associated with gentrification, with some benefits but predominantly harmful effects.

Keywords: gentrification; urban health; displacement.

Acknowledgments/Funding
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IL09 Demography and Migration: Discussing the Connection with an Eye on the Replacement Migration Concept

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ABSTRACT

Background: Contemporary demography is marked by two characteristics with enormous impact on global social and economic processes: ageing [1] and migration, being the latter a major element of fast demographic evolution, something marked by higher speed and unpredictability [2]. Although the different countries of the world experience these processes at a different pace and in distinct stages, (international) migration and ageing are global processes that can be identified almost everywhere. **Objective:** Departing from the global ageing process that is already associated to a slackening in the world population growth and will probably lead to a decline starting sometime between 2080 and 2100 [3], this presentation aims to explore: a) The progressive globalization of a second demographic transition where deaths regularly surpass births, considering its social and economic impacts; b) The relationship between migration, ageing and population evolution, exploring the role of migration in attenuating or stimulating population decline (and ageing); The hypothesis of Replacement Migration [4], that corresponds to the levels of international immigration that ageing societies would needed to offset declines in the demographic size and mitigate ageing. **Methods:** This analysis will be carried out with the support of academic and technical bibliography (reports) produced by international organizations, in addition to demographic statistics.

Keywords: (global) ageing; (international) migration; replacement migration

Acknowledgments/Funding

Though this is a single-authored presentation, it benefits from work developed in collaboration with Isabel Tiago de Oliveira, Daniela Craveiro, João Peixoto, Cristina Sousa Gomes, and Maria João Guardado Moreira. This research received no external funding.

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SESSION IV - Innovation for Sustainability & Environmental Transformation

IL10 GreenLabs Portugal: Promoting Sustainable Practices in Portuguese Research Institutions

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ABSTRACT

Greenlabs Portugal is a network of Portuguese grassroot green teams of scientist and staff of research institutes that advocate for sustainable research practices to reduce environmental impact of research in Portugal.
Our mission is to promote and support the implementation of green lab initiatives in Portugal and to foster collaborations, aiming to drive changes in Portuguese research community towards more environmentally aware and sustainable research practices. The Greenlabs Portugal platform was established in 2021 and steadily expanded since, currently connecting 11 research institutions across the country. As a network, we promote the exchange of knowledge and resources between members; organize and host meetings to share news, resources, and practices; assist in the creating of new green teams; promote common actions; connect Greenlabs to other European green teams and international networks. We expect to decrease the environmental impact of research by educating and providing tools to the scientist, so they can render their research more environmentally friendly without compromising research quality.

Keywords: Greenlab; sustainability; green research

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This research received no external funding.

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www.greenlabs.pt

IL11 Pioneering Change: The Role of Training in Environmental and Sustainable Transitions

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ABSTRACT

Background: As global environmental challenges intensify—ranging from climate change and resource depletion to biodiversity loss and pollution—organizations, governments, and communities are increasingly recognizing the urgent need for sustainable solutions. However, achieving meaningful progress toward sustainability goals requires more than just policy shifts or technological innovations. It demands a fundamental transformation in how individuals think, work, and collaborate across sectors. This transformation begins with education and capacity-building. Many professionals, regardless of their field, lack formal training in sustainability principles and practices. As a result, there's a growing demand for structured training programs that equip individuals with the knowledge, tools, and mindset needed to address complex sustainability challenges. These programs serve as a critical bridge between awareness and action, helping learners not only understand key concepts like circular economy, climate resilience, life cycle thinking, but also apply them within their professional and personal spheres. **Objective:** To show the outcomes of several training programs related to sustainability, held by a Research and Technology Organization, directed to industrial stakeholders. **Methods**: This work reviewed the contents, learning outcomes and feedback from the participants of 8 different programs, in a total of 25 editions (Figure 1). **Results:** One of the key insights gathered from feedback in circularity-focused courses is the vital importance of raising awareness. Many participants begin these programs with only a limited understanding of circularity. Through interactive learning experiences, they are introduced to core concepts such as resource efficiency, waste minimization, and life-cycle thinking. Educators often rely on real-world case studies to demonstrate how companies have effectively adopted circular practices. This exposure helps shift participants' perspectives and inspires them to spot circular opportunities within their own industries. In addition to theoretical insights, successful training programs

emphasize hands-on learning, equipping participants with practical skills essential for implementing circular solutions. Feedback consistently highlights the value of the sessions, which provide practical tools that enable participants to apply the techniques to real-life challenges, developing actionable skills. In doing so, they are better positioned to drive circular initiatives within their organizations. As circularity often demands collaboration across disciplines, including designers, engineers, managers, and policymakers, participants frequently emphasize the value of networking and teamwork opportunities. Many courses include group projects that simulate real-world circularity challenges, encouraging participants from varied backgrounds to co-create innovative solutions. This collaborative approach not only reflects the complexities of real-world implementation but also cultivates professional networks and promotes interdisciplinary thinking. **Conclusions:** As industries and societies increasingly shift toward sustainable models, the concept of circularity has gained prominence. While technical innovations play a vital role, it is the training and education of stakeholders that often become the driving force behind successful implementation. This work provides insights gathered from circularity-related courses highlighting how training empowers individuals, organizations, and communities to transition from linear practices to circular models.

Figure 1. INEGI's training programs rmijelated to sustainability

Keywords: circularity training, sustainability education, life cycle thinking

Acknowledgments/Funding
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IL12 Human Sustainability: The Engine of Organizational Performance

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ABSTRACT

Background: Organizations are facing increasing challenges related to employee well-being, engagement, and performance. Traditional productivity-driven approaches often neglect the fundamental role of human sustainability—the ability to sustain individuals' physical, emotional, and cognitive well-being in the workplace. **Objective:** This presentation explores how a human-centered approach enhances organizational performance by fostering resilience, motivation, and long-term effectiveness. **Methods:** Through a synthesis of research findings, case studies, and real-world applications, we analyze the impact of leadership, work culture, and well-being initiatives on employee performance and retention. Key factors such as psychological safety, purpose-driven work, and work-life balance are examined. **Results:** Evidence suggests that organizations prioritizing human sustainability report increased productivity, innovation, and employee satisfaction. Referring to the application of practical human sustainability measures, positive outcomes such as reduced burnout and improved employee engagement have been observed. **Conclusions:** Human sustainability is not just an ethical responsibility but a strategic advantage. Companies that invest in people as their core asset cultivate a thriving, high-performing workforce, ultimately driving organizational success.

Keywords: Human Sustainability; Organizational Performance; Workplace Well-being

Acknowledgments/Funding
This research received no external funding

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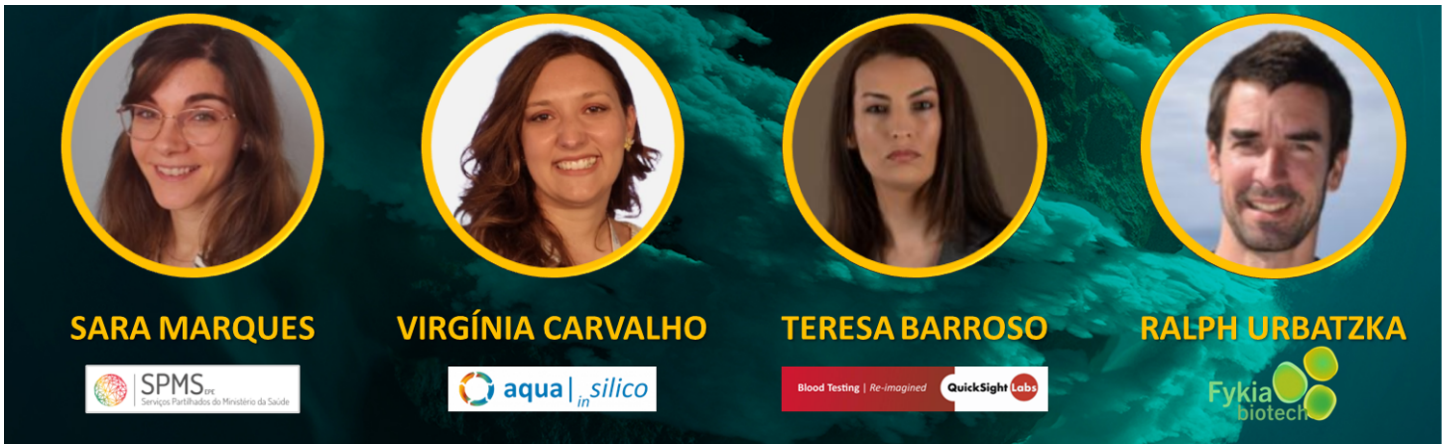
INNOVATIVE TALKS



IT01 Building Digital Transformation in Health

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ABSTRACT

This talk will address the impact of digital transformation to optimise healthcare services and improve patient outcomes [1], highlighting the role of the Shared Services of the Ministry of Health (SPMS) to this field [2]. The impact of the European Health Data Space (EHDS) [3] in facilitating access to health data for a more efficient and integrated provision of healthcare services at European level, as well as to advance research and medical innovation (e.g. improved diagnosis, personalized treatments, and clinical decision making) through health data reuse will be addressed. SPMS's active involvement in key international initiatives that promote digital health transformation and ensure alignment with the EHDS [2,4,5] will be presented, highlighting the pivotal role of cross-border collaboration to advance a digital ecosystem that promotes innovation and enhances citizens' healthcare and quality of life.Additionally, SPMS' contribution to the future of the EHDS will be covered, along with Portugal's commitment in building a global digital health ecosystem towards an optimized, patient-centered, innovation-driven healthcare system.

Keywords: Digital Health; EHDS; Health data (re)use

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IT02 Innovating Wastewater Management: The Power of Digital Twin Solutions

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IT03 MyVet and MyDoc – a laboratory in your phone

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ABSTRACT

Background: Quantifying erythrocytes and leukocytes is crucial in veterinary diagnostics. Traditional methods like flow cytometry, laser scattering, and impedance detection, while accurate, require significant sample volumes and complex preparation. Emerging point-of-care (POC) technologies have identified visible-near infrared (Vis-NIR) spectroscopy as a promising, minimally invasive alternative. **Objective:** This study aims to establish direct correlations between blood cell counts (erythrocytes, leukocytes, hemoglobin, and hematocrit)

and scattering coefficients from whole blood spectra, supporting the development of portable, reagent-free diagnostic tools for veterinary use. **Methods:** Fresh canine blood was collected via standard venipuncture at Anicura CHV - Veterinary Hospital Center. Spectral data were captured using a 4500K LED and Ocean Insight STS-vis spectrometer (300-800 nm). Scattering correction coefficients were calculated using an extended multiplicative scattering correction algorithm, and multivariate linear regression (MLR) models were used to assess the relationship between scattering data and hemogram parameters. Model performance was evaluated using Pearson correlation (R), mean absolute error percentage (MAPE), and coefficient of determination (R^2). **Results:** Fig. 1 presents the correlation plots for RBC, Hgb, HTC, and WBC, while Table 1 shows the scattering quantification. A moderate correlation between scattering coefficients and RBC counts (R=0.5739) was observed, with a standard error (SE) of 1.30×10^{12} and MAPE of 21.48%, suggesting that scattering data can reflect RBC levels despite notable variance. Hgb, which is closely linked to RBC, showed a similar correlation (R=0.5623), with a MAPE of 22.66% and SE of 32.51 g/dL, indicating potential for qualitative Hgb assessment. WBC correlations were weaker (R=0.4270) but demonstrated potential within the official recommended range. **Conclusions:** These findings confirm that scattering coefficients contain valuable information about blood cell counts, supporting the viability of Vis-NIR spectroscopy for non-invasive hemogram diagnostics. However, variability in the data highlights the need for self-learning artificial intelligence (SLAI) correction models to enhance accuracy, particularly for less abundant components like WBC. This approach holds promise for transforming POC testing into clinical and field settings.

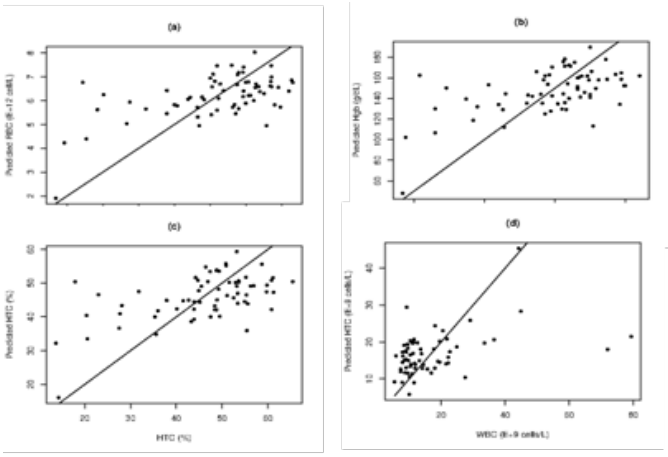

Figure 1. Correlation plots using scattering information for the quantification of: (a) RBC; (b) Hgb; (c) HTC; and (d) WBC.

Table 1. Benchmark results for scattering quantification of RBC, Hgb, HTC and WBC in dog blood.

Parameter	RBC (10^{12} cells/L)	Hgb (g/dL)	HTC (%)	WBC (10^9 cells/L)
SE	1.309	32.51	9.5	12.19
R2	0.3293	0.3161	0.3229	0.1823
MAPE (%)	21.48	22.66	21.99	49.87
R	0.5739	0.5623	0.5682	0.4270

Keywords: point-of-care; spectroscopy; hemogram; diagnosis; painless; reagentless; animal welfare; artificial intelligence

Acknowledgments/Funding

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IT04 New Solutions from Microalgae for Health and Agriculture

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IV 1H-TOXRUN INTERNATIONAL CONGRESS 2025

08-09 MAY, 2025

Porto, Portugal

ORAL COMMUNICATIONS

OC01 The effects of methylparaben exposure on drinking water bacteria virulence and tolerance to antibiotics

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ABSTRACT

Background: Parabens, widely used as preservatives in personal care products, pose an increased global concern due to their impact on environmental microbial communities, particularly in drinking water (DW), and potential risks to public health. Among different parabens detected in drinking water distribution systems (DWDS), methylparaben (MP) is the most prevalent at elevated concentrations, raising serious health concerns [1]. **Objective:** This study evaluated the effects of MP exposure at environmentally relevant concentrations of MP (15 µg/L) on bacterial tolerance to antibiotics and virulence. **Methods:** *Acinetobacter calcoaceticus* and *Stenotrophomonas maltophilia* both isolated from a DWDS were exposed to MP for a long period. The susceptibility to antibiotics (ceftazidime - CEF; levofloxacin - LEV; minocycline - MINO; and trimethoprim-sulfamethoxazole - TMP-SMX) was evaluated in the 5th and 10th weeks by the disk diffusion susceptibility methods according to the Clinical and Laboratory Standards Institute. Bacterial virulence was studied in terms of outer membrane vesicles (OMVs) production and properties [2], their ability to form biofilms and invade hosts cells [3 - human gingival fibroblasts (HGF). **Results:** Increased tolerance to TMP-SMX and CEF was observed to *A. calcoaceticus* after MP exposure for 5 and 10 weeks, respectively. The exposure to MP for 7 days was also able to change the properties of bacterial released OMVs, which are critical to bacterial virulence. MP-exposed *A. calcoaceticus* biofilm cells generated larger OMVs, while planktonic cells released smaller OMVs compared to their non-exposed counterparts. In *S. maltophilia*, MP exposure led to an increase in lipid content of biofilm-derived OMVs but resulted in a reduction in both lipid content and OMV concentration in planktonic cells. Additionally, MP exposure significantly enhanced biofilm formation in both species, further reinforcing bacterial virulence. Notably, MP-exposed *A. calcoaceticus* planktonic cells demonstrated an increased capacity to invade HGF, suggesting that MP contamination may exacerbate bacterial virulence. **Conclusions:** These findings reveal how MP exposure can drive bacterial adaptation by increasing antibiotic tolerance and enhancing interconnected virulence mechanisms - including OMV production, biofilm formation, and host cell invasion - posing significant health risks in DWDS.

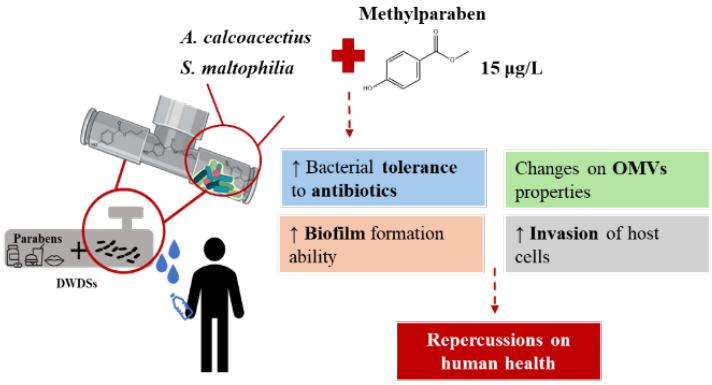

Figure 1. Effects of methylparaben exposure on drinking water bacterial pathogenicity.

Keywords: biofilms, host invasion, methylparaben

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OC02 Development of new phytochemical-based disinfectant formulations for the control of healthcare-associated bacterial infections

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ABSTRACT

Background: Healthcare-associated infections (HAIs) remain a critical public health challenge due to the persistence of multidrug-resistant (MDR) bacteria and the inefficacy of conventional disinfectants [1]. Biofilms further enhance bacterial resistance and limit the effectiveness of biocides [2]. Phytochemicals have emerged as natural alternatives due to their

antimicrobial and antibiofilm properties [3]. This study explored the potential of phytochemical-based formulations to enhance disinfection in healthcare settings. **Objective:** This research aimed to assess the antimicrobial efficacy of selected phytochemicals—eugenol (EUG), salicylic acid (SAL), shikimic acid (SHI), trans-m-coumaric acid (COU), and choline chloride (COL)—alone and in combination with widely used biocides, particularly benzalkonium chloride (BAC) and peracetic acid (PAA). **Methods:** The antimicrobial activity of phytochemicals and biocides was tested against *Staphylococcus aureus* and *Escherichia coli* following the European Standard EN 1276 (2019). For this, the dose-response and time-response effects, as well as the minimum bactericidal concentrations (MBCs), were determined. The Checkerboard assay, analyzed with Combenefit software, assessed synergism potential in dual and triple combinations through the Fractional Bactericidal Concentration Index (FBCI). Disinfection kinetics were characterized using the Chick-Watson and Weibull models. **Results:** Among the phytochemicals tested, SAL (with an MBC of 500 mg/L for both bacteria) and EUG (with an MBC of 1000 mg/L for *E. coli* and 1700 mg/L for *S. aureus*) showed the highest antimicrobial efficacy. For both bacteria, MBCs of 3 mg/L and 1 mg/L and high disinfection rates were obtained with BAC and PAA, respectively. BAC combined with EUG (750 mg/L) showed synergistic effects against *S. aureus* (FBCI = 0.309) and *E. coli* (FBCI = 0.350). A synergistic interaction was observed between BAC and EUG, while the other dual combinations exhibited additive effects. Triple combinations demonstrated promising bactericidal activity, effectively reducing bacterial culturability. Maximum reductions of (5.78 ± 0.07) log (CFU/mL) for *E. coli* and (5.82 ± 0.04) log (CFU/mL) for *S. aureus* within 30 minutes were obtained. **Conclusions:** This study demonstrated that phytochemicals could enhance the efficacy of conventional biocides, reducing the required concentrations and contributing to the development of effective and sustainable healthcare disinfection strategies.

Keywords: healthcare-associated infections; phytochemical-based disinfection; synergistic antimicrobial activity

Acknowledgments/Funding

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OC03 Identification of linezolid-resistant *Enterococcus* spp. in calves: findings from Portuguese high-yielding dairy farms

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ABSTRACT

Background: Antimicrobial resistance (AMR) is a global health threat requiring a One Health approach, as antibiotic-resistant bacteria can spread between animals and humans. *Enterococcus* spp., particularly *E. faecium* (*Efm*) and *E. faecalis* (*Efs*), are key AMR indicators due to their role as gut commensals and their potential as reservoirs of resistance genes. They are also opportunistic pathogens that can cause severe human infections. Cattle are a recognized reservoir of multidrug-resistant (MDR) *Enterococcus* spp., yet remain among the least studied food-producing animals in this context [1,2]. **Objective**: To assess if contemporary faecal samples from main cattle farms in Northern Portugal carry clinically relevant antibiotic-resistant *Enterococcus* spp. **Methods:** Thirty bovine fecal swab samples were collected from 10 high-yielding farms with Holstein-Friesian dairy cattle in 2 cities during 2023 [3]. Sample processing included pre-enrichment (37ºC/18h) without/with antibiotics (ampicillin 16µg/mL, vancomycin 8µg/mL or florfenicol 16µg/mL) followed by plating onto Slanetz–Bartley selective agar, without/with the same antibiotics (37°C/48h). Typical colonies were saved for identification (MALDI-TOF MS) and antibiotic susceptibility test (disk diffusion; EUCAST/CLSI). Prevalence percentages were calculated on a per-sample basis. **Results:** All samples contained *Enterococcus* (n=43) that were identified as *Efm* (n=18, 60%), *E. hirae* (n=14, 47%), *Efs* (n=9, 30%), *E. casseliflavus* and *E. durans* (n=1 each, 3%); and resistant to erythromycin (44%), tetracycline (39%), chloramphenicol (20%), ampicillin, linezolid, high-level streptomycin (17% each), ciprofloxacin (13%), and high-level gentamicin (7%). MDR isolates (23%) were mostly obtained from

calves rather than adults, and only found in *Efs* (67%) and *Efm* (50%) species. Linezolid-resistant isolates were only recovered from supplemented media with florfenicol while those resistant to ampicillin were better detected using culture medium with ampicillin or florfenicol. Ampicillin resistance was only detected in *Efm* while linezolid resistance was identified in both *Efm* and *Efs* (all calves, all MDR, 2 farms). **Conclusions:** Our study shows that dairy cattle carry MDR *Enterococcus* spp., including strains resistant to critically important antibiotics in the treatment of human infections (linezolid). These findings underscore the urgent need for sustained AMR surveillance and cross-sector collaboration within a One Health framework.

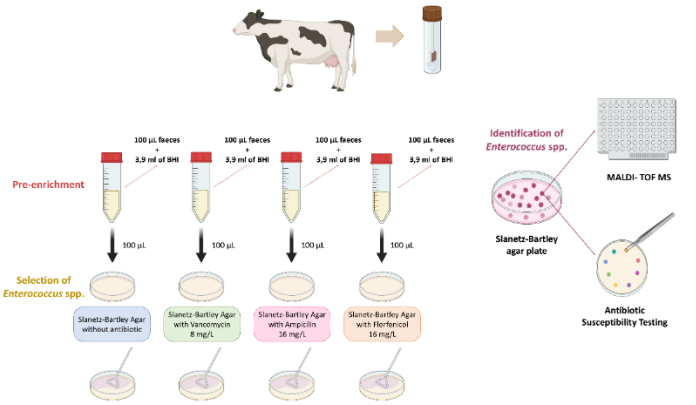

Figure 1. Schematic processing of cattle faecal swab samples towards the isolation, identification and characterization of *Enterococcus* spp.

Keywords: antimicrobial resistance; *Enterococcus* spp.; cattle

Acknowledgments/Funding
This work received financial support through the annual funding of 1H-TOXRUN of the University Institute of Health Sciences (IUCS-CESPU).

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OC04 MicroMundo@IUCS_CESPU (2022-2024): uncovering soil's hidden treasures and its impact on science and antimicrobial resistance awareness

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ABSTRACT

Background: MicroMundo@IUCS_CESPU (MMIC) is a service-learning pedagogical approach allowing university/school students to expand their knowledge on soil biodiversity, antimicrobial resistance (AMR) and experimental sciences [1]. School students experience a real research project by processing soil samples and looking for potential antibiotic-producing microorganisms [2,3]. **Objective:** To evaluate past MMIC editions' results (2022-2024) and to explore clinically-relevant antibiotic-resistant bacteria (CAB) in soils gathered within the project. **Methods:** University students (n=23), guided by professors, led 12 sessions for basic/secondary Cristelo school students (n=60). At MMIC end, promising soil colonies with antibiosis activity against *Escherichia coli* and/or *Staphylococcus epidermidis* were saved. A post-survey evaluated student experiences. After MMIC, soil samples underwent more analysis, including extended antibiosis assays using additional species and multidrug-resistant (MDR) strains, and screening for CAB using selective media for *Enterococcus* (Slanetz-Bartley with/without ampicillin or vancomycin) and *Enterobacterales* (MacConkey with/without cefotaxime, Coliform Chromogenic, Hicrome *Klebsiella* Selective agar) identification. Typical colonies were identified by MALDI-TOF and antibiotic susceptibility was tested by disk diffusion (EUCAST/CLSI). **Results:** Most students increased their interest in Science (86% on average) and AMR awareness (89% on average) (Fig.1). Of 26 soil samples processed, 310 colonies were tested, with two *Bacillus cereus thuringiensis* isolates from a moist agricultural soil in Cristelo showing antimicrobial activity against *E. coli*, *S. epidermidis*, *S. aureus,* besides MDR *E. coli* and *S. aureus*. Among CAB isolated from soils, *E. faecium* (n=11) showed tetracycline (64%) and erythromycin (9%) resistance. Within *Enterobacterales* (n=10; 30% MDR), species included *E. coli* (60%), *Citrobacter braaki* (20%), *Escherichia marmotae* (10%), and *Klebsiella aerogenes* (10%), with resistance to ampicillin and amoxicillin+clavulanic acid (40% each), cefotaxime and gentamicin (30%), aztreonam (20%), sulfamethoxazole+trimethoprim and tetracycline (10% each). **Conclusions:** The MMIC project is positively impacting students' AMR awareness. Our findings highlight soil as both a valuable reservoir of antibiotic-producing bacteria and a potential source of MDR bacteria, reinforcing its role in the One Health fight against AMR, and the revival of antibiotic discovery.

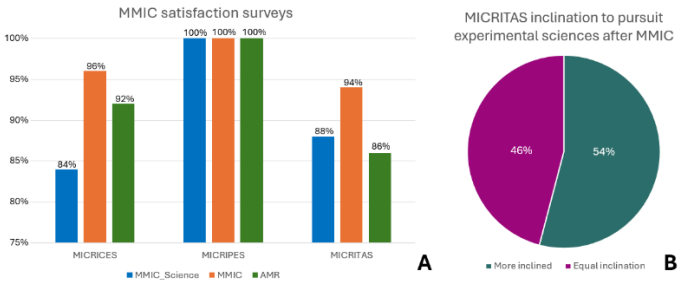

Figure 1. MMIC satisfaction surveys. A. MICRICES indicate school students, MICRIPES indicate school professors and MICRITAS indicate university students. B. MICRITAS inclination to pursuit experimental sciences after MMIC.

Keywords: MicroMundo; antibiotics; soil

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OC05 Characterisation of the poisoning profile in the emergency department of Lamego hospital

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ABSTRACT

Background: In clinical practice, the concept of poisoning refers to contact with substances that, depending on their physical and chemical properties, can disrupt the normal functioning of the body. Drug poisonings are becoming increasingly common and have become one of the most significant public health issues [1]. **Objective:** The main objective of this study was to characterize adult poisonings treated in the Emergency Department of Lamego Hospital (EDLH). **Methods:** This is an observational, descriptive, cross-sectional, and retrospective study. The data collected refer to cases of possible poisonings treated at EDLH between January 2021 and December 2022. A total of 136 possible poisoning cases were recorded at EDLH, of which 15 cases were excluded (13 because the individuals were underage and 2 due to incomplete data). Therefore, the study was based on a sample of 121 cases. **Results:** The majority of poisonings were alcohol-related (n=65, 53,7%), followed by drug poisonings (n=36, 29.8%), where in 2 cases there was concomitant ingestion of alcohol and drugs, with carbon monoxide poisonings (n=6, 5%) and chemical products (n=3, 2.5%) being less frequent. The predominant symptoms were related to the central nervous system (83.4%). A higher incidence was observed among males (52.1%), and the average age was 43 years. Regarding drug poisoning, the most involved pharmacotherapeutic group was anxiolytics, sedatives, and hypnotics (n=26, 68.4%). The predominant route of contact was oral, and most poisonings were voluntary. Individuals who suffered from drug poisonings were older (mean age 48 years) than those who suffered from alcohol poisonings (mean age of 38 years). Alcohol poisonings were more common in males (40.6%) compared to females (13.2%), while drug poisonings were more frequent in females (26.5%) compared to males (3.3%). Of the cases studied, 25% (n=30) of the individuals had concomitant diseases and were undergoing chronic treatment. In terms of treatment, fluid therapy was the most used intervention (n=79, 65%), and antidotes were administered in 8 cases (6.6%). **Conclusions:** The obtained results highlight the need for community health intervention strategies, raising awareness for the rational use of medications as well as for moderate alcohol consumption.

Keywords: poisonings; medicines and alcohol; hospital emergency

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OC06 Unveiling the toxicological risks of synthetic cannabinoid smoking: a GC-MS-based investigation

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ABSTRACT

Background: Synthetic cannabinoids (SCs) are a significant class of new psychoactive substances (NPS) commonly consumed via smoking. This process generates pyrolysis byproducts, some of which may exhibit increased toxicity compared to their parent compounds. The structural diversity and rapid emergence of SCs

and the use of different herbal products pose challenges in forensic toxicology, necessitating comprehensive chemical analysis of SC-infused herbal blends before and after combustion [1]. **Objectives:** This study aimed to identify SCs and their combustion byproducts in an herbal blend using gas chromatography-mass spectrometry (GC-MS). The goal was to assess potential structural modifications due to pyrolysis and evaluate their toxicological relevance. **Methods:** A commercially available SC herbal blend, *Esfinge*, provided by the Portuguese Judiciary Police, was analyzed before and after combustion. GC-MS analysis used headspace solid-phase microextraction (HS-SPME) and liquid extraction with dichloromethane and methanol. A controlled combustion apparatus was used to simulate smoking conditions. The detected compounds were identified through spectral library matching and/or by comparison with chemical standards and their toxicological potential was assessed using Globally Harmonized System (GHS) hazard statements. **Results:** Before combustion, JWH-210 was the primary SC identified, along with other organic compounds (i.e. Eucalyptol, Oplopanone). After combustion, several pyrolysis products emerged, including naphthalene, quinoline, indole, and acetophenone, which are known to have potential toxicological effects [2]. The transformation of SCs into novel pyrolytic derivatives suggests alterations in their pharmacological and toxicological profiles. **Conclusions:** The study highlights the formation of hazardous pyrolysis products from SC combustion, emphasizing the need for further toxicological evaluation. These findings contribute to forensic toxicology by improving the understanding of SC combustion products and their implications for public health and regulatory frameworks.

Keywords: synthetic cannabinoids (SCs); pyrolysis byproducts; gas chromatography-mass spectrometry (GC-MS)

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OC07 Alcohol consumption, depression, stress and anxiety in post-pandemic university students: exploratory study

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ABSTRACT

Background: The COVID-19 pandemic disrupted students' lives, impacting mental health and behaviors [1,3]. Increased anxiety, depression, and alcohol use were reported, influenced by social isolation and academic stress [1,2]. This study explores these effects on Portuguese university students in the post-pandemic period. **Objective:** This study aimed to investigate the impact of the post-COVID-19 pandemic period on alcohol consumption, as well as on stress, anxiety, and depression levels among university students, analyzing the possible influence on their mental health. **Methods:** The research followed a quantitative, descriptive-correlational, and cross-sectional design, with non-probabilistic convenience sampling. Participants included students aged 18 years or older, who were regularly enrolled in a higher education institution in Portugal. The study applied sociodemographic characterization questionnaires, the Alcohol Use Disorders Identification Test (AUDIT) to assess alcohol consumption, and the Depression, Anxiety, and Stress Scale (DASS-21). The collected data were analyzed using descriptive and inferential statistics, with a significance level of $p<0.05$. **Results:** A significant percentage of students increased alcohol consumption during the analyzed period, with 12.6% classified as engaging in harmful use or probable dependence. Regarding mental health, 41.4% reported moderate to extremely severe depressive symptoms, 27.5% had extremely severe anxiety, and 36.2% experienced moderate to severe stress. Factors like age, marital status, and year of study showed significant correlations with mental health indicators, suggesting these factors influence students' coping strategies. The findings highlight the need for prevention and intervention strategies to reduce harmful alcohol use and manage anxiety, depression, and stress. Higher education institutions should collaborate with healthcare professionals to create support programs, provide mental well-being workshops, and promote healthy lifestyles. Longitudinal studies are crucial to monitor these factors and assess intervention effectiveness. **Conclusions:** The post-pandemic period may have worsened alcohol consumption and mental health issues among university students. Adopting psychological support strategies and promoting healthy habits are crucial to minimizing negative impacts on academic performance and quality of life, fostering a safer university environment that supports personal and professional growth.

Keywords: alcohol consumption; depression; university students.

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OC08 From resistance to sensitivity in non-small cell lung cancer: newly established multidrug-resistant cancer cells contribute to identifying DNA damaging agents as collateral sensitizer drugs

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ABSTRACT

Background: Non-small cell lung cancer (NSCLC) is the most common form of lung cancer and presents a low 5-year survival rate [1]. Standard treatment of NSCLC includes chemotherapeutic drugs, such as taxanes and platinum-based agents. However, cancer cells may acquire resistance to multiple unrelated chemotherapeutic agents, known as multidrug resistance (MDR), which significantly limits treatment options [2]. Remarkably, it was discovered that some compounds present a stronger antitumor effect on MDR cells than on sensitive counterpart cells. This phenomenon, known as collateral sensitivity, may represent an important strategy to overcome MDR [3]. **Objective:** This work aimed to establish MDR cell lines from a sensitive NSCLC parental cell line and to identity collateral sensitizer drugs by using these newly established MDR cell sublines. **Methods:** Treatment of NSCLC A549 cells with progressively increasing concentrations of paclitaxel led to the establishment of two new MDR sublines. The MDR phenotype was confirmed using the Sulforhodamine B and Rhodamine123 accumulation assays. Further characterization of the cells included analysis of selected proteins expression levels by Western Blotting and evaluation of cell death and ROS production levels by flow cytometry. **Results:** The newly established cell lines were confirmed to be resistant to paclitaxel and also to other chemotherapeutic drugs from different drug classes. Moreover, the MDR phenotype of these cells was confirmed by the overexpression and increased activity of drug efflux pumps, such as P-glycoprotein. Curiously, MDR cells were more sensitive than the parental drug-sensitive cells to specific drugs involved in DNA damage, indicating that the MDR sublines presented a collateral sensitive effect to these drugs. The MDR cells presented higher DNA damage and increased ROS production than the parental sensitive cells, which could justify the higher susceptibility of the MDR cells to DNA damaging drugs. **Conclusions:** Two MDR NSCLC sublines which may become relevant models to study collateral sensitivity and to identify compounds to overcome MDR in NSCLC were successfully established and characterized. Most importantly, DNA-damaging drugs caused collateral sensitivity in these MDR cells, indicating that these drugs are good treatment options for NSCLC MDR patients.

Keywords: non-small cell lung cancer (NSCLC); multidrug resistance (MDR); collateral sensitivity.

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OC09 Development and validation of an HPLC-DAD analytical method for psilocybin and psilocin

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ABSTRACT

Background: "Magic mushrooms", also known as hallucinogenic mushrooms, belong to the genus *Psilocybe* and contain tryptamines, especially psilocybin and psilocin, with potential medicinal benefits for mental health [1]. Psilocybin is usually found in higher concentrations than psilocin and both are present in dried mushrooms at a total concentration of approximately 0.5 to 1.5%. *Psilocybe* mushrooms are used for religious, recreational, and therapeutic purposes due to their psychedelic effects [1]. The increasing use of these tryptamines and other psychoactive drugs led to regulatory legislation, specifically Portuguese Decree-Law

No. 15/93 of January 22, which aims to control the production, distribution, possession, and use of these substances. High-performance liquid chromatography (HPLC) coupled with UV-Vis, diode array (DAD) [2], or mass spectrometry (MS) [3] are commonly used analytical methods to quantify tryptamines. **Objective:** Development and validation of an HPLC-DAD analytical method for quantification of psilocybin and psilocin in *Psilocybe cubensis* extracts. **Methods:** Mushrooms were pulverised using a porcelain mortar and pestle and extracted with cold methanol by kinetic maceration on a magnetic stirrer plate. An Agilent 1260 Infinity II HPLC-DAD system with a Poroshell 120 EC-C18 3.0 x 150 mm, 2.7 µm column protected with a Poroshell 120 EC-C18 3.0 mm, 2.7 µm guard column was used for analytical determinations. The optimised chromatographic method was established with mobile phase solvents: 10 mM ammonium formate with 0.1% formic acid and acetonitrile; oven temperature: 40 °C; flow rate: 0.8 mL/min; total run time: 12 minutes; injection volume: 1 µL; and UV detection wavelength: 220 nm. **Results:** To optimise the HPLC-DAD analytical method, various parameters were evaluated: mobile phase gradients and solvents, oven temperature, flow rate, run time, post-run time, injection volume and UV detection wavelength. This analytical procedure was validated following the ICH Q2 guideline. Linearity was tested over a range of 9 concentrations (0.50-200 µg/mL for psilocybin and 0.25-100 µg/mL for psilocin), obtaining R^2 values > 0.999. Method precision (%RSD) was ≤ 10%, with an accuracy (%bias) ≤ 15% for both compounds. **Conclusions:** An HPLC-DAD analytical method for the detection and quantification of psilocybin and psilocin was optimised, validated, and effectively applied to *Psilocybe cubensis* extracts.

Keywords: psilocybin; HPLC-DAD; mushrooms.

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OC10 Binding of synthetic cannabinoids to human serum albumin: site characterization and recognition mechanisms

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ABSTRACT

Background: The use of new psychoactive substances (NPS) has been increasing since the beginning of the 2000's, with synthetic cannabinoids being one of the most reported groups. Consumers of these drugs typically seek their known psychoactive effects, such as relaxation and euphoria. Numerous reports of morbidity and mortality have been associated with the consumption of these substances [1,2]. Therefore, toxicokinetic and toxicodynamic studies are needed to better understand the behaviour of these NPS. **Objective:** This study aims to evaluate the binding affinity of a series of synthetic cannabinoids to human serum albumin (HSA), obtain insights into the binding sites and better understand the recognition mechanisms. **Methods:** Zonal elution chromatography was used to assess the binding affinity of five synthetic cannabinoids using a HSA-based column, CHIRALPAK® HSA. Mixtures of potassium phosphate buffer (67 mM, pH 7.0) and acetonitrile were used as mobile phases. Displacement experiments with concentrations ranging from 0-20 µM of well-known site-specific probes, including warfarin, (*S*)-ibuprofen and L-tryptophan were carried out. Both studies were performed using high-performance affinity chromatography (HPAC), which is a widely used and effective technique for evaluating intermolecular interactions between HSA and drugs [3]. **Results:** The binding percentages (*%b*) ranged from 98.7% to 99.9%. FUBIMINA showed the highest binding affinity, with a *%b* of 99.9%. Competition for site I was observed between warfarin and four synthetic cannabinoids. Molecular docking studies supported experimental findings, allowing to elucidate the recognition mechanisms, identify binding sites, and characterize their interactions with the protein. Surprisingly, the binding affinity of all synthetic cannabinoids increased in the presence of (*S*)-ibuprofen. **Conclusions:** The synthetic cannabinoids bounded to HSA with high affinity. Extrapolating this situation to real-world scenarios, particularly when synthetic cannabinoids are consumed, especially in uncontrolled high doses, alongside other drugs with high affinity to site I of HSA, a serious issue may arise. Additionally, all compounds showed increased retention to HSA in the presence of (*S*)-ibuprofen, resulting in a lower free fraction of these compounds. Consequently, (*S*)-ibuprofen may serve as a promising candidate for exploring potential strategies to mitigate synthetic cannabinoid's harmful effects.

Keywords: synthetic cannabinoids; high-performance affinity chromatography; displacement studies.

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Cardoso, T., et al., Enantioresolution and Binding Affinity Studies on Human Serum Albumin: Recent Applications and Trends. *Chemosensors* **2021**, 9, 304-338

OC11 Assessing the herbicidal potential of *Rhodopirellula rubra* LF2 extracts: a promising approach for sustainable weed control

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ABSTRACT

Background: The increasing global population and the corresponding rise in food demand require improvements in crop yields, as well as a reduction in the impact of weeds in agricultural fields. One potential sustainable solution may lie in the ocean—an area of the Earth that remains largely unexplored, yet harbors rich and diverse biodiversity. In our group, marine microorganisms have been found to produce bioactive compounds with antimicrobial, anti-obesogenic and antioxidant properties. [1,2] **Objective:** This study aims to screen extracts obtained from the *Planctomycetota* bacterium *Rhodopirellula rubra* strain LF2 as potential biofertilizers and/or bioherbicides. **Methods:** *R. rubra* LF2 was cultivated in three distinct culture media (M600, M607, and 1/10 M607), each varying in nitrogen and carbon concentrations. This approach was based on the premise that varying nutrient levels would activate distinct metabolic pathways. The cultures underwent extraction by exposure to ethyl acetate overnight. The resulting organic solution was dried, and the obtained powder was solubilized in 10% DMSO. The extracts were then tested in screening assays using three model plants: *Arabidopsis thaliana*, *Lactuca sativa*, and *Lolium perenne*. The plants were grown in solid Hoagland medium in the presence of the extracts for 2 to 3 weeks under controlled conditions (25°C, 16h:8h light:dark cycle). Biometrical parameters, including fresh weight, root length, and number of leaves, were measured to assess the effects of the extracts on plant growth. **Results:** *L. perenne* and *L. sativa* were unaffected by the *R. rubra* extracts in any of the parameters analyzed. However, *A. thaliana* was strongly affected by *R. rubra* extracts produced from M607 and 1/10 M607 cultures. These extracts completely inhibited seed germination. Extracts from the M600 culture significantly reduced root length, increased the number of leaves, and had no effect on the fresh weight of the plants. Additionally, M600 extracts reduced *A. thaliana* germination by more than 70%. **Conclusions:** This study provides valuable insights into *R. rubra* LF2 extracts, highlighting the potential of marine resources. The extracts clearly contain compound(s) that strongly inhibit the growth of *A. thaliana*, a common weed, while having no effect on the crop species *L. sativa* and the ryegrass *L. perenne*, both of economic interest. This selective activity suggests a potential bioherbicide capacity, which requires further investigation.

Keywords: bacterial extracts; bioherbicides.

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OC12 Citizen science biomonitoring of metal(loid) pollution in honey from Northwest England

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ABSTRACT

Background: Honey can be used to biomonitor contaminants, as honeybees can bioaccumulate both organic and inorganic (e.g., metal(loid)) contaminants from the total environment [1]. Honey chemical composition can be influenced by natural and anthropogenic activities [2]. **Objective:** Metal(loid) concentrations in honey samples collected by citizen scientist beekeepers in northwest England during fall 2018 were measured to assess potential sources from current and historical land use [3]. **Methods:** Spatial distribution of honey metal(loid) concentrations was assessed using a geographic information system (GIS) using postal codes to identify beehive locations of citizen scientists and analyzed using inductively coupled plasma mass spectrometry (ICP-MS). GIS tools were used to correlate metal(loid) concentrations with local pollution point sources and soil geochemistry databases [3]. **Results:** Metal(loid) concentrations in honey measured in the Greater Manchester area varied widely (**Figure 1**). The Greater Manchester area had higher mean As and Cd concentrations in honey (180 µg/kg and 398 µg/kg, respectively) compared to global means 25 µg/kg and 150 µg/kg, respectively), but mean honey Cu, Pb and Zn concentrations were lower (225 µg/kg, 260 µg/kg and 426 µg/kg, respectively) than global means (1005 µg/kg, 1470 µg/kg and 8545 µg/kg, respectively). Cd and Pb concentrations in honey measured in the Greater Manchester area were 398 and 260 µg/kg, respectively, which are up to two orders of magnitude higher than the recommended World Health Organization (WHO) and Food and Agriculture Organization (FAO) guidelines, which may warrant further study to determine potential health impacts from honey consumption. Landscape analysis showed no correlation between metal(loid) contaminants and beehive location. **Conclusions:** This baseline study demonstrates that honey collected by citizen scientists can be used as an effective environmental biomonitoring

tool to analyze metal(loid) contaminants in honey samples in urban areas.

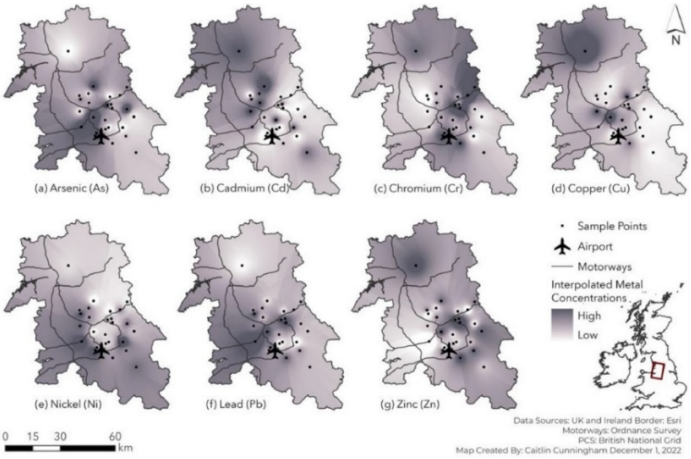

Figure 1. Patterns of interpolated relative concentrations of arsenic (a), cadmium (b), chromium (c), copper (d), nickel (e), lead (f), and zinc (g) across Manchester and surrounding area, UK. Sources for data layers on the map: UK and Ireland borders: Esri, Motorways: Ordnance Survey. Projected Coordinate System: British National Grid. Map created using ArcGIS Pro v. 3.0.2.

Keywords: honeybees; air pollution; contaminants.

Acknowledgments/Funding
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IV 1H-TOXRUN INTERNATIONAL CONGRESS 2025

08-09 MAY, 2025

Porto, Portugal

POSTER COMMUNICATIONS

PC01 Microscopic and molecular screening of myxosporeans in teleost fishes caught from Northeast Atlantic waters

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ABSTRACT

Background: Globally, the demand for fishery resources has increased significantly to meet the nutritional needs of the population. With the continuous increase in aquaculture and the introduction of new species, knowledge of the life cycle of infectious agents is essential, as it is crucial for public health and industry [1]. Myxosporeans are microscopic multicellular parasites belonging to the phylum Cnidaria Hatschek, 1888, which have as hosts fishes from different habitats with wide geographical distribution. They have a complex life cycle involving two hosts: an invertebrate (usually oligochaetes or polychaetes) and a vertebrate, typically fish [2]. **Objective:** This study aimed to expand knowledge of the diversity of myxosporeans parasitizing stocks of teleost species caught from Northeast Atlantic waters, with high commercial value and potential to be introduced into aquaculture, namely *Pagrus caeruleostictus* (bluespotted seabream), *Plectorhinchus mediterraneus* (rubberlip grunt) and *Sarpa salpa* (salema porgy). **Methods:** Specimens were necropsied and a myxosporean survey was carried out in internal and external tissues. Samples of tissue were analysed by light microscopy and, when infected, photographed for morphological characterization and prepared for histology and molecular procedures targeting the 18S rDNA. Positive PCR products were sequenced, and the consensus sequences were analysed by BLAST in MEGA11 software. **Results**: Morphological and molecular analyses revealed the presence of three myxosporean species. In *P. caeruleostictus*, an *Unicapsula* sp. was observed parasitizing the skeletal muscle; in *P. mediterraneus*, a *Lateroacaudata* sp. was found in the anterior and posterior kidney; and in *S. salpa*, a coelozoic parasite of the genus *Ceratomyxa* was present in gallbladder. **Conclusions:** In *P. caeruleostictus* a new occurrence of *Unicapsula pflugfelderi* Schubert, Sprague and Reinboth, 1975, previously described in two sparids species from Mediterranean [3], is reported; in *P. mediterraneus*, a potential new species is described having morphological similarity (inexistence molecular data) with the only other species of the genus *Laterocaudata* found in the gill of a freshwater fish in China [4]. Finally, in *S salpa*, molecular identity was provided for a species of *Ceratomyxa* previously reported in specimens captured from Tunisian waters [5].

Keywords: marine fishes; parasites; Myxozoa.

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PC02 Exploring cannabinoids as a novel strategy against antimicrobial resistance: a systematic review

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ABSTRACT

Background: Natural products have been widely researched for their potential antimicrobial properties in the fight against microorganisms' resistance to traditional drugs. Among these, cannabinoids have gained significant attention for their broad range of biological activities and their potential to enhance the effectiveness of traditional antimicrobial treatments [1]. **Objective:** This work presents a systematic review that provides a summary of research from 2020-2024 on the therapeutic potential of cannabinoids against various microorganisms, including bacteria, fungi, and viruses. **Methods:** A literature search was conducted in PubMed, Scopus and ScienceDirect databases following the PRISMA guidelines. References were screened and selected based on eligibility criteria, with full-text articles assessed for inclusion and summarized. **Results:** The final selection included 46 papers. The results demonstrated that cannabinoids have significant antibacterial activity, especially against Gram-positive bacteria including *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecium*, methicillin-resistant *Staphylococcus aureus* and vancomycin-intermediate resistant *Staphylococcus aureus*, as well as the oral cariogenic *Streptococcus mutans* [2,3]. Despite the limited antimicrobial activity against Gram-negative bacteria, due to the presence of the outer membrane, lipopolysaccharides and porins, some studies have demonstrated the antibacterial efficacy of cannabidiol (CBD) against *E. coli*, *Neisseria gonorrhoeae* and *Pseudomonas aeruginosa* [2]. Several cannabinoids have been shown to inhibit bacterial biofilm formation by reducing the expression of key biofilm-regulating genes, decreasing extracellular polysaccharide

production, disrupting quorum sensing and increasing ROS production [4]. CBD exhibits strong antiviral activity against SARS-CoV-2 variants by reducing viral entry, preventing early-stage replication, and modulating the immune response in later stages [5]. Additionally, CBD impacts cellular membranes, inhibiting the replication of Zika virus and other viruses, indicating its broad-spectrum antiviral potential. CBD also inhibited the growth and formation of *Candida albicans* biofilm and induced disorganization of mature biofilm, showing an antifungal activity [6]. Cannabinoids, when combined with traditional antimicrobial agents, show potential for synergistic interactions, making them a promising strategy in combating drug resistance. **Conclusions:** Cannabinoids hold potential as a novel approach to develop treatments for drug-resistant infections, reducing the need for traditional antimicrobial agents.

Keywords: cannabinoids; antimicrobial potential; antibacterial; antiviral; antifungal

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PC03 Antimicrobial photodynamic inactivation of *Pseudomonas aeruginosa* Biofilms: a synergistic approach with berberine, gentamicin, and colistin

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ABSTRACT

Background: Chronic wound infections caused by *Pseudomonas aeruginosa* biofilms are highly resistant to conventional treatments, necessitating innovative therapeutic strategies [1]. Antimicrobial photodynamic inactivation (aPDI) has proven to be a promising alternative [2]. However, its efficacy against Gram-negative bacteria remains suboptimal due to the restricted penetration of light and photosensitizers through the biofilm matrix, which hinders its full antimicrobial potential [3]. **Objective:** This study explores the potential of colistin (Col) at subinhibitory concentrations to enhance the photodynamic activity of a berberine-gentamicin (Ber-Gen) combination against *P. aeruginosa* biofilms. **Methods:** *P. aeruginosa* ATCC 10145 strain from the American Type Culture Collection was used. Col-induced membrane permeability changes were assessed by flow cytometry, and synergistic interactions within the Ber-Gen-Col combination were determined by checkerboard assay. Biofilms were exposed to blue light (420 nm, 30 mW/cm^2, 10 min) after one or three aPDI cycles (administered at 24 h intervals). Treatment efficacy was evaluated by quantifying biomass (crystal violet), metabolic activity (resazurin), and culturability (CFU/cm^2). The mechanism of action was examined by ROS production (fluorometry), membrane damage (microscopy), and biofilm structural changes (optical coherence tomography). **Results:** Colistin (4 µg/mL) increased membrane permeability by 30 %, significantly enhancing the photodynamic action of Ber-Gen-Col. The triple combination led to a nearly complete eradication of biofilm cells, achieving a 7-log CFU/cm^2 reduction and over 90 % decreases in biomass and metabolic activity. However, sustained suppression of biofilm regrowth was observed only after multiple irradiation cycles, while single-cycle treatments allowed biofilm recovery within 24 h. Regarding the mechanism of action of photoactivated Ber-Gen-Col, a significant disruption of the biofilm structure, increased reactive oxygen species (ROS) generation, and extensive membrane damage were observed. **Conclusion:** These findings demonstrate that integrating subinhibitory Col concentrations with repeated aPDI cycles represents a promising strategy for effectively eliminating *P. aeruginosa* biofilms in chronic wound infections.

Keywords: Antimicrobial photodynamic inactivation, berberine-gentamycin-colistin combination, *Pseudomonas aeruginosa* biofilms

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PC04 Targeting quorum sensing: ferulic and sinapic acids compromise *Pseudomonas aeruginosa* biofilm architecture and virulence

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ABSTRACT

Background: *Pseudomonas aeruginosa* is a Gram-negative pathogenic bacterium that is frequently associated with chronic infections in immuno-compromised individuals, such as those with cystic fibrosis or burns [1]. The pathogenicity and virulence of *P. aeruginosa* are primarily regulated by quorum sensing, with the *las* system playing a key role in this process. This system is essential for the formation and maintenance of the biofilm and for controlling various virulence factors [2]. Phenolic acids, such as ferulic and sinapic acids, are plant secondary metabolites, well known for their biological properties and have shown promise in modulating bacterial communication [3]. **Objective:** The aim of this study was to evaluate the potential of ferulic and sinapic acids to inhibit the *P. aeruginosa las* QS system and its underlying effects on biofilm structure and virulence factor production. **Methods:** The inhibitory effect on the *las* system was evaluated using bioreporter strains and bioluminescence-based assays. Biofilm architecture was analyzed using optical coherence tomography, while virulence factors (pyoverdine, pyocyanin, total proteases, lipases, gelatinases and siderophores) production and motility were investigated by absorbance measurement and plate agar method. **Results:** Ferulic and sinapic acids inhibited *las* QS activity by 90 % at a concentration of 1000 µg mL^{-1}. The N-3-oxododecanoyl-homoserine lactone production was reduced by 70 % at just 6.25 µg mL^{-1} of the phenolic acids. These compounds significantly changed biofilm architecture, reducing biofilm thickness from 25 µm to 9 µm. They also reduced the production of key virulence factors and impaired swarming motility. **Conclusion:** Ferulic and sinapic acids demonstrated strong inhibitory effects on the *las* QS system, leading to altered biofilm structure and reduced virulence. These findings support their potential as antipathogenic and antivirulence agents for prevention/treatment of *P. aeruginosa* biofilm-associated infections.

Keywords: biofilm architecture; quorum-sensing; *Pseudomonas aeruginosa*.

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PC05 Photodynamic inactivation of methicillin-resistant *Staphylococcus aureus* using harmine-antiseptic combinations

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ABSTRACT

Background: Antibiotic-resistant pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA), pose significant challenges in the treatment of chronic wound infections. Antibacterial photodynamic therapy (aPDT) offers a localized, resistance-independent alternative to antibiotics [1]. In aPDT, bacteria is targeted by molecules called photosensitizers (PSs) that are activated by light. Natural PSs are more eco-friendly, sustainable, cost-effective, and therefore, preferred over synthetic alternatives [2]. Harmine (HA), an alkaloid commonly found in various plants and animals, is a promising natural PS, with reported photodynamic efficacy against cancer cells [3]. **Objectives:** This study explores aPDT with HA as a PS and its combined action with wound antiseptics, namely octenidine dihydrochloride (OCT), polyhexamethylene biguanide (PHMB), and hydrogen peroxide, against *S. aureus* (MJMC568-B: MRSA) to enhance antiseptic efficacy and bacterial damage while minimizing concentrations. **Methods:** The antibacterial efficacy of HA, OCT, PHMB and H_2O_2 was independently evaluated by determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values using the microdilution and plating agar methods, respectively. An evaluation of the capacity of HA-antiseptics combinations to boost the antibacterial activity was conducted by a disk diffusion assay,

and a checkerboard assay. The photodynamic activity of HA was assessed independently and in combination by irradiating with UV light (365 nm) using a light-emitting diode (LED) system and quantification through colony-forming unit (CFU) analysis. **Results:** Antibacterial efficacy of HA individually and in combination with antiseptics against *S. aureus* was assessed, and an increase in the antibacterial activity when combined was observed. UV-A irradiation of 19.8 J/cm^2 (5.5 mW/cm^2, 60 min) enhanced the antibacterial capacity of HA (250µg/ml), significantly reducing *S. aureus* culturability (3.85±0.39 CFU/cm^2 logarithmic reduction compared to a 0.10±0.17 CFU/cm^2 logarithmic reduction when not irradiated), highlighting its potential for aPDT applications. **Conclusions:** These findings highlight HA potential as a natural PS for aPDT and its role in enhancing antiseptic efficacy against MRSA. The combined aPDT-antiseptic approach offers a promising strategy for chronic wound management, reducing antibiotic reliance and mitigating resistance development.

Keywords: antibacterial photodynamic therapy; antiseptics; harmine.

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PC06 From In to Out: assessing antimicrobial-resistant *Escherichia coli* across treatment stages of wastewater

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ABSTRACT

Background: Wastewater treatment plants (WWTPs) are critical points for the selection and transmission of antimicrobial resistance (AMR), which has become a major global public health concern [1,2]. The One Health approach is essential in addressing this issue, as AMR affects both human and environmental health [3]. **Objective:** To evaluate the role of WWTPs as reservoirs and/or sources of antimicrobial-resistant *Escherichia coli*, a key indicator of fecal contamination. **Methods:** Samples were collected from a WWTP located in northern Portugal, designed primarily for domestic sewage treatment. The sampling covered various stages of the treatment process: influent (untreated water), effluent (treated water), biological sludge, and dehydrated sludge, across four seasons (Spring, Summer, Autumn, and Winter). *Escherichia coli* levels were quantified using MUG-EC microplates (with and without ciprofloxacin, 1 µg/mL) for influent and effluent samples. Bacterial isolation and enumeration were performed using Chromogenic Coliform Agar (CCA) for all sample types, with isolate identification via 16S rRNA analysis. Antimicrobial susceptibility testing (AST) was conducted using the disk diffusion method (CLSI/EUCAST). **Results:** *E. coli* levels showed a reduction by a factor of approximately 10^1 (Autumn and Winter) to 10^2 (Spring and Summer) between influent and effluent. The study with ciprofloxacin showed that the levels of resistant strains increased in Spring but decreased in the other 3 seasons. In general, the enumeration of *E. coli* using CCA correlated with the MUG-EC results. The 16S rRNA gene affiliation resulted in non-discriminatory results between *Escherichia* spp. and *Shigella* spp., leading to difficult AST interpretation. Despite this, variable susceptibility to gentamicin, tetracycline, ciprofloxacin, and imipenem was observed. Of concern, bacteria released into the environment via effluent (discharged directly into water bodies) and dehydrated sludge (used in agriculture) exhibited higher AMR profiles, including multidrug resistance (MDR). **Conclusions:** This study demonstrates that WWTP treatment effectively reduces microbial load. However, the presence of antimicrobial-resistant bacteria in effluents and sludge, particularly with MDR profiles, raises concerns regarding environmental dissemination. Given the seasonal variability in AMR levels and the small sample size, further studies are needed to comprehensively assess AMR throughout WWTPs and its environmental impact.

Keywords: multidrug resistance; WWTPs; influent; effluent; sludge; One Health.

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PC07 The potential of selected natural products as biofilm control agents and antibiotic resistance modifiers

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ABSTRACT

Background: The emergence of multidrug-resistant bacteria, especially within biofilms, poses a serious public health threat and represents a major challenge for effective infection management. Traditional antibiotics often fail to effectively treat bacteria and biofilm-associated infections, prompting the need for novel strategies to combat bacterial resistance and biofilm formation. Natural compounds, such as phytochemicals and natural deep eutectic solvents (NADES), have emerged as promising candidates due to their diverse bioactive properties and potential synergistic effects with existing antibiotics. **Objective:** This study aimed to evaluate the antibiofilm and antibiotic resistance-modifying activities of selected natural compounds, including phytochemicals and NADES, in combination with antibiotics. **Methods:** *In vitro* assays were performed using two monoterpenes (menthol and linalool) and a choline chloride-raffinose NADES, combined with ten antibiotics (methicillin, amoxicillin, oxacillin, erythromycin, ciprofloxacin, mupirocin, fusidic acid, tetracycline, tobramycin, and gentamicin). The effects were tested on *Escherichia coli* CETC 102 and *Staphylococcus epidermidis* ATCC 35984. Minimum inhibitory and bactericidal concentrations (MIC and MBC, respectively) of the phytochemicals were determined. Antibiotic susceptibility was evaluated via disc diffusion, and biofilm control was assessed by biomass and metabolic activity quantification, and cell culturability. **Results:** Menthol displayed a MIC and MBC of 800 µg/mL against *E. coli* and MIC against *S. epidermidis*. Linalool exhibited a MIC of 800 and 400 µg/mL against *E. coli* and *S. epidermidis*, respectively. Disc diffusion indicated a potentiation effect of both molecules on erythromycin against *E. coli*, and of menthol on amoxicillin against *S. epidermidis*. Biofilm control studies showed that both molecules applied individually reduced more than 90% of the biofilm's metabolic activity, yielding, in some cases, a total reduction of culturable cells. Moreover, the dual combinations with antibiotics enhanced their activity up to 40% in biofilm metabolic inactivation, 13% in biomass removal, and 2.8 logs in culturability reduction. **Conclusions:** This study has revealed promising results, showing the potential of these monoterpenes and NADES as antibiotic resistance modifiers, and supporting menthol and linalool as strong antibiofilm agents.

Keywords: antimicrobial resistance; phytochemicals; natural deep eutectic solvents.

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PC08 Insect peptides as novel compounds against *Candida* spp. infections

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ABSTRACT

Background: *Candida* spp. infections are increasing all over the world, as well as antifungal resistance, highlighting the urgent need for new therapeutic strategies. Previously, several *in silico* studies have identified insect peptides as potential antifungal drugs – BLAP-6 (from *Blaps rhychopetera*) and Gomesin (from *Acanthoscurria gomesiana*) against different fungal species. **Objective:** The present study aimed to evaluate the antifungal activity of both peptides on *Candida* spp.. **Methods:** The evaluation was conducted using several methods, including disk diffusion, minimum inhibitory concentration (MIC), minimum biofilm eradication concentration (MBEC) (EUCAST guidelines), and biofilm biomass quantification by crystal violet staining. **Results:** MIC and MBEC assays showed that Gomesin has potent antifungal activity at lower concentrations (270 mg/L), achieving total biofilm eradication for most species, except C. *glabrata*. BLAP-6 exhibited moderate antifungal effects, with some tolerance/resistance profiles observed. **Conclusions:** BLAP-6 and Gomesin are promising drug candidates suitable for the treatment of *Candida* spp. infections. Furthermore, some species showed some tolerance/resistance to BLAP-6; therefore, the study of mechanisms involved should be seen as an important future perspective.

Keywords: *Candida* spp.; *Candida albicans*; *Candida tropicalis*; *Candida glabrata*; *Candida parapsilosis*; insect peptides; antifungal resistance; antifungal activity

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PC09 Exploring antimicrobial resistance in autochthonous Portuguese hens: a study of Escherichia coli on eggshells

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ABSTRACT

Background: The widespread use of antibiotics in intensive animal farming significantly contributes to antimicrobial resistance (AMR), posing a serious global public health threat [1]. Despite this, little is known about the role of autochthonous Portuguese laying hens as reservoirs of antibiotic-resistant *Escherichia coli*. Raised in extensive farming systems with reduced antibiotic exposure, these indigenous breeds offer a unique opportunity to study AMR dynamics under absent selective pressure. Investigating their role could yield valuable insights into the natural ecology of resistance and inform strategies for more sustainable livestock management [2]. **Objective:** To investigate the presence of antibiotic-resistant *E. coli* and antibiotic resistance genes on the 124 eggshells of autochthonous Portuguese laying hens. **Methods:** A total of 46 *E. coli* isolates previously obtained from *Preta Lusitânica* (n=15), *Amarela* (n=9), *Branca* (n=12), and *Pedrês Portuguesa* (n=10) eggshell swabs were confirmed by PCR. Antimicrobial susceptibility testing for 10 antibiotics was assessed following EUCAST/CLSI guidelines [3]. ESBL phenotype was searched using the Double-Disk Synergy Test. Antibiotic resistance genes (*bla*$_{TEM}$, *bla*$_{CTX}$, *bla*$_{OXA-1}$, *bla*$_{SHV}$) were searched by PCR in 44 of the 46 *E. coli* isolates. **Results:** *Escherichia coli* eggshell isolates exhibited resistance to gentamicin (96%), tetracycline (41%), ampicillin (24%), amoxicillin/clavulanic acid (4%), amikacin (4%), and trimethoprim/sulfamethoxazole (2%). Resistance to at least one antibiotic was found in 98% of the eggshell isolates, and a multidrug-resistant phenotype was identified in 17% of the isolates (*Preta Lusitânica, Branca, Pedrês Portuguesa*). Only 2% of the *E. coli* isolates showed pan-susceptibility (*Amarela*). ESBL phenotype was not detected. The *bla*$_{TEM}$ and *bla*$_{CTX}$ genes were detected in 36% and 11%, respectively, of the 44 *E. coli* isolates tested, while the *bla*$_{OXA-1}$ and the *bla*$_{SHV}$ genes were not detected. **Conclusion:** This study provides insights into AMR in autochthonous hens, highlighting the potential role of environmental factors, such as soil, water, feed, and even human contamination, in AMR transmission to the eggshells. The findings contribute to the understanding of AMR in extensive farming systems and underscore the importance of considering environmental sources in efforts to mitigate AMR, increase consumer confidence, and support the development of sustainable poultry practices.

Keywords: One Health; antimicrobial resistance; native breeds; laying hens; *Escherichia coli*.

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PC10 Diagnostic pitfalls in *Staphylococcus* spp. surveillance: lessons from a multinational university student cohort

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ABSTRACT

Background: *Staphylococcus aureus* is a leading cause of severe and hard-to-treat human infections, particularly when resistant to cefoxitin due to the presence of the *mec*A gene [1]. However, diagnostic challenges arise from the misidentification of *S. aureus* and related species when relying on classical identification methods (mannitol fermentation; coagulase production), as well as from the detection of strains carrying the *mec*A gene but phenotypically susceptible to cefoxitin - known as "stealth" strains [2]. **Objective:** Building upon a previous collection of *S. aureus* from healthy students' nares [3], we aimed to expand this collection with new samples, assess the occurrence of "stealth" isolates, and further investigate cases of incongruent identification. **Methods:** Nasal swab samples (n=557) from 507 students (median-23-years; 9 countries) attending a large university (Porto district) were collected between March 2022 and November 2024. They were inoculated onto mannitol-salt agar and, in parallel, enriched in brain-heart-broth with 6.5% NaCl further plated onto ChromID® MRSA-SMART. Isolates deriving from mannitol-salt (only fermenting colonies) and chromogenic (all typical colonies) agar media were stored for species identification (MALDI-TOF MS), cefoxitin-susceptibility (disk-diffusion), and *mecA* gene screening (PCR). **Results:** *Staphylococcus aureus* was identified in 46% (256/557; 6 countries) of cases. Other *Staphylococcus* species included *S. haemolyticus* (n=5), *S. capitis* (n=3), *S. warneri* (n=3), *S. saprophyticus* (n=1), *S. simulans* (n=1), and *S. ureilyticus* (n=1). These isolates expressed variable coagulase production (7 positive, 7 negative). On another hand, a non-fermenting *S. aureus* was detected (cromogenic medium). Ten (1.8%) students were colonized with methicillin-resistant staphylococci species carrying *mec*A including *S. aureus* (n=6), *S. haemolyticus* (n=3), *S.ureilyticus* (n=1). The *mec*A gene was also detected in 4/16 (25%; 3 Portuguese, 1 Italian) *S. aureus* susceptible to cefoxitin, the so-called "stealth" strains. Screening is ongoing in more isolates. **Conclusions:** Our study highlights the importance of integrating both phenotypic and genotypic methods for *Staphylococcus* accurate identification. Furthermore, the detection of stealth strains in healthy students underscores the need for robust community-based screening, as *S. aureus* carriage may be underestimated. Future studies will unveil if these strains are capable of reversion to resistance.

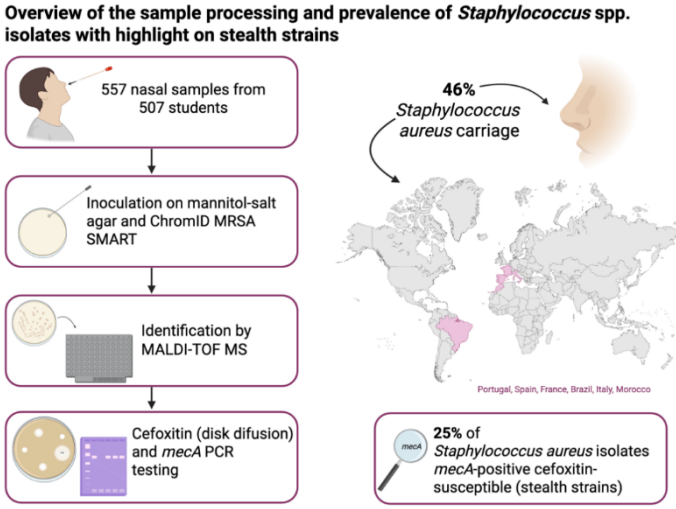

Figure 1. Overview of the sample processing and prevalence of *Staphylococcus* spp. isolates with highlight on stealth strains.

Keywords: *Staphylococcus* spp.; *mec*A; university students.

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PC11 Matrix metalloproteinases in focus: an exploratory epidemiological study in a northern Portuguese population

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ABSTRACT

Background: Matrix metalloproteinases (MMPs) are zinc-dependent enzymes that regulate extracellular matrix degradation and remodelling. Among them, MMP-8 and MMP-9 are key mediators of inflammation, tissue remodelling, and wound healing. They have been implicated in both type 1 diabetes

mellitus (T1DM) and periodontal disease (PD) [1]. The literature indicates a bidirectional association between T1DM and PD: chronic hyperglycemia exacerbates periodontal inflammation, while periodontal disease can impair glycemic control [2,3]. However, the immune mechanisms underlying this this association are not yet fully understood. **Objective:** To evaluate the levels of MMP8 and MMP9, as mediators of the inflammatory process, in a North Portuguese adult population with T1DM and varying severities of PD. The assessment is carried out in saliva, a fluid that reflects the environment of the oral cavity and the immunological status associated with both clinical conditions. **Methods:** The study included 74 individuals, comprising 44 patients with T1DM and 30 control subjects. Both groups included individuals with healthy periodontal status, gingivitis, or periodontitis. Demographic characteristics, smoking habits, oral hygiene practices and clinical parameters were recorded. Salivary levels of MMP-8 and MMP-9 were quantified using Human MMP-8 ELISA (Abcam) and Human MMP-9 Sandwich ELISA (BioLegend), respectively. Data analysis was conducted using IBM® SPSS® Statistics for Windows, version 29.0. **Results:** Association between periodontal health status and the presence of T1DM revealed significant differences ($p < 0.001$). Periodontal health was seen in 90.5% of controls, but only 9.5% of diabetics. The prevalence of gingivitis and periodontitis was higher in individuals with T1DM (75.0% and 81.8% respectively) compared to controls (25.0% and 18.2%). Stage IV periodontitis was found exclusively in the diabetic group ($p=0.046$). Periodontal indices showed significant differences between T1DM and control groups. T1DM individuals had significantly higher salivary levels of MMP-8 [63.55 ng/mL (23.05–96.80)] and MMP-9 [875.0 ng/mL (320.0–1875.5)] compared to controls ($p < 0.001$). **Conclusion:** This exploratory study shows that T1DM individuals present higher levels of MMP-8 and MMP-9 in saliva, which was also associated with more severe periodontal disease. Further studies should assess HbA1c levels and explore their correlation with MMP expression.

Keywords: periodontal disease, MMP-8/9, epidemiology.

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PC12 A comparative analysis of the structural diversity of biosynthetic gene clusters in the phylum *Planctomycetota*

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ABSTRACT

Background: *Planctomycetota* are bacteria with a unique cell biology known to possess important potential for the production of new molecules with biological activities. This potential has only recently started to be unveiled, motivated by reported antimicrobial and anticancer activities [1-3]. Three novel secondary metabolites have already been characterized [4,5]. Enzymes involved in the synthesis of bioactive compounds are often encoded in biosynthetic gene clusters (BGCs), which harbor biosynthetic genes and genes encoding transporter and regulator proteins [6]. Previous studies reported high numbers of up to 13 BGCs present in their genomes, with little to no similarity to characterized clusters in other phyla that belong to known compounds [7]. However, no information regarding the genomic structure of these BGCs is currently available. The discovery of new drugs can be arduous, costly and time-consuming. Bioinformatics analysis can address some of these problems, particularly in underexplored bacterial phyla such as *Planctomycetota*. In that regard, *in silico* analyses can accelerate the identification of putative novel clusters and compounds [8]. **Objective:** This study aimed to compare the presence and genomic organization of BGCs in *Planctomycetota* using bioinformatics tools**. Methods:** 129 planctomycetotal reference genomes were analyzed in a genome mining approach using antiSMASH. Similarity networks of the 987 detected BGCs were constructed using Biosynthetic Gene Similarity Clustering and Prospecting Engine, and Clinker was then used to visualize the similarities between BGCs and the genomic structure of the clusters. **Results:** Our data indicate that the predicted BGCs can vary between and within genera. The genomic organization of BGCs appears to be more conserved within certain genera of the class *Planctomycetia*: *Gimesia*, *Blastopirellula* and *Paludisphaera*, which seem to have more conserved BGC structures. The presence of similar organized BGCs in more distantly related taxonomic groups may point towards horizontal gene transfer events followed by the preservation of these genes within the groups. **Conclusions:** Our results provide one of the first insights into the structure of BGCs of *Planctomycetota*, which can be a key factor for the identification and production of novel compounds. The data also showed that members from the same genera may produce similar compounds, as the genomic structure of BGCs seem to be more conserved within genera

Keywords: *Planctomycetia*; biotechnological potential; genomic structure.

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PC13 Assessing awareness of human identification techniques among professionals and students at Fernando Pessoa University

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ABSTRACT

Background: The identification of cadavers is one of the main areas of Forensic Medicine (FM), particularly in cases of advanced decomposition or mass disasters [1]. The use of radiographs can also be useful, as it allows us to observe changes that would otherwise not be visible [2]. Primary identification methods include DNA, fingerprint analysis, and dental examination. In the case of Forensic Dentistry (FD), techniques such as cheiloscopy, palatal rugoscopy and bite mark analysis can be used as effective identification methods [2]. Maintaining a legible and updated dental record over time is highly valued. **Objective:** This study aimed to assess the knowledge of these techniques among fifth-year students, recent graduates, and faculty members of the Fernando Pessoa University (UFP). **Methods:** An online questionnaire was used, targeting fifth-year students of the Integrated Master's Degree in Dental Medicine, recent graduates, and members of UFP. Inclusion and exclusion criteria were applied allowing data analysis through Microsoft Excel version 14.1.0 and the statistical software IBM SPSS Statistics version 22.0. **Results:** A total of 50 responses were obtained, with 7 responses excluded due to being incomplete or unanswered. Participants in this study were characterized according to their age group, being the majority between 20 and 30 years old. We had 17 answers from fifth-year students, 15 from newly graduated and 11 from professors. The first aspect examined was if any participants had taken any course or subject on FM, to which the majority responded affirmatively [3]. Concerning knowledge of different FM techniques, DNA was the only method known by all individuals, followed by fingerprint analysis [3]. Among the FD techniques, bite marks and palatal rugoscopy were the most well-known, while cheiloscopy was less familiar, with only 8 respondents reporting having used any of these techniques. Regarding radiographs and clinical records, it was generally considered that all respondents acknowledged their importance in FM, particularly in FD [3]. Some difficulties were encountered due to the small sample size; however, the authors intend to conduct a broader study on the topic. **Conclusions:** FD plays a significant role in human identification. The results showed that most respondents understood the different identification methods. Additionally, radiographs and clinical records were given great importance, which is highly beneficial for future evaluations in the field of FD [1-3].

Keywords: forensic medicine; human identification; forensic dentistry.

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PC14 Knowledge, attitudes, and perceptions about child abuse among dental students

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ABSTRACT

Background: Dentists are in a privileged position to detect child maltreatment, abuse, and neglect [1,2], and it´s important to sensitize and train professionals for this task [3], which is so relevant from a social and public health point of view. Detection, signaling, and reporting are crucial steps for the protection and treatment of these children [1]. **Objective:** Evaluate a population of students of the Integrated Master's Degree in Dental Medicine on knowledge, attitudes and perception about child maltreatment, abuse and neglect. **Methods:** Approved by the Ethics Committee, data collection was carried out through a questionnaire that was applied by email, where each potential participant was invited to participate in the research, informed of its objectives and, if they accepted to participate, giving their informed consent. **Results:** The sample included 342 students from all academic years (77%

female, 23% male). Participants most recognized figurative injuries as signs of physical abuse, while traumatic alopecia with skull deformation was least identified. For psychological abuse, aggressive behavior and/or self-mutilation were most recognized, whereas changes in sphincter control were less acknowledged. Regarding sexual abuse, Sexually Transmitted Infections (STI) - related injuries were more recognized, while regressive behaviors were less identified. For neglect, poor hygiene was widely recognized, while developmental and social delays were less considered. A significant association ($p<0.05$) was found between the dentist´s (MD) role in diagnosing/reporting maltreatment and the student's year of study and gender. More 5th-year students identified the MD as key for diagnosis and knew legal reporting duties. Interestingly, while more female students viewed the MD as a favored professional for diagnosis, more male students claimed to understand legal reporting responsibilities. Overall, 71.1% reported cases to authorities, with the National Commission for Child Protection and Security Forces being the most cited. Fifth-year students showed greater knowledge compared to others. **Conclusions:** We emphasize the importance of establishing future interventions to improve dental students' knowledge and confidence in identifying and reporting these situations promptly, which may help to combat the perpetuation of these situations.

Keywords: dental student; maltreating; child.

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PC15 The upcoming problematic of 3D-printed firearms: analysis of european news reports

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ABSTRACT

Background: Initially emerging in North America, 3D-printed firearms have become a growing security concern in Europe. The increasing availability of open-source blueprints, as well as accessibility to 3D printers and improved composite materials, have enabled individuals to manufacture firearms with minimal control or technical expertise. Their danger lies in the rapid and unregulated nature of production, complicating law enforcement and regulatory agencies' efforts to track and control their spread. The European Union acknowledged the issue in its Action Plan on Firearms Trafficking (2020–2025), emphasizing the need to monitor new technologies and the potential for their misuse by terrorist and criminal groups [1]. **Objective:** To identify European news agencies' reports on 3D-printed firearms attacks and apprehensions, elucidating the characteristics of suspects and 3D-printed. **Methods:** Data was collected from European news agencies and the report from the Third Constructive Dialogue on Firearms held by the United Nations Convention against Transnational Organized Crime [1]. The cases span from 2017 to 2025. A mixed-methods approach, combining quantitative and qualitative analyses, was employed. **Results:** In a total of 16 3D-printed firearms' apprehensions across 12 European countries (e.g., Germany [1], the Netherlands [1], Sweden [1,2], the United Kingdom [1,3], Finland [1,4] and Spain [1,5]) law enforcement agencies seized fully assembled 3D-printed firearms, 3D-printed accessories and parts, 3D-printers, firearm parts, fully functional firearms, converted firearms, ammunitions, blueprints for firearm models and an array of far-right extremist and Nazi memorabilia. Among the seized firearms, the most common type of firearm is the PKC (parts kit completion), a 3D-printed firearm made with a 3D-printed receiver (or frame) and multiple commercially available, factory-made parts for the pressure-bearing components [1-5]. **Conclusions:** The seized far-right items demonstrate an increased interest in this type of weapon by extremist groups [1], due to the ease of its access and production. To tackle this trend, European authorities have updated legislative frameworks, namely by the EU Firearms Directive, to address the risks of 3D-printed firearms. Also, operational measures, such as Europol/Cepol-led conferences, awareness campaigns, and coordinated cross-border law enforcement actions that have been implemented to improve detection, prevention, and response to their production and use.

Keywords: illicit firearms manufacturing; parts kit completion/conversions (PKC); untraceable firearms.

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PC16 Ozone: eliminating pathogens and safeguarding evidence in forensic sciences and other sectors

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ABSTRACT

Background: Microbial contamination poses significant risks to biodiversity, food safety, and public health (fig 1) [1]. Traditional microbicide methods that rely on harmful chemicals are associated with risks to environmental health (e.g., toxicity to non-target organisms and resistance to pathogens) [2-4]. In forensics, microbial exposure decomposes biological evidence, staining or altering documents and textiles, corroding metals, weakening polymers, and introducing contamination that compromises analytical accuracy and evidence integrity. **Objective:** Given the urgent need for sustainable alternatives, this systematic review examines the ozone as an ecological and effective solution for decontamination [5,6]. **Methods:** A systematic search was carried out in databases such as PubMed, ScienceDirect, and Google Scholar using the keywords "ozone decontamination," "ozone concentration," "application methods and environments," and "fungal load reduction." Inclusion criteria were based on thematic relevance, scientific rigor, and date of publication (2020-2025), while articles outside the specified period and articles not peer-reviewed were excluded. **Results:** Ozone, applied in its gaseous or aqueous forms, significantly reduces pathogens load, with an average efficacy rate of over 85% in the case of fungal contamination. Higher ozone concentrations and longer exposure times are consistently associated with more significant reductions. Therefore, ozone is an effective microbicide agent that mitigates pathogen contamination while presenting minimal environmental impacts. **Conclusions:** After application, ozone rapidly reverts to oxygen, highlighting its advantage as a residue-free decontaminant. This is particularly relevant in forensics, where maintaining evidence's integrity free of chemical contamination is essential. Ozone's ability to effectively disinfect surfaces and atmospheres and prevent microbial growth on stored evidence and forensic laboratories allows for accurate analysis and preservation of biological evidence. Creating standardized ozone application protocols in various sectors, including forensic science, improves decontamination efforts and security measures. Future research will focus on optimizing ozone application mechanisms and investigating long-term impacts on materials and environments to leverage its potential in the military and civilian domains.

Figure 1. Main sectors affected by microbial contamination.

Keywords: pathogens control; forensic evidence preservation; technological innovation.

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PC17 Cyberattacks on critical infrastructures: the role of Forensic Sciences in prevention and investigation

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ABSTRACT

Background: Critical infrastructures (e.g., national security, energy systems, transport systems/supply chain, health, and telecommunications) (fig. 1) have been the target of constant cyber-attacks that debilitate the population's sense of security and have had profound economic, social, and public safety impacts [1,2]. Also, cyber-physical systems in smart cities face complex challenges that require robust cyber-resilience and digital forensic incident response strategies [3]. **Objective:** To understand the role of digital forensic techniques in preventing and investigating these cyberattacks. **Methods:** A systematic literature review was carried out (Association of Computing Machinery, PubMed, Scopus and IEEExplore) of articles published between 2020 and 2025, using the keywords: “critical infrastructure”, “cyberattack”, “digital forensics” and “incident response”. The papers obtained were selected based on predefined inclusion criteria (thematic relevance, scientific rigor, and date of publication) and exclusion criteria (articles outside the specified period and not peer-reviewed). **Results:** Initially, 146 articles were identified, 45 of which were included in the review after applying the inclusion/exclusion criteria. The main results show a growing use of advanced digital forensic investigation techniques, including automated and artificial intelligence tools (examples: facial recognition with AI, genetic analysis with machine learning, language analysis, and behavioral profiling), for the rapid identification of incidents and proactive prevention. Methods for forensic analysis in SCADA systems, smart grids, computerized hospital systems, and telecommunications were highlighted. The literature also pointed to significant challenges in preserving digital evidence and the difficulty in attributing authorship and accountability for attacks. The results show significant advances in digital forensic techniques applied to protecting and investigating attacks on critical infrastructures, primarily through AI and automation. However, vital and considerable challenges persist (e.g., the systems' complexity, threats' continuous evolution, and the legal difficulties associated with digital evidence collection and validation). **Conclusions:** Investments in specialized research and development will strengthen the ability of forensic teams to respond to emerging cyber threats and ensure the security and resilience of critical infrastructures.

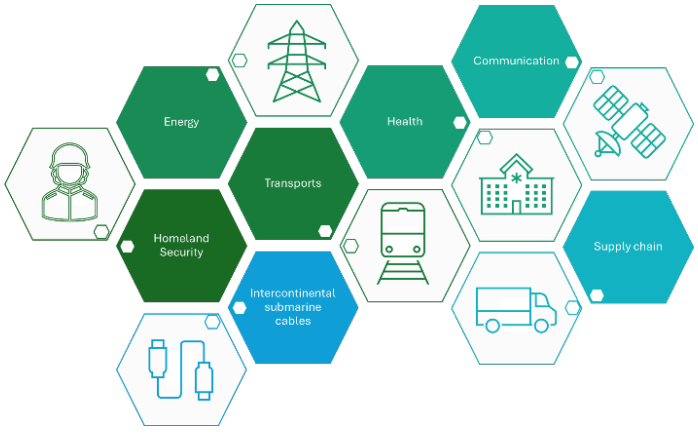

Figure 1. Examples of critical structures.

Keywords: digital awareness; forensic analysis; resilience

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PC18 Forensic Sciences and refugee children: identification and family reunification in humanitarian crisis scenarios

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ABSTRACT

Background: Armed conflicts, ethnic persecution, and humanitarian disasters (Fig. 1) have led to the displacement of millions of people, including many children who, during these journeys, are often separated from their parents or guardians [1-3]. Forensic sciences have proved to be fundamental in identifying unaccompanied minors and reuniting families, using techniques such as forensic genetics, biometrics, and document analysis [4-6]. **Objective:** Review of recently published literature on forensic science for unaccompanied refugee children identification, examine the applied techniques, collaborative efforts, ethical challenges, and the need for standardized protocols and training. **Methods:** A systematic review of the scientific literature was carried out (Scopus, PubMed, and IEEExplore databases) using the keywords “forensic science”, “child refugees”, “family reunification”, “genetic identification”, and “humanitarian crisis”. Studies published between 2020 and 2025 and peer-reviewed were considered, and articles outside this period, without access to the full text or which did not directly address the topic, were excluded. **Results:** Of the 391 articles identified, 106 met the inclusion criteria. These articles mainly deal with the use of genetic profiles (e.g., STRs, mitochondrial DNA), biometric systems (e.g., fingerprints, facial recognition), and shared international databases to identify minors (e.g., INTERPOL - Missing Children Database and the Child Sexual Exploitation Database - ICSE, EUROPOL - AP Twins/Child and Offender Identification System in the EU). There has been a growing trend toward collaboration between government bodies, NGOs, and forensic institutions. However, several studies warn of the ethical and legal difficulties in using genetic data on vulnerable populations, especially without proper consent from parents or guardians. **Conclusions:** Forensic science is essential for protecting refugee children in crisis scenarios. Technological advances enable increasingly rapid and accurate identification, contributing to family reunification and

international human rights application. However, significant challenges remain, such as the need for clear ethical standards, shared international protocols, and specialized training for professionals involved in this type of research. Therefore, forensic science must be guided by principles of humanity, safety, and respect for children's rights as the rule of law.

Figure 1. Main causes of child refugees.

Keywords: forensic databases; humanitarian crisis; international humanitarian law

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PC19 The power of DNA and fingerprint databases

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ABSTRACT

Background: Recent advancements in various scientific fields (e.g., next-generation DNA sequencing, AI-driven biometrics, rapid DNA, enhanced fingerprint systems, cloud-based digital forensics) resulted in the emergence of different branches within Forensic Sciences, which provide comprehensive support to the justice system [1-3]. The present work specifically focuses on two areas of criminalistics: Forensic Genetics (DNA profiling) [4,5] and Lophoscopic Analysis (mainly fingerprint analysis), both essential for human identification. **Objective:** Analyze the evolution of forensic databases in Portugal and other countries. **Methods:** A systematic review was carried out in publicly available databases (PubMed, Scopus, Google Scholar) using the following keywords: "Fingerprint analysis", "Technological advances in forensic science", "DNA databases", "Forensic genetics", "Forensic legislation", and "Personal data protection". **Results:** The worldwide rising trends in crime rates reflect the necessity for reliable forensic databases, often aided by advanced software and technologies, that expedit Justice and enhance crime resolution due to the database's ability for high search speed and accuracy in identifying individuals. Over the years, the observed evolution in technology and legislation aimed to counterbalance investigative efficiency and personal data protection. These efforts differ among countries: the US and the UK favor technology and oversight, while the EU prioritizes privacy. Moreover, if China rapidly expands with minimal safeguards, developing nations such as India are still building their legal frameworks. Despite all efforts, operational challenges related to security, access control, and citizens' privacy persist. Therefore, law enforcement authorities are constantly implementing stricter data protection laws, encrypting sensitive information, enforcing access controls, enhancing audit trails, and promoting transparency through oversight bodies to overcome these challenges. **Conclusion:** The continuous development of DNA and Fingerprint databases, coupled with clearer regulations and technological advancements, strengthens the Judicial System and ensures Justice through the conviction of the guilty and the exoneration of the innocent.

Keywords: biological criminalistics; legal framework; individual identification

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PC20 Under pressure leadership: ethical challenges and critical decisions in Forensic Science

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ABSTRACT

Background: Leadership in forensic science is essential for ensuring the quality, ethical rigor and reliability of decisions relating to forensic investigations [1]. Forensic experts often encounter ethical dilemmas, institutional pressures, judicial expectations [2], as well as workplace-related factors (management, supervision, backlogs, high number of cases) or personal related factors (family, medical, financial) necessitating sensitive and highly responsible decision-making [3-5]. **Objective:** This work identifies and analyses forensic experts' main challenges when making critical decisions. **Methods:** The review was conducted following the PRISMA guideline and included a search of the PubMed, ScienceDirect, and IEEExplore databases for articles published between 2020 and 2025. The search utilized keywords such as "forensic leadership", "ethical decision-making", "forensic science management", and "crisis response". Only peer-reviewed articles addressing leadership and decision-making issues in forensic contexts were included. Articles outside this timeframe or that did not meet the criteria of scientific rigor were excluded. **Results:** Of the 227 articles initially identified, 20 met the inclusion criteria, revealed a lack of formal leadership training among forensic professionals and highlighted ethical conflict management, institutional pressure, and effective communication as key competencies. These findings underscore the importance of collaborative leadership in crisis situations and when faced with controversial decisions. **Conclusions:** The results point out to an urgent need for leadership training within the forensic community (e.g., dedicated coursework, case-based learning, interdisciplinary projects, mentorship, workshops, internships, simulations, reflective assessments, and lectures from field experts) and communication and team management skills (e.g., clear communication protocols, leadership role designation, standardized reporting formats, regular cross-functional training and simulations, reliable communication technology, culture of trust and accountability, and crises debrief to improve future responses). Promoting informed and responsible leadership could enhance the integrity of forensic decisions and increase trust in the institutions that rely on these professionals.

Keywords: critical decision-making; professional ethics; team management

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PC21 Development of an assessment tool to identify the best practices and protocols for CBRN threats

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ABSTRACT

Background: Portugal's ability to respond to terrorist threats remains challenging and complex. According to Vegar [1], the intricate nature of the Portuguese legal system, which regulates each institution's working competencies, interactions, and chain of command, is too strict. These regulations and legal attributions present several difficulties in fulfilling the mission of responding to and combating terrorism, especially when the threat is chemical or biological [1-3]. **Objective:** This work aims to design/elaborate/prepare a questionnaire to be filled in by all the responder entities (e.g., Civil Protection Authorities, GNR, Instituto Dr. Ricardo Jorge, Armed Forces Hospital, among others) in case of an event. **Methods:** This questionnaire will contain twenty questions designed to gather information from institutions identified in the CBRN nº3 Operational Directives [2]. Fisher's Exact Test will be applied to the statistical analysis for the Closed questions to identify associations between variables. The weighted average will be calculated for the prioritization questions, and Kendall's Concordance Coefficient (W) will be used to assess agreement between respondents. For the free response questions, a content analysis will be carried out to identify recurring themes and patterns, followed by a Cluster Analysis to identify specific profiles or patterns. **Results:** The main goal is to evaluate the readiness of each officially deployed institution when faced with biological and chemical threats. By accurately identifying the institutions involved, we can clarify the response hierarchy, map their interconnections, and detail the procedural protocols for their intervention. Additionally, it is crucial to specify the types of threats each laboratory or institution

is prepared to manage. Creating multidisciplinary assessment tools to identify the procedures adopted is fundamental to complementing the information previously obtained in the literature review. **Conclusions:** The results will allow the development of a checklist of multidisciplinary guidelines, raise awareness among authorities/entities, and promote investment in improving protocols to increase preparedness and mitigate the consequences.

Keywords: CBRN threat and response; homeland security; terrorism; terrorism awareness

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PC22 The role of Forensic Anthropology in identifying human commingled remains: a literature review

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ABSTRACT

Background: Forensic Anthropology is a critical tool in war crimes investigations, particularly in analyzing mass graves where human remains are often commingled and fragmented. It enables victims' identification while supporting human rights violations documentation [1-3]. This review focuses on the Minimum Number of Individuals (MNI), a central method for analyzing such remains. **Objective:** To examine the role of Forensic Anthropology in the investigation of mass graves, with a specific focus on MNI estimation, and to evaluate the methodologies applied to improve MNI accuracy and their implications for identification and legal outcomes. **Methods:** A systematic search was conducted from 2022 to 2025 in PubMed, Scopus, and Web of Science. Keywords included "war crimes", "Forensic Anthropology", "Bones", "Mass disasters", "Mass graves", and "Minimum number of individuals". Inclusion criteria were peer-reviewed research articles, full-text articles directly addressing the topic. Papers outside the timeframe or lacking methodological rigour were excluded. Sixteen studies were selected for review. **Results:** The 17 reviewed studies emphasized recurring challenges in estimating MNI in mass grave contexts. Most relied on traditional osteological methods—classifying bones by type, side, size, and morphology—to identify the minimum number of distinct individuals. Taphonomic factors such as decomposition and environmental exposure often hindered visual assessments. To increase precision, researchers used demographic indicators (e.g., age and sex) to differentiate remains. DNA analysis was applied as a complementary technique in complex cases involving damaged or incomplete bones. Though resource-intensive, molecular methods proved valuable in verifying or refining MNI estimates. The findings reflect the complexity of victim identification in mass graves and underscore the importance of integrating classical anthropological approaches with advanced technologies. **Conclusions:** MNI determination is essential for scientific and legal processes, since Forensic Anthropologists convert physical evidence into reliable documentation of atrocities, supporting justice and historical record.

Keywords: Forensic Anthropology; mass graves identification; minimal number of individuals

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PC23 Forensic Anthropology: assessing reliability and sources of bias

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ABSTRACT

Background: Forensic anthropology is critical in human identification, mainly in extreme situations (e.g., mass disasters when other methods fail or in criminal cases where the victim's body is in an advanced decomposition state) [1]. Forensic anthropology has legal and medico-legal implications for individuals' identification. The methods used ensure that the conclusions are coherent, reliable and subject to bias, even though a comprehensive study analyzing the reliability and potential biases of the discipline's methods has yet to be conducted [2]. Moreover, additional contextual information influences morphological analysis in forensic anthropology. A study involving 52 experienced osteologists divided into two groups,

measured a human femur with or without additional information, and found that human cognitive processes are susceptible to biases and errors. Consequently, metric analyses can be affected, particularly when individuals are exposed to a specific context beforehand [3]. In contexts with commingled remains, metric analysis is an important technique to determine the minimal number of individuals involved and distinguish between different subjects. Therefore, deciding which measures are more robust and provide the most reliable results is of the utmost importance. **Objective:** To determine which measurements are more easily repeated and reproduced in the humerus and the femur. **Methods:** Right and left humeri (20 each) and right and left femurs (20) were measured twice by the same observer (a week apart) and then by a second observer. The measurements performed were in the humerus: a) maximal length (h1); b) minimal diaphysis circumference (h2); c) epicondyle distance (h3); and d) head circumference (h4). In the femur were: a) maximal length (f1); physiological length (f2); maximal diaphysis circumference (f3); and d) head circumference (f4). Samples belonged to the XXI Collection of Identified Skeletons from CESPU. Results were analyzed using the intraclass correlation coefficients (ICCs). **Results:** For inter-observer error, the mean ICC analysis in the humerus was h1- 1, h2-0.882, h3-0.775, and h4-0.775; in the femur was f1-0.789, f2-0.788, f3-1; f4-1. Regarding the intra-observer error analysis, the mean ICC values were 1 for all variables. Altogether, the values of the coefficients indicate excellent reliability and reproducibility. Nevertheless, some bones were severely damaged, making some measurements impossible to collect. The femur was systematically better preserved, and the diaphysis displayed less damage than the epiphysis. **Conclusions:** Although all the selected measures displayed perfect repeatability and excellent reproducibility, it was clear that some bone structures are more prone to being damaged. The development of methodologies to work in reassembling skeletons in a commingled remains context should have this under consideration.

Keywords: Forensic Anthropology; human identification; medico-legal implications

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PC24 Decomposition of intestine: contribution of *Escherichia coli* in cadaveric phenomena

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ABSTRACT

Background: The determination of the *postmortem* interval (PMI) can provide valuable information and can be assessed through the study of cadaveric phenomena, which involves changes in the microbial load of deceased tissues. *E. coli* is a commensal microorganism commonly found in the intestine, making it potentially significant. **Objective:** This study aimed to explore whether the death of tissues promotes or suppresses the growth of intestinal bacteria. It also examined whether environmental factors, such as heat and dryness, can significantly influence microbial proliferation and lead to noticeable tissue changes. The findings are expected to offer deeper insights into the relationship between cadaveric decomposition and (necro)microbial activity. **Methods:** For pre-inoculum preparation, *E. coli* was cultured on LBA and incubated for 24 h at 37°C. For the inoculum, the cells were inoculated in LB broth and incubated for 18 h at 37°C. After 16 h, the OD of the inoculum was adjusted to $OD_{600} = 1$, corresponding to $10E^8$-$10E^9$ cells/mL. Fresh pig intestine was used. The procedure was conducted in an *in vitro* simulation, at 37°C and 5% relative humidity. The inoculum was placed under conditions mimicking a hot and dry environment using an incubator. Intestinal pieces were placed in 6-well plates with RPMI-1640 medium, allowing bacterial growth. For each designed time point (0, 5, 24, 48 and 120h), 20µL was pipetted, and dilutions were made (from -1 to -8) in a 96-well plate previously filled with 180µL of PBS. The plates were then inoculated for CFU counting by pipetting 10µL onto LBA solid agar plates for the -6, -7 and -8 dilutions. **Results:** Results were analyzed by counting CFU, and the plates were photographed. In the *E. coli* trial, proliferation was observed to be so extensive across all evaluated time points that precise quantification could not be achieved. To minimize the risk of reporting inaccurate data and drawing uncertain conclusions, it was conservatively concluded that *E. coli* continued to replicate beyond the 120h mark. **Conclusions:** *E. coli* may prove useful in estimating longer PMI; however, an increased number of dilutions will be required to achieve accurate and reliable quantification. Further in-depth and large-scale studies will be necessary to draw definitive conclusions.

Keywords: *E. coli*; forensic; PMI

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PC25 Bacterial load and dynamics: exploring their potential as biomarkers for postmortem interval estimation

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ABSTRACT

Background: Establishing the *postmortem* interval (PMI), the time since death, is vital in forensic investigations but remains challenging due to multiple factors like the cause of death, body location, and environmental conditions [1]. Traditional PMI estimation methods include *Algor Mortis*, *Rigor Mortis,* and *Livor Mortis*, along with complementary approaches from entomology, botany, and microbiology, though all carry some degree of inaccuracy [2]. Recently, microbiology and *postmortem* biochemistry (thanatochemistry) have gained attention, as microbial succession and biochemical changes offer promising, though still limited, insights into PMI estimation [3]. **Objective:** This study aimed to quantify the total bacterial load and map two bacterial species, *Escherichia coli* (Ec) and *Enterococcus faecalis* (Efs), two gut microbiota species, across various organs (lungs, heart, kidneys, liver, and brain) at varying PMIs under controlled, pathogen-free conditions. **Methods:** Male C57BL/6J SPF mice were analyzed at six *postmortem* timepoints (0, 12, 24, 48, 72, and 96h). Feces and organs (n=3 animals/assay) were collected, thoroughly resuspended in buffered peptone water, and then seeded onto non-selective (Blood agar) and selective (MacConkey-MC for Ec and Slanetz-Bartley-SB for Efs) media. After routine aerobic incubation, colony-forming units (CFU) were quantified per gram of tissue to assess total and species-specific bacterial loads. **Results:** The total bacterial load increases at later *postmortem* timepoints. The heart and lungs (highly vascularized organs) showed a detectable bacterial load at the initial timepoint (0 h). Organs typically considered more sterile, such as the kidneys and brain, showed low or undetectable bacterial loads initially, although with a gradual increase over time. In SB, used for the quantitative detection of Efs, bacterial growth emerged at later timepoints. On MC, selective for Enterobacteriaceae, and used for the detection of Ec, bacterial growth was organ-dependent and detectable only after 24 h. Notably, *E. coli* was absent from liver samples on this medium. Biochemical analyses are ongoing to complement microbiological analysis. **Conclusions:** This culture-based quantitative study shows how PMI impacts the growth and dynamics of *E. faecalis* and *E. coli*, suggesting their potential as traceable biomarkers for PMI in forensic contexts. Nonetheless, further studies are required to validate and extend these findings to more complex scenarios.

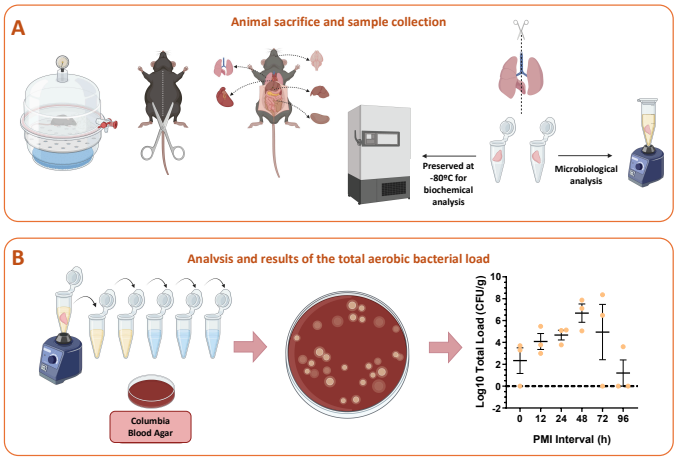

Figure 1. (A) Schematic representation of animal sacrifice using an isoflurane chamber followed by cervical dislocation and sample collection. Tissues were preserved at -80 ºC for biochemical and microbiological analysis. (B) Workflow for microbiological quantification of collected organ samples, including homogenization, culture on Columbia Blood Agar, and total bacterial load assessment. The graph on the right shows total aerobic bacterial load (Log10 CFU/g) for lungs across different *postmortem* intervals.

Keywords: *postmortem* interval; thanatomicrobiome; bacterial translocation

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PC26 Exploring the potential of dichloroacetate in targeting oral cancer metabolism

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ABSTRACT

Background: Oral cancer represents a significant global health burden, and its incidence is steadily rising [1]. A hallmark of cancer is metabolic reprogramming - most notably, a shift toward aerobic glycolysis, commonly known as the Warburg effect. This altered metabolic phenotype supports rapid proliferation, enhances survival under hypoxic conditions, and contributes to

resistance to conventional therapies. As such, targeting dysregulated metabolic pathways has emerged as a promising therapeutic strategy [2]. Dichloroacetate (DCA), a small-molecule inhibitor of pyruvate dehydrogenase kinase (PDK), has attracted attention for its ability to reactivate mitochondrial oxidative phosphorylation [3]. **Objective:** This study aims to evaluate the therapeutic potential of DCA in oral cancer, focusing on its cytotoxicity and its ability to target key components of tumor cell metabolism. Specifically, we investigate DCA's interaction with monocarboxylate transporters (MCTs) and ATP-dependent efflux pumps involved in multidrug resistance. **Methods:** The cytotoxic activity of DCA was evaluated using the sulforhodamine B (SRB) assay to determine the GI_{50} in SCC09 and SCC25 human oral cancer cell lines. A non-tumor cell line (HOK, human oral keratinocytes) was also included. The expression of MCTs and ATP-dependent efflux pumps in oral cancer cells was evaluated, both in the presence and absence of DCA treatment, at the mRNA transcript and protein levels using qRT-PCR and Western blotting, respectively. The UALCAN and GEPIA databases were used to analyze the expression of the targets and correlate them with clinicopathologic indicators from head and neck squamous cell carcinoma tissue samples. **Results:** We found that DCA decreases oral cancer cell viability after 24 hours of treatment, and the cytotoxic effect on SCC09 and SCC25 cancer cells could modulate the protein expression of monocarboxylate transporters. From the UALCAN analysis, we observed an increase in the expression of MCT1 and MCT4 transporters in head and neck squamous cell carcinoma tissue samples compared to normal tissues. Moreover, we found a positive correlation between tumor stage and the overexpression of MCT1 and MCT4. Similar results were obtained for the P-gp efflux pump and CD147. Overall survival and disease-free survival were also analyzed with GEPIA. **Conclusions:** The results highlight the potential of DCA as a promising cytotoxic agent in oral cancer that deserves further investigation.

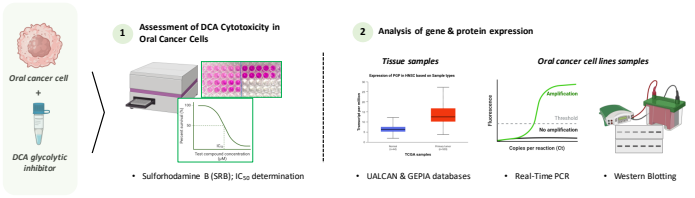

Figure 1. Schematic representation of the experimental approach to evaluate the effect of the glycolytic inhibitor dichloroacetate (DCA) on oral cancer cells.

Keywords: oral cancer; tumor metabolism; dichloroacetate

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PC27 The oral health implications of changes in the diabetic oral microbiome

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ABSTRACT

Background: Diabetes mellitus (DM) significantly impacts oral health by modulating periodontal microbiota, impairing salivary defense mechanisms, and altering gingival fluid dynamics [1]. Evidence suggests a bidirectional relationship between DM and periodontitis, characterized by dysbiosis and exacerbated inflammatory responses [2], underscoring the need for precision-targeted therapeutic strategies. **Objective:** The present investigation aims to ascertain the impact of DM on alterations in the oral microbiome, and its clinical ramifications for oral health, particularly with regard to periodontal complications and xerostomia. **Methods:** A rigorous bibliographic search was conducted in scientific databases, namely PubMed, Google Scholar, Web of Science, B-on, and Scielo. The PICO method was applied to formulate a precise clinical question by establishing clear inclusion and exclusion criteria. Observational, case-control, cross-sectional and descriptive studies published after 2004 and written in Portuguese, English or French were considered. A total of 1254 articles were previously identified, and after a strict application of the criteria and a complete reading of the texts, only 8 studies were considered eligible and included in this review. **Results:** Research consistently demonstrates that individuals with diabetes exhibit a higher prevalence and greater severity of periodontal disease, as evidenced by increased clinical periodontal parameters [2,3]. These include mean periodontal probing depth (PPD), clinical attachment loss (CAL), a higher gingival bleeding index and higher gingival crevicular fluid volume (GCF-V), in comparison to individuals without DM. With regard to xerostomia, the prevalence was found to be significantly higher among diabetic patients, as well as a marked reduction in salivary flow and an increase in salivary glucose levels, with a positive correlation with inadequate glycemic control as assessed by glycated hemoglobin (HbA1c) [1]. **Conclusions:** The analysis of the scientific literature demonstrates that DM is associated with significant changes in the oral microbiome that increase the risk and severity of oral complications, particularly periodontal disease and xerostomia [1-3]. These complications are caused by multiple factors, including altered inflammatory responses, reduced salivary flow and specific metabolic deregulation, which severely compromise oral health and consequently the quality of life of diabetic patients.

Keywords: diabetes; oral microbiome; periodontal disease

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PC28 Isoquinolinequinone N-oxides as potent antitumor compounds against pancreatic cancer cell lines: preliminary results

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ABSTRACT

Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive malignancies, being the 7th leading cause of cancer-related mortality worldwide. PDAC remains associated with an extremely poor prognosis, owing to drug resistance and to the lack of alternative therapeutic options. Standard chemotherapy typically involves gemcitabine, either alone or combined with paclitaxel. Moreover, Chitinase-3-Like 1 (CHI3L1) was identified as a key protein responsible for reducing PDAC drug response *in vitro* [1]. Thus, the identification of new molecules with antitumor potential and that can counteract drug resistance will be of great significance, as new therapeutic agents to be used either alone or in combination with chemotherapy. Interestingly, a family of Isoquinolinequinone (IQQ) *N*-oxides with antitumoral activity against counterpart pairs of sensitive and multidrug-resistant cell lines from non-small cell lung cancer and colorectal cancer has been recently identified [2,3]. **Objective:** The aim of this study is to explore the antitumor and chemosensitizing activity of a family of seven IQQ *N*-oxides in a panel of PDAC cell lines. **Methods:** The cytotoxicity effect of seven IQQ *N*-oxides is being assessed with the Sulforhodamine B (SRB) assay in four PDAC cell lines: PANC-1, BxPC3, Miapaca-2 and Capan-2. Gemcitabine was used as a positive control [1]. The impact of these compounds on the expression levels of CHI3L1, β-catenin and other proteins involved in relevant cancer-related pathways, such as ERK signalling, will be analysed by Western blotting. Furthermore, the therapeutic combination of the most promising IQQ *N*-oxides with gemcitabine will be evaluated by SRB. **Results:** Preliminary results demonstrated that the GI_{50} concentrations of three IQQ *N*-oxides were around or below 2 μM, in both PANC-1 and BxPC3 cell lines. **Conclusion:** This preliminary work highlights the antitumor potential of these isoquinolinequinone *N*-oxides in PDAC cell lines.

Keywords: antitumor compounds; isoquinolinequinone *N*-oxides; pancreatic cancer

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PC29 Patient-reported outcomes in oral oncology: new approaches, contemporary visions

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ABSTRACT

Background: Emerging trends in Patient Reported Outcomes (PROs) are transforming healthcare by centering the patient experience and emphasizing Quality of Life (QoL) in clinical decision-making [1]. **Objective:** Analyze the benefits of using PROs in daily critical practice based on the experience of our research group and literature review. To explore new strategies to apply results more efficiently in clinical decisions. **Methods:** A systematic literature review was conducted across PubMed, Cochrane Library, B-On, and Scielo, targeting publications from 2020-2025 in English and Portuguese, resulting in the analysis of 83 studies. Study selection followed the PICO framework and PRISMA guidelines. Consecutive outpatients at the Otorhinolaryngology and Head and Neck Departments of IPO-Porto completed PRO questionnaires prior to clinical consultations, as part of routine care. A digital platform facilitated the assessment of QoL. Inclusion criteria encompassed relevant keywords in the publication. Exclusion criteria included duplicate articles and those not aligned with the topic. Data analysis employed Item Response Theory using the Rasch model (WinRasch software), enabling the construction of a hierarchical measurement scale and evaluation of item fit statistics. **Results:** Monitoring PROs in head and neck oncology at IPO-Porto has

proven valuable for clinical decision-making, identifying patient needs, and supporting stepped-care models. It aids in risk stratification and prognosis prediction, enhancing clinical practice, research, and strategies for optimizing patients' QoL [2]. Technological advances, including smartphones, wearables, and AI, enable real-time data collection, disease monitoring, personalized treatment, and early intervention. The transition to electronic PROs improves data accuracy, efficiency, and adaptability, facilitating culturally sensitive tools that promote patient understanding, adherence, and shared decision-making [3]. **Conclusions:** Integrating information technology in PROs assessment is crucial for standardizing clinical evaluations and decision-making, thereby enhancing the organization and efficiency of clinical research. This approach improves communication and the transfer of evaluative data into clinical practice, enriching the clinical understanding and supporting informed decision-making in healthcare.

Keywords: patient-reported outcomes; oral cancer; clinical decision; quality of life; e-PROs

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PC30 Ivermectin decreases the expression of ALDH1 in ovarian cancer cell lines in combination with chemotherapy

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ABSTRACT

Background: Cell Microarray (CMA) technology has been established as an essential tool for advancing cancer studies, allowing for the simultaneous protein analysis of multiple samples [1]. This method facilitates the exploration of various biomarkers associated with diagnosis, prognosis, and therapeutic response, making evaluation processes more efficient and comprehensive [1]. Carboplatin and paclitaxel are commonly used chemotherapy drugs for ovarian cancer treatment. Numerous studies have demonstrated that patients often develop resistance, underscoring the need to discover more effective therapeutic options to improve patient outcomes [2]. In oncology, drug repurposing has shown promising results in achieving this goal. Ivermectin, an antiparasitic drug, has been shown to enhance the efficacy of carboplatin and create a synergistic effect when combined with paclitaxel [3]. Aldehyde Dehydrogenase 1 (ALDH1) is an aldehyde catalyzer that plays a crucial role in drug metabolism. The study of this biomarker could be vital to the understanding of treatment resistance found in advanced ovarian cancer. **Objective:** Using the CMA approach, the aim is to explore the ALDH1 expression pattern in two ovarian cancer cell lines, after *in vitro* treatment with carboplatin, paclitaxel and ivermectin. **Methods:** Two ovarian cancer cell lines were used: OVCAR8, characterized by resistance to Carboplatin, and OVCAR8 PTX R C, which exhibits resistance to both Carboplatin and Paclitaxel [2]. The cells were exposed to chemotherapeutic agents for 48 hours, administered either alone or in combination with Ivermectin. After the incubation period, the cells were collected, formalin-fixed and embedded in Histogel®. Then the samples were paraffin-embedded and cut into glass slides to perform ALDH1 protein detection using immunocytochemistry. **Results:** In general, ALDH1 expression was found in the cytoplasm of the cells with a dot pattern. Our results show that in both OVCAR8 and OVCAR8 PTX R C cell lines, the treatment with the combinations of carboplatin/paclitaxel plus Ivermectin, whether in 2D or 3D environments, leads to a notable decrease in ALDH1 expression compared to treatments in monotherapy. **Conclusions:** High levels of ALDH1 are associated with chemoresistance. The low levels of ALDH1 found in ovarian cancer cells treated with Ivermectin plus carboplatin or paclitaxel reveal a more sensitive profile, which could be a promising alternative for ovarian cancer treatment.

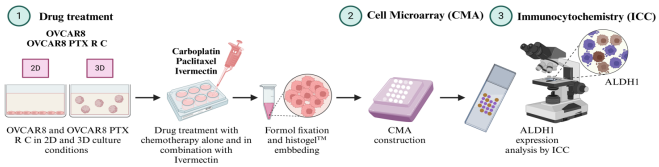

Figure 1. Evaluation of ALDH1 expression in drug-treated cells. (1) Drug Treatment: OVCAR8 and OVCAR8 PTX R C cell lines were cultured in 6-well plates under 2D (flat) and 3D (spheroid or aggregate) culture conditions. Cells were incubated at 37 °C with 5% CO_2 for 96 hours. After this period, the cells were treated with different drugs, both alone and in combination. After 48 hours of treatment, cells were harvested, fixed in formalin, and embedded in Histogel® for further analysis. (2) CMA: All treatment conditions from (1) were included in a single CMA block, allowing the analysis of multiple experimental conditions in one format. (3) Immunocytochemistry: ALDH1 expression in drug-treated cells was analyzed by immunocytochemistry using specific antibodies for ALDH1. Images show the distribution and intensity of ALDH1 expression in the treated cells, highlighting the effects of the treatments on the modulation of this protein expression. The figure was created with Biorender.com.

Keywords: cell microarray; drug repurposing; ovarian cancer; chemoresistance; ALDH1

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PC31 Lymphocyte population characterization in ovarian cancer microenvironment

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ABSTRACT

Background: Ovarian cancer (OC) is one of the most common gynecological cancers, with poor prognosis and high mortality [1]. A key early sign of OC is the presence of ascites [2], indicating transcoelomic metastasis and tumor spread within the peritoneal cavity [3]. The accumulation of this serous fluid facilitates migration of tumor cells, which detach from the primary tumor (PT) in multicellular aggregates, migrate through ascitic fluid, and establish metastatic implants (MI) [3]. Ascites also contains acellular components and other cells, such as immune cells. In fact, the interactions between all these components play a crucial role in disease progression [2,3]. Since transcoelomic metastasis involves changes in tumor cell differentiation, characterizing and comparing the tumor immune microenvironment (TIME) in both PT and MI is crucial to assess whether therapies targeting PT are also effective against MI. **Objective:** We aimed to a better understanding of the TIME in OC patients, evaluating and comparing the populations of T lymphocytes, B lymphocytes and macrophages in PT and their respective MI. **Methods:** We used a tissue microarray with samples of PT and MI from each patient, which included 13 cases with histological diagnosis of high-grade serous carcinoma that have not undergone chemotherapy treatment to: conduct a qualitative assessment of matrix elements using histochemical techniques (trichrome staining for collagen, orcein staining for elastin and silver staining for reticulin); perform a semi-quantitative assessment of different leucocyte populations through IHC staining with anti-CD3 (T lymphocytes), anti-CD20 (B lymphocytes) and anti-CD163 (macrophages); evaluate and quantify the immune cells using two different methods (manual semi-quantitative evaluation under LM and automatic quantitative evaluation using QuPath, **Figure 1**). **Results:** Collagen fibers were observed in the stroma of both PT and MI, whereas no elastic fibers were observed; reticular fibers were present in rich lymphoid tissue areas. The presence of T and B lymphocytes, as well as macrophages, was higher in MI than in PT. There was a greater abundance of T lymphocytes than B lymphocytes in the stroma. Finally, the macrophage population was more abundant than the lymphocyte population, both in PT and MI. **Conclusions:** The differences observed between PT and MI show an immune system adaptation that can influence the progression of the disease, its prognosis, and treatment response.

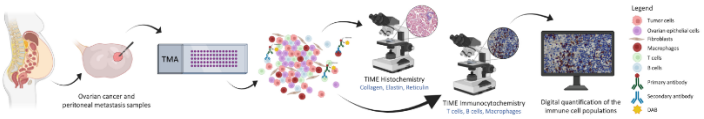

Figure 1. Overview of the experimental approach used to evaluate the populations of T lymphocytes, B lymphocytes and macrophages in PT and MI.

Keywords: tumor immune microenvironment; peritoneal metastasis; immunohistochemistry

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PC32 Gadoteric acid and gadolinium exposure – what is the impact on kidney gene expression?

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ABSTRACT

Background: The nephrotoxicity of gadolinium [Gd (III)] has been reported, raising concerns about the safety of gadolinium-based contrast agents (GBCA). Gd (III) exposure, in renal tubular cells (HK-2), causes apoptosis, and leads to upregulation of genes related to lipogenesis/lipolysis and to signaling pathways related to inflammation/hypoxia [1]. Gadoteric acid (Gd-DOTA), a macrocyclic GBCA, appears to be one of the more stable. Recently, we reported that, in healthy rats exposed to a single dose of Gd (III) or Gd-DOTA, the kidneys' transcriptome, compared to controls, presented distinct differential gene expression patterns [2]. **Objective:** To evaluate the short- and long-term effects of exposure to Gd (III) and Gd-DOTA on the kidney's gene expression of genes specifically related to apoptosis, inflammation, hypoxia and lipid metabolism. **Methods:** In short- and long-term studies (2 days and 20 weeks after exposure, respectively), male Wistar rats were divided in 3 groups/study (*n*=10/group) and exposed to a single dose (0.1 mmol/kg) of Gd (III), Gd-DOTA or vehicle (control). At the end of the protocols, renal tissue was collected to evaluate the kidney gene expression of *casp3, bcl2*, *spp1*, *sqstm1*, *nfkb1, nfe2, nlrp3, il6, tgfb1, il1b, hif1a, acaca* and *cpt1a,* through qPCR. **Results:** Two days after exposure, Gd (III) group presented higher levels of gene expression of *il6*, *tgbf1*, *nfkb1* and *hif1a* than the controls and, compared to Gd-DOTA group, *tgbf1* and *acaca* mRNA levels were increased; the Gd-DOTA group presented increased levels of *il6* and *cpt1a,* compared to the control group. Twenty weeks after exposure, Gd (III) group presented decreased gene expression of *tgbf1* and *acaca* compared to the controls; the Gd-DOTA group presented lower *tgbf1* mRNA levels than the control group. **Conclusions:** Short-term exposure to free Gd (III) was associated with upregulation of genes related to hypoxia and inflammation, while exposure to Gd-DOTA was only associated with upregulation of *il6* (encoding interleukin-6) and *cpt1a*, which encodes for carnitine palmitoyltransferase 1A, an enzyme involved in fatty acid oxidation. Over time, these alterations seem to reduce or even revert for both compounds, since in the long term, only gene expression downregulation was observed. Gd-DOTA revealed a safer profile, however, further studies are warranted to evaluate its true safety, especially in cases of repeated exposures and/or pre-existing renal function impairment.

Keywords: inflammation; hypoxia; apoptosis; lipid metabolism

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PC33 Inflammatory and senescence-related effects of polyethylene microspheres on dermal cells

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ABSTRACT

Background: Microplastics (MPs) have been raising environmental and human health concerns [1]. Polyethylene (PE) is a synthetic organic polymer and is one of the main constituents of plastics [2]. PE MPs are widely used in cosmetics and personal care products due to their cost-effectiveness, versatility and durability [3]. However, their effect on skin cells remains unclear. Exposure of the dermis to these particles may induce several cellular and molecular changes, contributing to skin ageing and disease. **Objective:** Investigate the potential cytotoxic impact of PE MPs in normal human dermal fibroblasts (NHDFs) and murine macrophages (RAW 264.7), focusing on cell viability and induction of inflammatory and senescence responses. **Methods:** RAW 264.7 and NHDF cells were incubated with different concentrations of the MPs (25-500µg/mL) during two different time-points (24 and 48 hours). Cellular metabolic activity was measured in both cell lines using the resazurin assay. In macrophages, nitric oxide (NO) production was quantified using the Griess assay, interleukin-1 beta (IL-1β) secretion was measured in the supernatants by ELISA and the expression of pro-IL-1β and inducible nitric oxide synthase (iNOS) was analysed by Western blot (WB). In fibroblasts, the mRNA levels of collagen were measured by RT-PCR analysis and the morphology of these skin cells was analysed by microscopy. The senescence markers H2Ax and Lamin B1 were monitored by immunocytochemistry and the activity of the lysosomal enzyme senescence-associated β-galactosidase was quantified by a cytochemical assay. **Results:** Preliminary findings indicate that exposure to PE MPs compromises the cellular metabolism in both cell models, with a significant decrease in macrophages and an increase in fibroblast cells. Upon incubation with the MPs, increased NO production and a slight decrease in the expression of pro-IL-1β were detected in RAW 264.7 macrophages, while no changes in iNOS content were observed. In addition, the concentration of secreted IL-1β was higher. In cultured skin fibroblasts, alterations in cell morphology, as well as in the levels of senescence markers, were triggered by exposure to PE MPs. **Conclusions:** Our data suggest that PE MPs can trigger an inflammatory response and can affect the morphology and function of fibroblasts in the dermis, contributing to their senescence. Further research is needed to clarify their role in promoting skin ageing.

Keywords: microplastics; skin exposure; cellular ageing

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PC34 Beer enriched with "Lapins" cherry extracts: antioxidant activity and liver toxicity

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ABSTRACT

Background: Beer can be considered a functional beverage and integrate innovative ingredients, namely sweet cherries, with different properties, such as antioxidant activity [1,2]. **Objective:** To evaluate the antioxidant activity, *in vitro*, and liver toxicity, in human hepatocarcinoma cells (HepG2), in beer after incorporation of aqueous (CAE) and ethanolic (CEE) extracts of cherry variety "Lapins". **Methods:** CAE and CEE (1mg/mL) were incorporated into commercial bottles of Imperial Stout beer (IS-N). The total phenolic content (TPC), expressed in mg of gallic acid equivalents (GAE)/g, was determined. The antioxidant capacity was evaluated by the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) neutralization and hydrogen peroxide (H_2O_2) assays, both expressed in the concentration required to inhibit the activity by 50% (IC_{50}). Also, the ferric reducing antioxidant power (FRAP) assay was performed and expressed in µmol of trolox equivalent (TE)/mg. Cell toxicity was studied in HepG2 cells, with assessment of metabolic activity by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Data were analysed using GraphPad Prism software, and significant differences were considered for $p < 0.05$. **Results:** The incorporation of CEE to IS-N beer significantly decreased TPC (4.2 ± 0.1 mg GAE/g for IS-N + CEE; 8.3 ± 0.2 mg GAE/g for IS-N; $p < 0.05$). There was an increase in the antioxidant capacity by the ABTS assay (IC_{50} = 80.1 ± 1.1 µg/mL for IS-N; IC_{50} = 60.5 ± 1.5 µg/mL for IS-N + CAE; IC_{50} = 48.0 ± 0.6 µg/mL for IS-N + CEE); however, the incorporation of cherry extracts was not promising in the H_2O_2 (27.0 ± 1.5 µg/mL for IS-N; IC_{50} = 58.7 ± 0.4 µg/mL for IS-N + CEE; IC_{50} = 78.8 ± 1.6 µg/mL for IS-N + CAE;) and FRAP (44.4 ± 0.0 µmol/g for IS-N; 42.7 ± 0.0 µmol/g for IS-N + CAE; and 39.7 ± 0.0 µmol/g for IS-N + CEE) assays. Furthermore, IS-N + CEE showed greater antioxidant capacity than IS-N + CAE. After the incorporation of cherry extracts, cytotoxicity was observed in concentrations higher than 10 mg/mL (for IS-N + CAE, 24h incubation) and at the concentration of 500 mg/mL (for IS-N + CAE and IS-N + CEE, 48h incubation). IS-N + CEE showed the greatest increase in cell viability. **Conclusions:** The addition of cherry extracts to beer increased the antioxidant capacity by the ABTS assay, while TPC was reduced with the addition of CEE. The incorporation of both extracts showed promising potential, with low cytotoxicity in HepG2.

Keywords: antioxidant activity; liver toxicity; sweet cherry

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PC35 Immune microenvironment of the omentum in appendicitis: a phenotypic perspective in cell populations

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ABSTRACT

Background: The omentum plays a crucial role in managing inflammation and combating infections, particularly in acute appendicitis [1]. This fat-rich fold of the peritoneum contains lymphoid structures known as "Milky Spots", which house several types of phenotypically and functionally distinct immune cells, including macrophages and lymphocytes, that contribute to its protective function [1,2]. Among these, T cells (CD3+), including helper (CD4+) and cytotoxic (CD8+) subtypes, B cells (CD20+) and macrophages (CD163+), are known to participate in different aspects of immune regulation and response [1,3]. Understanding the specific roles of those cells in the omental tissue can provide a more detailed insight into the nature and dynamics of the inflammatory response orchestrated by the omentum. The omentum's mobility allows it to adhere to and potentially encapsulate necrotic and infected areas, facilitating pathogen clearance and tissue repair [1,3]. This process is particularly relevant in conditions such as surgical wounds, intestinal ulcers and inflamed appendices [1,2]. **Objective:** This study aims to conduct a phenotypic characterization of the immune response in omental samples from three acute appendicitis patient groups: Group I without peritoneal blockage, Group II with peritoneal blockage, and the control Group III without appendicitis. **Methods:** Omentum samples (n = 3 each group) were analyzed through immunohistochemistry to identify macrophages, lymphocytes and lymphocyte subpopulations, using primary antibodies against CD163, CD20, CD3, CD4, and CD8. **Results:**

Observations revealed clear distinctions between the studied groups. Groups I and II showed an increased presence of T cells (CD3+, CD4+, CD8+) and B cells (CD20+), as well as macrophages (CD163+), compared to their minimal presence in the control group. Notably, Group II exhibited the highest levels of CD8+ T cells and CD163+ macrophages, while CD3+ and CD4+ T cells were found at moderate levels. **Conclusions:** The results from this study suggest an association between peritoneal blockage and heightened immune cell infiltration. CD8+ T cells play a key role in immune defense against intracellular pathogens, especially viruses and some intracellular bacteria, and tumor surveillance. Their presence in the omentum of appendicitis patients reinforces the organ's involvement in this inflammatory condition. Also, the high levels of CD163+ macrophages unveil a remodeling response concomitant with a prolonged inflammatory response.

Keywords: milky spots; immunohistochemistry; immune cells

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PC36 Potential skin benefits of incorporating *Prunus avium* Lapins extracts into a commercially available Portuguese India Pale Ale craft beer

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ABSTRACT

Background: A commercially available Portuguese India Pale Ale craft beer (ALM-IPA) has shown potential skin benefits in a previous study [1]. However, the incorporation of cherry extracts into bottled beers lacks scientific evidence. **Objective:** To evaluate, *in vitro*, the benefits of incorporating aqueous (ACE) and ethanolic (ECE) cherry extracts into ALM-IPA beer, in terms of antioxidant and photoprotective activity and the viability of human keratinocytes (HaCaT cells). **Methods:** Experimental study, with the incorporation of ACE (infusion, 1:10) and ECE (70%) (1 mg/mL) into ALM-IPA bottles. Total phenolic content (TPC) was determined. The antioxidant potential was assessed using the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), hydrogen peroxide (H_2O_2) and iron-reducing antioxidant power (FRAP) assays. The photoprotective potential was estimated by determining the sun protection factor (SPF) and the ultraviolet absorption capacity (UV-AC). The viability of HaCaT cells was assessed using the 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Data were analysed using the one-way ANOVA test and significant differences were considered for $p < 0.05$. **Results:** Regarding antioxidant activity, and comparing both extracts, ALM-IPA+ACE presented the lowest value of IC_{50} for ABTS assay (86.16 ± 5.33 µg/mL) and the highest FRAP value (27.58 ± 0.42 µmol of trolox equivalents/g), which is related with the highest TPC observed (15.10 ± 0.16 mg of gallic acid/g). ALM-IPA+ECE presented the lower IC_{50} (43.27 ± 2.14 µg/mL) compared to ALM-IPA+ACE (65.08 ± 1.69 µg/mL) for H_2O_2 assay. However, ALM-IPA beer showed higher antioxidant activity (IC_{50} = 55.21 ± 4.68 µg/mL for ABTS; IC_{50} = 23.54 ± 1.53 µg/mL for H_2O_2; FRAP = 53.74 ± 1.27 µmol of trolox equivalents/g). Regarding photoprotective potential, both extracts presented photoprotective potential (SPF > 6) [2]. Analyzing the viability of HaCaT cells after incubation with both extracts, ALM-IPA+ACE and ALM-IPA+ECE presented cytotoxicity, for the 24 h and 48 h incubation period, only for concentrations higher than 100 µg/mL (cell viability > 80%) [3]. In the 24 h incubation period, ALM-IPA cell viability was higher than ALM-IPA+ACE and ALM-IPA+ECE, and generally, ALM-IPA+ECE was superior to ALM-IPA+ACE. **Conclusions:** More studies are needed regarding the incorporation of plant extracts into commercially available beers, particularly in other stages of brewing or different styles of beer.

Keywords: craft beer; *Prunus avium* Lapins; keratinocytes

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PC37 Gamification in e-Health: strategies for oral health

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ABSTRACT

Background: Gamification in healthcare is an innovative approach that uses game elements to motivate and engage patients

in healthy behaviors. Gamification in oral health uses various game elements to create engaging and motivating experiences for patients [1-7]. Some of the most common elements include goals and challenges to achieve, rewards to obtain, competition, feedback, and the creation of an engaging narrative [1-7]. **Objective:** To analyze the benefits of using gamification to promote Oral Health. To present implementation models that have been successful in the last 3 years. **Methods:** A search was made in PubMed, Cochrane Library, B-On, and Scielo, considering articles published between 2020 and 2025, in English and Portuguese. The study selection strategy followed the PICO framework (Population: oral health patients; Intervention: gamification in oral health promotion; Comparison: other oral health promotion strategies; Outcome: improved oral health practices). The selection and analysis of studies were carried out according to PRISMA recommendations. **Results:** Gamification in healthcare can bring several benefits, both to patients and healthcare professionals. Some of the main benefits include: increased adherence to treatments: gamification can make treatments more fun and engaging, which can increase patient adherence; promotion of healthy habits: gamification can encourage physical exercise, healthy eating, optimized oral hygiene habits, dentist monitoring and other oral health-promoting behaviors; improved management of chronic diseases: gamification can assist in the management of chronic diseases, such as diabetes, hypertension or periodontal disease, by making monitoring and treatment more fun and engaging; reduction of stress and anxiety by providing a fun and relaxing experience; improved communication between patients and healthcare professionals. **Conclusions:** Gamification and e-health are interdependent: gamification enhances user engagement in e-health through game elements, while e-health broadens the application and personalization of gamification in healthcare. In oral health, gamification improves motivation, facilitates learning, encourages long-term hygiene habits, and reduces dental anxiety, representing a key strategy in global oral health promotion.

Keywords: gamification; e-health; oral health; oral health promotion; literacy; games

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PC38 Analysis of multiple pesticide and endocrine disruptor residues in drinking water using SPE and GC–MS/MS

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ABSTRACT

Background: The presence of pesticide residues and endocrine disruptors in sources used for drinking water abstraction has become a significant concern nowadays [1,2]. These substances pose risks to the environment, biodiversity, and public health. Consequently, monitoring these compounds in drinking water is crucial to maintaining their quality and safety. Therefore, there is a need to develop sensitive analytical methods that can detect these compounds even at very low concentrations [1-3]. **Objective:** This study aims to optimize a solid-phase extraction (SPE) and gas chromatography-tandem mass spectrometry (GC–MS/MS) method for determining a specific group of pesticides (alachlor, atrazine, chlorofenvinphos, isoproturon, pentachlorophenol, methiocarb, and simazine) and endocrine disruptors (bisphenol A, estradiol, estrone, ethynylestradiol, nonylphenol, and octylphenol) in drinking water. **Methods:** Sample preparation processes such as extraction and preconcentration procedures (i.e., SPE sorbent, conditioning solvent, clean-up, elution solvent and volume, as well as adjustment of derivatization procedure) were optimised to reliably monitor these residues in aqueous matrices. In addition, GC-MS/MS chromatographic conditions were optimized for analyte analysis using multiple reacting monitoring. Recovery rates were assessed using ultrapure water spiked with known concentrations of analytes. Performance was evaluated based on signal response, method sensitivity, and recovery efficiency. **Results:** The optimized SPE-GC-MS/MS method demonstrated high selectivity and sensitivity for the target compounds. Pesticides and endocrine disruptors exhibited recoveries above 64% and 70%, respectively, demonstrating the method's reliability. **Conclusions:** The developed method proved effective for analyzing pesticides and endocrine disruptors in ultrapure water samples, with satisfactory analyte recovery (average of 67%). Further analysis using drinking water matrix will be conducted to assess matrix effects and validate the analytical method before sample analysis. The use of GC-MS/MS provided improved selectivity and lower detection limits compared to gas chromatography-mass spectrometry (GC-MS). Subsequently, this method could be applied for routine drinking water quality monitoring, ensuring compliance with regulatory standards.

Keywords: water quality assessment; environmental contaminants; chromatographic analysis

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PC39 Enantioselective liquid chromatography method for the simultaneous determination of chiral and achiral fungicides in aqueous matrices

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ABSTRACT

Background: Fungicides are organic compounds, many of them chiral, used in various applications such as medicines, personal care products, agrochemicals and industry. Although they have beneficial effects in controlling fungal plagues and treating diseases, their widespread use has led to their detection in the environment [1-3]. As a result, fungicide contamination is increasingly a global environmental concern, due to the risks it poses to non-target organisms and to human health. Given that enantiomers of chiral fungicides can show different bioactivity, toxicity and degradation, monitoring the enantioselective occurrence of these compounds in the environment is very important to assess the toxicity and adverse effects of each enantiomeric form [3-5]. **Objective:** The aim of this study is to develop and validate an enantioselective chromatographic method for analyzing a group of five chiral fungicides (ipconazole, metconazole, penconazole, tebuconazole, and tetraconazole) as well as an achiral one (fluconazole) in aqueous matrices. **Methods:** The chromatographic method was developed using the chiral polysaccharide column Lux i-cellulose 5 [cellulose tris(3,5-dichlorophenylcarbamate)] under reversed elution mode in a liquid chromatograph with a diode array detector. Different compositions and proportions of the mobile phase, various column temperatures and different flow rates were tested. **Results:** An enantioselective chromatographic method was optimized, allowing to enantioseparate the enantiomers of ipconazole, metconazole, penconazole, tebuconazole, and tetraconazole and of the achiral fungicide fluconazole. **Conclusions:** This optimized method will be validated and used for the determination of the enantiomers of each chiral target fungicide – ipconazole, metconazole, penconazole, tebuconazole, and tetraconazole – as well as the achiral fungicide fluconazole in water matrices.

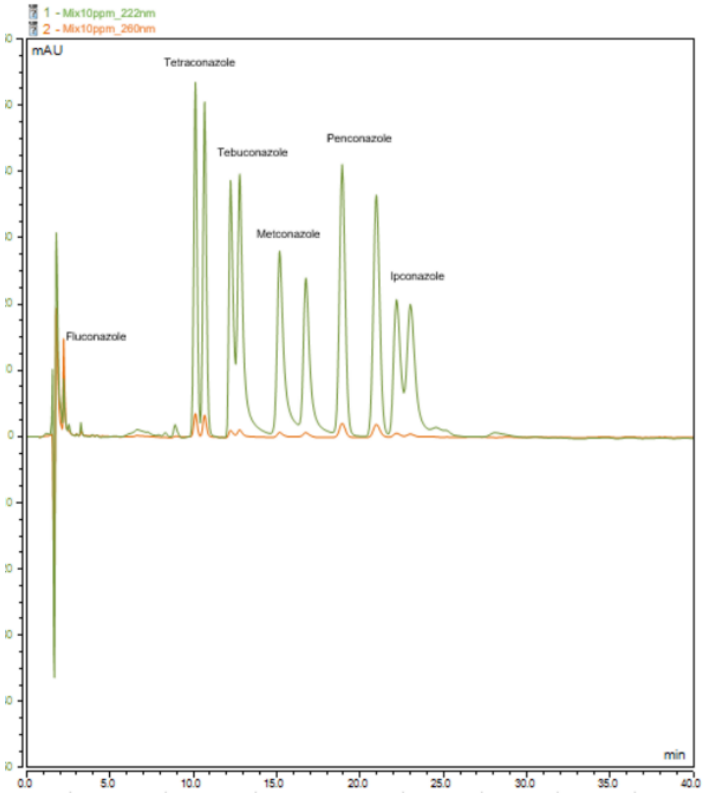

Figure 1. Chromatogram of a mixture of target fungicides, each at 10 mg L^{-1}, at two different wavelengths (222 nm and 260 nm).

Keywords: environmental contaminants; fungicides; water monitoring; chiral chromatography

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PC40 Monitoring azole antifungals in water: a step toward environmental and human health protection

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ABSTRACT

Background: Water is an essential resource for all living organisms and human activities. However, the presence of organic micropollutants (OMPs) at residual levels (ng/L–µg/L) in aquatic environments raises significant concerns. Among these, Priority Substances (PSs, Directive 2013/39) and Contaminants of Emerging Concern (CECs, Watch Lists of Decisions 2015/495, 2018/840, 2020/1161, 2022/1307, and 2025/439) have been detected in surface water (SW) and drinking water (DW) [1], prompting regulatory action within the European Union (EU). Monitoring these compounds is crucial for risk assessment and environmental protection. Azole antifungals, recently included in the EU Watch Lists, represent an emerging threat due to their potential ecotoxicological risks and their role in promoting antifungal resistance. Their presence in aquatic environments may contribute to the spread of resistant pathogens, such as *Candida auris* and *Aspergillus fumigatus*, which pose direct risks to human health [2]. **Objective:** To address this challenge, this study focuses on optimizing a solid-phase extraction (SPE) method using carbon-based adsorbents for the detection of azole antifungals in SW and DW matrices. **Methods:** Key parameters, including different carbon materials, sample pH, and elution solvents, were evaluated to enhance extraction efficiency. **Results:** The results suggest that carbon-based SPE cartridges have potential for monitoring azole compounds, with the added advantage of being reusable for at least three cycles without loss of performance. Further optimization is required to improve extraction efficiency and ensure reliability in routine analysis. **Conclusions:** This work contributes to the development of advanced analytical methods for water quality assessment and supports efforts to mitigate environmental and human health risks associated with emerging contaminants.

Keywords: contaminants of emerging concern; analytical methods; antifungal resistance

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PC41 Fluoroquinolones: a preliminary study on optimized extraction and chromatographic methods

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ABSTRACT

Background: Fluoroquinolones (FQs) are a widely prescribed class of antibiotics, used in both human and veterinary medicine, commonly administered for prophylactic and therapeutic purposes [1]. Due to their extensive use and excretion in both unmetabolized form and metabolites, FQs and their metabolites persist in aquatic environments. As a result, these compounds are frequently detected in surface and groundwater worldwide, with concentrations ranging from ng L^{-1} to µg L^{-1}. This persistence contributes significantly to the spread of antibiotic resistance among microbial populations, posing risks to both ecosystems and human health [1,2]. Therefore, monitoring water matrices is crucial for assessing both ecological risks and potential human health impacts [2]. **Objective:** This study aimed to optimize a sample preparation procedure and develop an enantioselective method for the analysis of FQs, namely Ciprofloxacin, Enrofloxacin, Nadifloxacin, *N*-Desmethyl Ofloxacin, Ofloxacin, and Ofloxacin *N*-oxide in aqueous samples. Solid-phase extraction (SPE) was employed for sample preparation, followed by an enantioselective analytical method based on high-performance

liquid chromatography (HPLC), coupled with a fluorescence detector (λ_{Ex}: 290 nm, λ_{Em}: 460 nm), except for Nadifloxacin that was analysed using a UV detector (λ = 291 nm). **Methods:** Different conditions were tested using Oasis® MAX Extraction cartridges (Waters) for sample preparation, and different mobile phases were assessed to optimize the chromatographic separation on a Lux® 3 µm Cellulose-2 chiral column. **Results:** The performance of MAX cartridges under different conditions was assessed based on recovery efficiency. The method demonstrated high efficiency, with recoveries higher than 70% for most of the FQs, except for Ofloxacin *N*-oxide and *N*-Desmethyl Ofloxacin. Optimal separation conditions were achieved using the aforementioned chiral column, under reverse elution mode. **Conclusions:** The optimized analytical method will be employed to quantify the levels of FQs in ecotoxicological assays. Further method validation is required to ensure accurate quantification of their presence in culture media samples.

Keywords: antibiotics; environmental contaminants; enantioselective methods

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PC42 Analytical techniques for microplastics analyses

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ABSTRACT

Background: Pollution caused by plastic waste is an emerging environmental problem. Micro- and nanoplastics are omnipresent in various ecosystems. This threat requires the use of instrumental techniques for the analysis of these xenobiotics. Moreover, there is an urgent need to develop methods enabling the analysis of increasingly smaller particles. **Objective:** The aim of the study is to present a review of analytical techniques for microplastic determination. Furthermore, the poster will present the comparative analysis of real samples using two complementary analytical techniques, pyrolysis gas chromatography-mass spectrometry (Pyr-GC/MS) and laser direct infrared imaging (LDIR). **Methods:** A review of the latest scientific literature was done to compare instrumental analytical methods used for the determination of micro- and nanoplastics. Additionally, Pyr-GC/MS and LDIR were used to analyze the same real samples, to compare the obtained results. **Results:** The analytical process of microplastics analysis is affected by the sample preparation, extraction, quantification and quality assurance/quality control (QA/QC). The sample preparation stage is crucial and depends on the sample matrix and the location from which it was collected (air, water, soil, sediments, or biota). This step is followed by the extraction of particles. The methods commonly used to extract microplastics in the environment include visual inspection, flotation, density separation, size separation (like sieving and filtration), digestion, biological removal, and chemical treatments. At present, many techniques for microplastic analysis are used, such as Fourier transform infrared spectroscopy, Raman spectroscopy, laser diffraction, scanning electron microscope, thermal analysis, or pyrolysis gas chromatography coupled to quadrupole mass spectrometry. **Conclusions:** All methods used for micro- and nanoplastics determinations have their advantages, disadvantages, and limitations. Hence, the information on microplastics obtained through one detection method is usually unstructured and inconsistent. In order to obtain reliable and complete results, it is necessary to use methods that complement each other. The poster will present a summary of the most efficient techniques for more precise and accurate microplastic determinations in complex environmental samples.

Keywords: microplastic; analytical methods; analysis

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PC43 Microplastics in rice: an invisible threat

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ABSTRACT

Background: Humans are exposed to various dangers in their daily lives, including exposure to contaminants through food. Microplastics are increasingly being studied as an environmental contaminant in both marine and terrestrial systems [1]. They are present in air, water, and soil, leading to their internalization by organisms and, ultimately, to the contamination of the human food chain [2]. Moreover, microplastics can act as vectors for chemical contaminants (e.g., metals) and pathogenic microorganisms [3]. The contamination of microplastics in rice, one of the most consumed foods worldwide, is understudied but should be further investigated considering the potential impacts on human health. Understanding human exposure is important for risk assessment. **Objective:** This study aimed to test analytical methods of extracting and quantifying microplastics in rice. **Methods:** Commonly used methods were applied to rice (Carolino variety, acquired from local markets), including digestion of 10 g with solutions such as 10% KOH, 30% H_2O_2, 15% H_2O_2 + Fe, 30% HNO_3 (with both raw and cooked rice), and 65% HNO_3 for 24 h at 60 ºC. After digestion, samples were filtered using a glass fiber filter (1.2 µm pore). Density separation was also performed using a NaCl solution with a density of 1.2 g/cm³, mixed for 1 min with 50 g of rice, left to settle for 1 h, followed by filtration with a glass fiber filter (1.2 µm pore). After drying, the filter membranes were weighed. **Results:** The tested methods were not effective in extracting microplastics. The presence of starch clogged the filter and concealed microplastics. Similarly, in density separation, the supernatant solution intended for filtration became heavily filled with starch, which did not settle. **Conclusions:** This study concluded that rice presents specific challenges that hinder the applicability of analytical methods commonly used to extract microplastics. Starch is one of the main obstacles, as it interferes with the effectiveness of these techniques, which may explain the scarcity of published studies on the contamination of rice with microplastics.

Keywords: microplastics; food contaminants; analytical methods

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PC44 Optimization and validation of an HPLC-DAD method for the identification of 14 cannabinoids: application in Cannabis sativa L extracts

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ABSTRACT

Background: *Cannabis* sp. plant has its origins in East Asia, where it was initially used for recreational and religious purposes. This plant encompasses hundreds of chemical compounds, including phytocannabinoids, terpenoids, and flavonoids. The cannabinoids of greatest medicinal interest are Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD), which are present in the plant in their acid form [1]. Sativex® is an oromucosal spray containing standardized extract with Δ^9-THC and CBD already approved as a treatment option for neuropathic pain associated with multiple sclerosis [2]. With cannabis increasing recognition as a medicinal option, there are growing concerns over how to extract, detect and quantify cannabinoids properly and efficiently. High-performance liquid chromatography (HPLC) coupled with ultraviolet (UV) detection is considered the gold standard for cannabinoid analytical assessment included in cannabis monographs present in several pharmacopeias [3,4]. **Objective:** The main goal of this project was to optimize and validate an HPLC-DAD analytical method for the quantification of 14 cannabinoids in cannabis extracts. **Methods:** Flower pulverization was accomplished with a Retsch MM 400 ball mill. Extraction was performed according to the European Pharmacopoeia [4]. Chromatographic separation of cannabinoids was achieved on an Agilent 1260 Infinity II HPLC-DAD system, using an InfinityLab Poroshell 120 EC-C18 (3.0 x 150 mm, 2.7 µm) column protected with a Poroshell 120 EC-C18 3.0 mm, 2.7 µm guard column. The gradient elution was performed using methanol with 0,05% formic acid and deionized water with 0,1% formic acid mixtures, with a flow rate of 0,5 mL/min, run time of 30 min, and injection volume of 5 µL. **Results:** The optimized method resulted from adjusting chromatographic conditions: mobile phases (solvents, gradient, pH and flow rate: 0,5 – 1 mL/min), column length (50 – 150 mm) and temperature (30 – 50 ºC). Diode array analysis was performed for specificity assessment and UV quantification was performed at 224, 230, 260, 272 and 280 nm. To demonstrate that the analytical method fits its purpose, accuracy, precision, linearity, and range were established based on regulatory guidelines – ICH Q2. **Conclusions:** The developed and validated method successfully

separates 14 cannabinoids, as well as other compounds present in the cannabis extracts tested.

Keywords: cannabis; Δ^9-THC; HPLC-DAD

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PC45 Analysis of terpenes in cannabis by GC-MS: method development and its application to Cannabis sativa L extracts

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ABSTRACT

Background: In addition to cannabinoids, key bioactive compounds in cannabis, terpenes and terpenoids, also have therapeutic importance, potentially working synergistically through their entourage effect for the medicinal efficacy of this plant [1,2]. Besides their therapeutic advantages, they are also responsible for the characteristic aroma of numerous varieties of cannabis, ranging from citrus to woody aromas [3]. Gas Chromatography-Mass Spectrometry (GC-MS) has become a powerful and reliable analytical tool for precisely identifying and quantifying terpenes in cannabis. Understanding the terpene profile is essential for optimizing strain selection, which may enable the development of targeted therapies for specific medical conditions [4]. **Objective:** This study aims to develop a GC-MS-based analytical method for the separation and quantification of terpenes in cannabis extract. **Methods:** Dried cannabis flowers were pulverized in a Retsch MM 400 ball mill and extracted using a modified European Pharmacopoeia method [5]. 40 mL of ethyl acetate was added to the ground cannabis sample, and the mixture was stirred for 15 minutes at room temperature and centrifuged at 4500 rpm for 5 minutes. The supernatant was transferred to a volumetric flask and extraction was repeated twice with 25 mL. The final volume was adjusted to 100 mL followed by filtration. The extract was diluted to 1:10 in ethyl acetate and analyzed by GC-MS. For the chromatographic separation, a capillary column containing 5% diphenyl 95% dimethylpolysiloxane (30 m × 0.25 mm × 0.25 µm) was used, and the injector temperature was programmed to 280 ºC. A typical run started at a temperature of 60 ºC, raising to 280 ºC at a helium flow of 1 mL/min with a total run time of 42 min. **Results:** Several chromatographic parameters were studied to enhance the separation of terpenes, namely the starting run temperature, ramp profile, and running times. The extraction from cannabis flowers was performed using two solvents, dichloromethane and ethyl acetate, to evaluate the efficiency of the extraction. The chromatographic conditions established made it possible to separate and identify the nine compounds in the same run, both in a mixture of standards and in the extracts. **Conclusions:** A GC-MS analytical method was developed, allowing the separation, identification, and quantification of 9 terpenes simultaneously.

Keywords: medicinal cannabis; phytochemistry; mass spectrometry; gas chromatography

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PC46 Enantiomeric profiling of ketamine in street samples for recreational use

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ABSTRACT

Background: While ketamine (KET) has gained increasing scientific attention for its novel therapeutic applications, there has also been a parallel global rise in its non-medical, recreational use. [1]. Due to its effects on the central nervous system—such as dissociation, perceptual alterations, and its well-known anesthetic properties—KET is frequently used recreationally for its psychoactive effects. [2]. As concerns over its use outside the medical settings and its potential for addiction continue to grow, many governments have classified KET as a strictly regulated substance in numerous countries. This chiral compound was traditionally found in its racemate form. However, the prevalence of enantiomerically pure forms, or different enantiomeric compositions has been increasing in recreational samples. Enantiomers exhibit distinct potency and affinity for the N-methyl-D-aspartate receptor with (S)-KET exhibiting fourfold greater affinity for NMDA receptors [2,3]. The discrimination of the enantiomeric fraction (EF) present in seized and or consumed samples is essential due to the different biological activities of enantiomers, which may have potential toxicity implications for consumers. Additionally, it may allow for the determination of origin, precursors used, and possible synthesis processes, relevant for forensic investigation. **Objectives:** The present work aims to determine the enantiomeric fraction (EF) of KET in recreational samples using an enantioselective liquid chromatography method [4]. **Methods:** A previously developed enantioselective method using liquid chromatography coupled with diode-array detection was adapted for the analysis of powder samples provided by consumers [4]. Enantiomers were separated using an analytical Lux® 3 µm cellulose-4 column (150 × 4.6 mm internal diameter) under isocratic elution conditions. The optimized method employed a mobile phase of ammonium acetate in ultrapure water (with 0.1% diethylamine) and acetonitrile (70:30, v/v), with a flow rate of 1 mL/min and detection at 220 nm. **Results:** The findings revealed that all analyzed samples remained in racemic form, highlighting the continued dominance of this composition in illicit drug markets. **Conclusions:** Given the distinct pharmacological and toxicological properties of KET enantiomers, further monitoring of enantiomeric profiling in seized substances is crucial for forensic investigations, public health assessments, and regulatory measures.

Keywords: chiral psychoactive drugs

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PC47 GC-MS-Based Study of 14 Cannabinoids Separation in Cannabis sativa L. Extracts using a Derivatization Approach

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ABSTRACT

Background: Cannabinoids from *Cannabis sativa* L. are increasingly studied due to their potential as medicinal drugs, offering therapeutic benefits such as pain relief and anti-inflammatory effects, while also raising concerns as substances of abuse due to their psychoactive properties [1,2]. Gas chromatography-mass spectrometry (GC-MS) is a powerful tool for identifying and quantifying cannabinoids, offering high sensitivity and specificity. However, due to the thermal instability of cannabinoids, derivatization is a crucial step to improve their detectability and chromatographic behavior in GC-MS analysis. **Objective:** This study aims to develop a derivatization protocol and a GC-MS-based analytical method for cannabinoid detection in extracts of the *Cannabis* sp cultivar ZF plant. **Methods:** Extraction of cannabinoids from dried cannabis flowers was achieved following the European Pharmacopoeia protocol [3]. The standards and extracts were derivatized with 120 µL of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide with 1% of trimethylchlorosilane (BSTFA + 1% TMCS), 80 µL of pyridine in 200 µL of anhydrous ethyl acetate. The mixture reacted for 30 min at 60 ºC, then cooled to room temperature and injected directly into the GC-MS for analysis. The chromatographic conditions were established using a capillary column containing 5% diphenyl 95% dimethylpolysiloxane (30 m x 0.25 mm x 0.25 µm), injector temperature set to 280 ºC followed by a temperature ramp from 180 up to 280 ºC at a helium flow rate of 1 mL/min to a total run of 25 min. **Results:** Several derivatization conditions were tested to allow high yields of the derivatized cannabinoids while preventing decarboxylation of the acidic forms. Hence, reaction time and temperature, the quantity of derivatizing agent, the use or not of pyridine, and the use of solvents like ethyl acetate, dichloromethane, and acetonitrile were studied. Chromatographic conditions were also optimized to allow the simultaneous separation and detection of 14 compounds in the same run. **Conclusions:** The optimized derivatization conditions ensured the stability of the different cannabinoids avoiding decarboxylation of the acidic forms and formation of byproducts. The established chromatographic conditions provided an adequate separation and peak resolution of a total of 14 cannabinoids. The GC-MS-based analytical method was successfully applied to the identification and detection of these cannabinoids in cannabis extracts.

Keywords: medicinal cannabis; phytochemical analysis; gas chromatography; mass spectrometry

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PC48 Enantioseparation of 3-chloromethcathinone by liquid chromatography at the milligram scale

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ABSTRACT

Background: The most prominent synthetic cathinone (SCAT) is 3-chloromethcathinone (3-CMC), accounting for 34% and 63.41% of the total seized new psychoactive substances (NPS) in Europe in 2021 and 2022, respectively [1, 2]. Over the latest years, since the first identification on the European drug market in September 2014 in Sweden, 3-CMC has gained significant popularity among the younger drug users [3]. Moreover, 3-CMC is chiral and its enantiomers can show different biological activity, highlighting the importance of the enantioselectivity studies in clinical, forensic and ecotoxicological context. **Objective:** The aim of this study was to optimize a chromatographic method for the enantiomeric separation of 3-CMC at the milligram scale for further use in *in vitro* and ecotoxicity assessments. **Methods:** The enantioseparation as well as the enantiomeric purity evaluation of the 3-CMC were performed by liquid chromatography coupled to the ultraviolet-visible detector (UV/Vis), using a CHIRALPAK® AD-H 10 x 250 mm, 5 µm, a semi-preparative column. A Dionex Ultimate 3000 automated fraction collector was used for fractions collection. Data was analyzed by Chromeleon 7.0 software. For method conditions optimization, a solution at 100 µg mL^{-1} of 3-CMC in ethanol with diethylamine was used. **Results:** The optimized method allowed the separation of the enantiomers of 3-CMC at final concentration of 3.7 mg mL^{-1}, with an enantiomeric purity of 98 % and 95 % for the first and second eluted enantiomer, respectively. The determination of the absolute configuration of the enantiomers is ongoing by electronic circular dichroism. **Conclusions:** The isolated enantiomers will be used for the enantioselective evaluation of the 3-CMC ecotoxicity. The determination of the absolute configuration of the enantiomers will enable correlating the ecotoxicity of each enantiomer.

Keywords: 3-chloromethcathinone; enantioseparation; synthetic cathinones

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PC49 Cebranopadol, a first-in-class investigational opioid: Insights from preliminary cytotoxicity assays

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ABSTRACT

Background: Cebranopadol, a promising opioid analgesic undergoing phase III trials, was developed for the treatment of acute and chronic moderate-to-severe pain. Because it is a non-selective ligand acting at more than one member of the opioid

receptor family, combining a dual agonist action on mu-, delta-, and kappa-opioid receptors (MOR, DOR, and KOR) and nociceptin/orphanin FQ peptide (NOP) receptors, it has improved efficacy and tolerability over currently available prescription opioids [1,2]. The combined NOP and opioid receptor agonism make cebranopadol a potent analgesic for neuropathic and nociceptive pain, with an optimized safety profile, no significant side effects, and low tolerance and abuse potential [1,3]. Due to the novelty of this molecule, there are very few studies on its toxicological and cytotoxic potential. In this context, this study evaluated cebranopadol cytotoxicity in different cell lines representative of its metabolizing and target tissues. **Objective:** The present study aimed to assess the cytotoxicity profile of cebranopadol in three different cell lines: BV-2 (murine microglia), HepG2 (human hepatocellular carcinoma), and SH-SY5Y (human neuroblastoma). **Methods:** BV-2, HepG2, and SH-SY5Y cells were exposed to increasing cebranopadol concentrations (up to 2.64 µM) for 48 hours and assayed for cytotoxicity through the sulforhodamine B (SRB) assay, whereby the IC_{50} value was determined for each cell line. **Results:** Cebranopadol was found to inhibit HepG2 and SH-SY5Y cell growth with IC_{50} values of 1.81 ± 0.08 µM and 2.28 ± 0.07 µM, respectively; as for BV-2 cells, there was no growth inhibition within the concentration range tested (IC_{50} > 2.64 µM). These values are above the therapeutic concentration range reported for humans [2], supporting cebranopadol safety when used as prescribed. Cytotoxicity may arise in overdose situations. **Conclusions:** The results add information on cebranopadol effects on hepatic and central nervous system cells, demonstrating cell line-dependent sensitivity and low cytotoxicity potential, and corroborating its safety as an alternative therapeutic option for pain control. Metabolic and molecular studies are needed to fully clarify its effects at therapeutic and supratherapeutic concentrations.

Keywords: cebranopadol; cytotoxicity; prescription opioids

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PC50 Dual-action breakthrough: Fiscalin derivatives targeting amyloid burden and acetylcholinesterase for Alzheimer's therapy

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ABSTRACT

Background: Alzheimer's disease (AD) is the most common age-related dementia, characterized by neurodegeneration associated with the accumulation of amyloid-beta (Aβ) peptides and hyperphosphorylated Tau, mitochondrial dysfunction, and oxidative stress, among other pathological features [1]. Moreover, reduced acetylcholine levels contribute to the cognitive impairment and memory deficits characteristic of the disease. With life expectancy increasing globally and the current treatments providing only symptomatic relief, AD prevalence is projected to more than triple by 2050 [2]. Therefore, the development of novel compounds targeting these mechanisms could help restore brain function or slow disease progression. In this regard, fiscalins, a class of valine-derived alkaloids with an indolyl and tricyclic anthranilic acid core, have previously demonstrated neuroprotective, antimicrobial, and anticancer properties, making them promising candidates for further investigation in AD treatment [3]. **Objective:** The main objective of this work was to evaluate the cytotoxicity and neuroprotective effects of six synthetic fiscalin derivatives (Figure 1), and their ability to inhibit acetylcholinesterase (AChE), the enzyme that degrades acetylcholine, using SH-SY5Y cells differentiated into a cholinergic phenotype. **Methods:** The cytotoxicity of the compounds (0–50 µM) was assessed after 24 h of exposure by the neutral red uptake and MTT reduction assays, to select non-cytotoxic concentrations. Neuroprotection was further tested against β-amyloid peptide (Aβ; 50 µM), assessing cell viability by the MTT reduction assay after 24 h of exposure in the absence or presence of fiscalin derivatives (10 and 25 µM). Additionally, compounds' ability to inhibit AChE activity was evaluated using Ellman's assay. **Results:** All the tested compounds were non-cytotoxic for concentrations up to 25 µM. Three of the six tested fiscalin derivatives significantly reduced Aβ-induced cell death, while five compounds significantly reduced AChE activity, when compared with control cells. **Conclusions:** These findings highlight the potential of these compounds to counteract Aβ toxicity and reduce AChE activity, two critical features of AD. Nevertheless, further studies are needed to elucidate their neuroprotective mechanism(s).

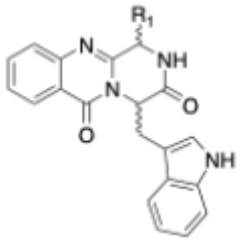

Figure 1. Chemical structure of fiscalin derivatives.

Keywords: Alzheimer's disease; fiscalin derivatives; β-amyloid; acetylcholinesterase activity

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PC51 Efficacy and safety of semaglutide for obesity: A systematic review of phase 3 clinical trials

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ABSTRACT

Background: Obesity is a chronic disease with significant metabolic and cardiovascular complications, yet effective long-term pharmacological treatments remain limited. Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) have emerged as a promising therapeutic class for weight management due to their effects on appetite regulation and energy balance. Semaglutide (SMG), a potent GLP-1 RA originally developed for type 2 diabetes, has demonstrated substantial weight-loss benefits, leading to its investigation as an obesity treatment. **Objective:** This review evaluates phase 3 clinical trials assessing the efficacy and safety of both subcutaneous and oral SMG in individuals with obesity or overweight, with and without type 2 diabetes. **Methods:** A search was conducted using PubMed, Web of Science, and ClinicalTrials.gov databases from inception through to December 31, 2024. Inclusion and exclusion criteria were designed to identify clinical trials evaluating the efficacy and safety of SMG for weight management. **Results:** The STEP (Semaglutide Treatment Effect in People with Obesity) program, targeting individuals with obesity or overweight with comorbidities, evaluated subcutaneous SMG 2.4 mg, demonstrating superior weight loss compared to placebo and liraglutide. Key strengths include well-designed randomized controlled trials demonstrating significant weight loss and metabolic benefits. The OASIS (Oral Semaglutide Treatment Effect in People with Obesity) studies explored higher-dose oral SMG (up to 50 mg) in obesity, achieving weight loss comparable to subcutaneous SMG 2.4 mg. Results showed that SMG achieved a significantly higher reduction in mean body weight (–15.5 kg and –15.1%) compared with placebo (−2.5 kg and –2.4%). Gastrointestinal adverse events, primarily nausea and vomiting, were common but typically mild to moderate and transient. **Conclusions:** SMG, in subcutaneous and oral formulations, has proven to be highly effective in significantly reducing body weight. Clinical studies confirm the superiority of SMG over most GLP-1 RAs, promoting safe and effective weight loss. Collectively, these studies underscore SMG's efficacy and acceptable safety profile, positioning it as a transformative option in obesity pharmacotherapy.

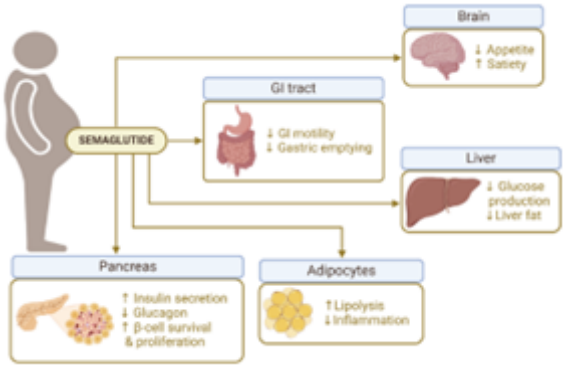

Figure 1. Mechanism of semaglutide for the management of obesity (Created in BioRender. Carvalho, M. (2025) https://BioRender.com/l84z782).

Keywords: semaglutide; GLP-1 receptor agonist; obesity; weight loss therapy

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PC52 Antibiotic prescription in dentistry: Systematic and critical evaluation

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ABSTRACT

Background: Antibiotics have been widely used to treat bacterial infections. In dentistry, their use has been generalized for the treatment of dental infections. This raises major concerns related to their overuse and to the resistance that develops [1]. A critical evaluation of their efficacy in comparison with other therapeutic options is therefore necessary. **Objective:** Critically evaluate the effectiveness of the use of systemic antibiotics in the treatment of dental infections, compared to other therapeutic options. Additionally, this review aimed to explore the motivations behind antibiotic prescriptions in dentistry, with a view to promoting the appropriate use of these drugs and improving patient education regarding adherence to prescribed regimens. **Methods:** A search was made in PubMed, Cochrane Library, B-On and Scielo, considering articles published between 2013 and 2023, in English and Portuguese. The study selection strategy followed the PICO framework (Population: patients with dental infections; Intervention: systemic antibiotic therapy; Comparison: other therapeutic options; Outcome: improved antibiotic prescribing practices). The selection and analysis of studies were carried out according to PRISMA recommendations, using the ROBINS-I tool to assess the risk of bias of the included studies. **Results:** Of the 384 records initially identified, 12 studies were selected that met the inclusion criteria. These studies covered different geographical regions all over the world [1,2]. The results showed that there is a global trend towards the inappropriate prescription of antibiotics in odontogenic cases, especially in conditions such as pulpitis (25.5%) and dental abscesses without systemic involvement (68%) [2,3]. Amoxicillin was identified as the most frequently prescribed antibiotic, followed by the amoxicillin/clavulanic acid combination, while clindamycin was the preferred antibiotic in patients allergic to penicillin [2,3]. **Conclusions:** This review highlights a worrying practice of excessive and inappropriate prescription of systemic antibiotics in dentistry, particularly in cases where local treatment would be sufficient [1]. The results underline the imperative need for rigorous implementation of existing international guidelines, as well as reinforcement of ongoing training for professionals in the field. These measures are key to ensuring the rational use of antibiotics, preventing the rise of bacterial resistance and ensuring better clinical outcomes for patients.

Keywords: Dental infection; Antimicrobial therapy; Bacterial resistance to antibiotics

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PC53 Cannabis-based therapies in chronic pain management: A systematic review of clinical trials

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ABSTRACT

Background: Chronic pain affects millions worldwide, driving the need for effective treatment alternatives. Cannabis-based therapies have gained attention for their potential analgesic effects, but their efficacy and safety remain a topic of debate [1]. **Objective:** This review examines clinical trials assessing the effectiveness of cannabis-derived treatments, including FDA-approved cannabinoid medications such as CBD (Epidiolex®), a combination of Δ^9-THC and CBD (Sativex®), and synthetic cannabinoids like nabilone (Cesamet®) and dronabinol (Marinol® and Syndros®). **Methods:** A search was conducted using PubMed, Web of Science, and ClinicalTrials.gov databases from inception through to October 31, 2024. Inclusion and exclusion criteria were designed to identify clinical trials evaluating only randomized double-blind clinical trials that compared the effects of cannabinoids with a placebo or standard treatment, lasted at least 4 weeks after the start of treatment, and used one of the internationally validated pain intensity scales. **Results:** While some studies report reductions in pain intensity and improvements in associated symptoms, others show limited or no significant benefit. The outcome variability highlights the need for further research to determine optimal formulations, doses, and patient populations that may benefit most. **Conclusions:** While cannabis-based treatments hold promise for chronic pain management, clinical evidence remains inconsistent. This review highlights the urgent need for more rigorous clinical trials to establish definitive safety and efficacy profiles before these therapies can be widely adopted in clinical practice.

Keywords: *Cannabis sativa*; cannabinoids; chronic pain; clinical trials

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PC54 Cytotoxic effects of cannabidiol on human metastatic melanoma cells: a potential therapeutic strategy?

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ABSTRACT

Background: Melanoma is the most aggressive form of skin cancer, responsible for the majority of skin cancer-related deaths due to its high metastatic potential and resistance to treatment [1]. The limited efficacy of systemic therapies in treating this disease underscores the need to explore novel therapeutic strategies. Cannabidiol (CBD), a bioactive compound from *Cannabis sativa*, has shown anticancer properties in various cancer models [2,3]. However, its effects on melanoma cells and its potential selectivity remain under investigation. **Objective:** This study evaluates the cytotoxic effects of CBD on human metastatic melanoma cells. **Methods:** Two melanoma cell lines (A375 and MeWo) and a normal human keratinocyte cell line (HaCaT) were treated with CBD (0.05-100 µM) for 24 and 48 hours. Cell viability was assessed by the MTT assay, and morphological changes were analyzed by inverted light microscopy. **Results:** CBD reduced cell viability in a concentration-dependent manner in all cell lines. HaCaT cells were the most sensitive with IC_{50} values at 24 h of 4.1 µM, followed by A375 (IC_{50} 5.1 µM) and MeWo (IC_{50} 5.8 µM) cells. Similar values were observed at 48 h ($p > 0.05$), indicating no significant time-dependent effect. Morphological changes were observed in all cell lines, becoming more pronounced with increasing CBD concentration and exposure time. **Conclusions:** CBD is a promising candidate for melanoma management. However, its non-selective cytotoxicity toward melanoma cells remains a major challenge for safe clinical application. Ongoing research aims to elucidate the molecular mechanisms underlying CBD-induced cytotoxicity and to explore strategies for improving selectivity, potentially enhancing its clinical applicability in melanoma treatment.

Keywords: cannabinoid; anticancer; skin cancer; therapy

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PC55 Hidden dangers of synthetic cathinones: unveiling the role of oxidative stress in the cardiotoxicity of methylone and 3,4-DMMC

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ABSTRACT

Background: Synthetic cathinones (SCs) are the second most reported class of new psychoactive substances worldwide, according to the United Nations Office on Drugs and Crime (UNODC), with over 100 analogues identified [1]. These recreational stimulants exhibit pharmacological properties similar to amphetamines [2] and have been associated with serious cardiac events, including myocardial infarction and sudden cardiac death [3]. However, the mechanisms underlying their cardiotoxicity remain unclear. **Objective:** This study investigates the cardiotoxic effects of two commonly abused SCs, namely 3,4-methylenedioxymethcathinone (methylone) and 3,4-dimethylmethcathinone (3,4-DMMC), and examines the role of oxidative stress in their toxicity. **Methods:** Rat H9c2 cardiomyoblasts were exposed to methylone (0.01-4 mM) and 3,4-DMMC (0.0005-0.8 mM) for 24 and 48 hours. Cell viability was evaluated using the MTT assay, while oxidative stress was assessed by measuring reactive oxygen and nitrogen species (ROS/RNS) production at multiple timepoints (0.5 to 24 h) at the EC_{50} concentration. Additionally, the protective effects of antioxidants, including ascorbic acid (AA, 0.1 mM), N-acetylcysteine (NAC, 1 mM), and Trolox (TRX, 0.2 mM), were assessed 24 hours after incubation with EC_{40}. **Results:** Both substances decreased cell viability in a concentration-dependent manner, but this effect was not significantly affected by incubation time. 3,4-DMMC showed greater cytotoxicity than methylone at 24 h (EC_{50}: 0.28 mM vs. 0.98 mM, p=0.0013) and 48 h (EC_{50}: 0.18 mM vs. 1.04 mM, $p < 0.0001$). ROS/RNS levels increased over

time, reaching statistical significance at 3 h for 3,4-DMMC ($p < 0.05$) and 4 h for methylone ($p < 0.01$), indicating the involvement of oxidative stress. Among the antioxidants tested, only AA effectively attenuated SC-induced toxicity, while NAC and TRX showed no protective effect. **Conclusions:** Our findings demonstrate that SCs induce significant cardiotoxicity *in vitro*, with 3,4-DMMC being more toxic than methylone. Oxidative stress contributes, at least in part, to the cardiotoxic effects of these substances. Notably, AA offers potential protection against SC-induced damage. These results highlight the need for further research to elucidate the precise mechanisms of SC-induced cardiotoxicity and to explore therapeutic strategies.

Keywords: cathinones; myocardial damage; toxicity mechanisms

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PC56 Chalcone Derivatives with Potential Antimitotic Activity

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ABSTRACT

Background: Cancer remains one of the leading causes of mortality worldwide, with incidence rates rising in recent years [1]. Among various therapeutic strategies, microtubule-targeting agents have demonstrated significant efficacy in cancer treatment. However, their clinical application is often limited by high toxicity and tumor resistance [2]. Therefore, the discovery of novel small molecules with antitumor activity and improved efficacy is crucial. Several studies have shown that the presence of a 3,4,5-trimethoxyphenyl fragment is a key structural feature for interacting with tubulin, a microtubule subunit protein essential for mitotic spindle formation, and consequently for cell division and proliferation [3]. In this study, 16 chalcone derivatives (compounds 1–16) were synthesized, and their *in vitro* cytotoxic activity was evaluated in different cancer cell lines. **Objectives:** This study aimed to assess the antitumor and antimitotic activity of 16 chalcone derivatives and investigate the cellular mechanism of action of the most promising compounds. **Methods:** The chalcone derivatives were synthesized through Claisen-Schmidt condensation. Their cytotoxic activity was evaluated using the sulforhodamine B (SRB) assay to determine the GI_{50} in cancer cell lines, including melanoma (A375-C5), breast adenocarcinoma (MCF-7), and non-small cell lung cancer (NCI-H460). Antimitotic activity was assessed by indirect immunofluorescence, analyzing DNA (stained with DAPI) and spindle morphology (through microtubule labeling with an anti-α-tubulin antibody). Additionally, Annexin V/PI double staining followed by flow cytometry was performed to assess apoptotic cell death induction. **Results:** Among the 16 studied compounds, 4 exhibited promising antitumor activity ($GI_{50} < 10$ µM) and were selected for further mechanistic characterization. These 4 compounds displayed potent growth-inhibitory activity and disrupted microtubule dynamics during mitosis, leading to spindle assembly defects. This instability resulted in prolonged mitotic arrest, ultimately triggering apoptosis. **Conclusions:** The studied chalcone derivatives demonstrated promising potential as antitumor and antimitotic agents, impairing cell division and promoting cancer cell death. These findings support their potential for further development in cancer therapy.

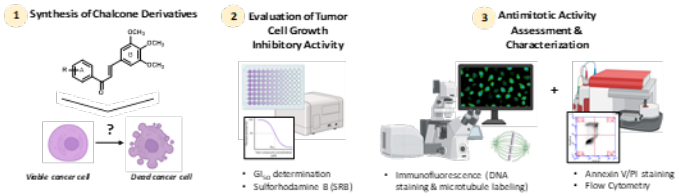

Figure 1. Graphical representation of the approach used to evaluate the potential of chalcone derivatives as antitumor and antimitotic agents.

Keywords: chalcone derivatives; anticancer; antimitotics

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PC57 Assessment of the Antitumor Potential of Flavonoid-Amino Acid Conjugates

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ABSTRACT

Background: Cancer is a complex disease characterized by multiple alterations that promote uncontrolled cell proliferation and resistance to cell death. Multidrug resistance to conventional chemotherapy often results in treatment failure, highlighting the need for alternative therapeutic strategies [1]. In this context, natural products and their derivatives have attracted significant interest for their potential anticancer properties. Of particular interest, chiral derivatives of flavonoids (CDFs) have demonstrated the ability to inhibit the growth of specific human tumour cell lines, emphasizing the critical role of stereochemistry in their biological activity [2, 3]. **Objective:** This study aimed to screen a small library of previously synthesized CDFs in a panel of cancer cell lines to identify the most promising compounds for further study as potential chemotherapy drugs. Additionally, this study also aimed to assess their effects on key aspects of cancer biology, including metabolic processes and the mechanisms of cell death. **Methods:** Cell viability assays were conducted on three tumour cell lines: A375-C5 (melanoma), MCF-7 (breast), and NCI-H460 (lung), using synthesized CDFs. The most effective compound was further analysed based on several parameters of cancer cells, including extracellular levels of glucose and lactate. Furthermore, Annexin V/PI double staining in conjunction with flow cytometry was conducted to evaluate the induction of apoptotic cell death. **Results:** The cytotoxic effects of nine CDFs were assessed, leading to the selection of one compound for further investigation due to its GI_{50} values being below 20 µM in all cell lines. In terms of metabolism, the quantification of glucose and lactate revealed unexpected results, as both glucose consumption and lactate production increased. The CDF 6HF-DTrp induced significant changes in lactate production across all three cell lines. However, its effect on glucose consumption was only significant in the A375-C5 cell line. Moreover, apoptotic cell death of cancer cells was observed. **Conclusions:** The findings indicate that selected CDF exhibit encouraging antitumor effects; however, additional mechanisms beyond metabolism should be activated by this compound.

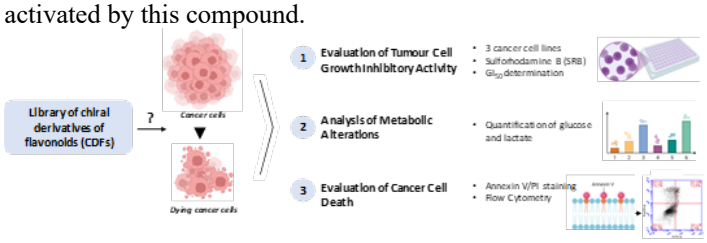

Figure 1. Schematic overview of the experimental approach for evaluating the anticancer properties of chiral flavonoid derivatives (CDFs).

Keywords: chiral derivatives of flavonoids; cancer; multidrug resistance

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PC58 Novel Edaravone Derivatives as Neuroprotective Agents for the Treatment of ALS

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ABSTRACT

Background: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease (ND) with limited treatment options [1]. Although with some drawbacks, Edaravone is one of the available drugs for ALS that targets oxidative stress (OS), a key factor in the pathophysiology of this disease [1]. **Objective:** This work aimed to evaluate the cytotoxic effects and neuroprotective potential of a new series of Edaravone derivatives (MS's), designed to target mitochondria and enhance its biological activity, against OS inducers and mitochondrial disruptors. **Methods:** Cholinergically differentiated SH-SY5Y cells were treated with MS's (0–100 µM) for 24h to assess their cytotoxicity using the NR uptake and MTT reduction assays. To evaluate the potential neuroprotective effects of MS's, SH-SY5Y cells were simultaneously exposed to *tert*-Butyl Hydroperoxide (*t*-BHP, 0–40 µM), Iron(III) (0–1000 µM, in the form of a FeNTA complex) or Phenazine Methosulfate (PMS, 0–5 µM), in the presence and absence of non-cytotoxic concentrations of MS's (0–25 µM). Cellular viability was then assessed by the NR uptake assay, 24h after exposure. The production of reactive oxygen/nitrogen species (RS) was also measured in SH-SY5Y cells upon exposure to *t*-BHP, Iron(III) or PMS, in the presence and absence of MS's for 24h, using the DCFH-DA probe. In all assays, edaravone was used as a model drug. **Results:** The novel edaravone derivatives generally exhibited no significant cytotoxicity at concentrations below 50 µM. Under OS conditions induced by *t*-BHP, all test compounds significantly reduced RS production. However, only four compounds significantly reduced *t*-BHP-induced cytotoxicity. In the presence of Iron(III) or PMS, six of the nine developed MS compounds notably reduced RS overproduction and significantly reduced both Iron(III)- and PMS-induced

cytotoxicity, with three MS compounds showing more pronounced cytoprotective effects, with greater increase in cell viability observed for all tested concentrations and in a concentration-dependent manner. **Conclusions:** These Edaravone derivatives showed promising neuroprotective properties, exhibiting enhanced antioxidant activity compared to the parent compound. Given these findings, these innovative derivatives hold significant potential for further studies to assess their applicability in the treatment of ND, particularly ALS.

Keywords: Amyotrophic Lateral Sclerosis; Oxidative Stress; Neuroprotection

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PC59 Dietary habits and health conditions associated with microplastics in human feces

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ABSTRACT

Background: Microplastics (MPs) are emerging environmental contaminants that have been detected in various human biological samples, raising concerns about their potential health impacts [1]. Given the increasing presence of MPs in food, beverages, and the environment, understanding exposure pathways and human health effects is crucial. **Objective:** This review aims to evaluate recent epidemiological studies on MP contamination in human feces, focusing on the potential association with dietary habits and health conditions. **Methods:** A literature review was conducted in March 2025 on Web-of-Science, Scopus and Google Scholar with variations of keywords “microplastics”, “feces”, “human” and “diet”, spanning from 2018 to 2025. Five studies were identified. **Results:** MPs were consistently detected in human feces, with concentrations varying based on dietary habits and environmental exposure [2,3]. Higher MP levels were observed in individuals with frequent consumption of packaged and take-out foods. One study found a significant correlation between MP concentrations and inflammatory bowel disease (IBD) severity, suggesting a potential role in disease progression. Additionally, MPs were linked to alterations in gut microbiota, metabolic disruptions, and increased body mass index (BMI), highlighting their possible role in obesity development. Of the five studies, four found a positive correlation between the consumption of packaged foods and the presence of MPs in human feces. **Conclusions:** Reviewed studies collectively indicate ubiquitous contamination of human feces with MPs, with potential implications on gut health, inflammation, and metabolic disorders. However, causality remains uncertain, and further research is necessary to determine whether MPs actively contribute to metabolic changes or are merely a byproduct of dietary habits. Indeed, consumption of foods and drinks packaged in plastic seem to contribute to human exposure. Further research on exposure and toxicity mechanisms is essential for developing effective public health strategies.

Keywords: microplastics; plastic packaging; epidemiology; human exposure

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PC60 Heavy Metal Profiles in Teas and Herbal Infusions: A Cross-Country Analysis

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ABSTRACT

Background: Teas and herbal infusions (THIs) are valued for their health benefits but may contain heavy metal impurities, posing health risks due to bioaccumulation and toxicity [1]. Heavy metal contamination in THIs is linked to anthropogenic sources like pollution, soil contamination, irrigation, fertilizers, and food

processing equipment [2]. With the rising consumption of THIs, monitoring heavy metal contamination is needed and has become a public health issue [3]. **Objective:** This study aimed to determine the concentrations of heavy metals in THIs available in Portuguese, Spanish, French, and Italian markets. **Methods:** THIs samples were purchased from different European countries: Portugal (n=23), Spain (n=11), France (n=9), and Italy (n=3). Infusions were prepared and analyzed using inductively coupled plasma mass spectrometry (ICP-MS) with an iCAP™ Q instrument. The heavy metals assessed included arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb). Statistical analysis was performed using JASP 0.19.3.0. **Results:** From the 46 samples analyzed, 43.5% were herbal infusions (HI), 41.3% tea, and 15.2% herbal mixtures with flavors (Mix). Tea samples had the highest levels of As (0.72 µg/L) and Pb (0.84 µg/L), while HI showed the highest Hg concentrations (0.04 µg/L), and Mix samples had the highest Cd levels (0.42 µg/L). The heavy metal profiles of THIs from Portugal and Spain were similar, following the order: As > Pb > Cd > Hg. In contrast, those from France and Italy followed the pattern: Pb > As > Cd > Hg. Among the analyzed samples, the French THIs had the highest Cd (0.36 µg/L) and Pb (0.81 µg/L) levels, while the Spanish samples contained the highest As concentration (0.88 µg/mL). Portuguese THIs exhibited the highest Hg levels (0.37 µg/L). Significant differences were observed in Hg and Pb concentrations between teas and infusions (p=0.044 and p<0.001, respectively). Additionally, Hg levels varied significantly between THI samples from Italy and Portugal (p=0.026). **Conclusions:** This study highlights the presence of heavy metals in THIs available in the Portuguese, Spanish, French, and Italian markets, with varying contamination profiles among countries. These findings raise concerns regarding food safety and emphasize the need for regulations and continuous monitoring to minimize heavy metal exposure through THI consumption.

Keywords: Tea and herbal infusion; Heavy metals; ICP-MS

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PC61 Exploring the Microbiota of Eucalyptus globulus Leaves: Identification of Potential Beneficial Microorganisms

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ABSTRACT

Background: The most common eucalyptus species in Portugal, *Eucalyptus globulus* Labill., has been planted as a source of wood for producing high-quality paper pulp since the mid-20th century [1]. Over the past 70 years, the area of eucalyptus plantations has steadily expanded, significantly impacting productivity and leading to an increased reliance on chemical fertilizers to sustain soil fertility across multiple growth cycles. Thus, eco-friendly bio-resources are a promising trend to meet more sustainable agriculture/forestry needs [2,3]. **Objective:** This study aims to isolate and identify the microbiota community present on Eucalyptus globulus leaves in pure culture, to screen them for plant growth promoting effects. **Methods:** The isolation from leaves surface and extracts, in Luria-Bertani medium (LB), PYGV AGAR (DSMZ Medium 621) and potato dextrose agar media (PDA), with subsequent identification based on the 16S rRNA gene allowed the isolation of 45 strains. **Results:** The most common genus obtained was *Bacillus*, but *Curtobacterium*, *Frigobacterium*, *Pseudomonas*, *Priestia*, *Staphylococcus*, *Paenibacillus*, *Rossellomorea*, *Acetobacter* sp (a potential new species) and the yeast *Rhodotorula* were also detected. Furthermore, to assess the biofertilizer potential of the species, the indole-3-acetic acid (IAA) production and phosphorus solubilization capacity of all the isolates were evaluated. Strains like *Pseudomonas azotoformans*; *Curtobacterium flaccumfaciens* and *Bacillus* sp. demonstrated the ability to produce IAA and solubilize phosphorus. Moreover, *Priestia megaterium, Bacillus* sp*, Rossellomorea* sp, *Rhodotorula* sp and *Frigoribacterium* sp produces IAA and *Staphylococcus warneri* and *Bacillus* sp, solubilizes phosphorus. **Conclusions:** Therefore, this approach represents a significant step toward the development of a biofertilizer consortium that will promote *E. globulus* growth, enabling a more sustainable management of this hardwood species.

Keywords: Biofertilizer, IAA, Phosphorus solubilization

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PC62 The selective bioherbicide action of a microalgae isolated from a Portuguese soil

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ABSTRACT

Background: To meet the food needs of the world's expanding population without compromising environmental quality, it is necessary to use eco-friendly bio-resources to boost agricultural production and soil fertility. The eukaryotic microalgae and cyanobacteria present in biological soil crusts are highly adaptable photoautotrophic microorganisms [1,2]. Their ability to produce a wide range of bioactive compounds with potential biostimulant or bioherbicide allows for various agricultural uses. Despite their significance for sustainable agricultural growth, there are few studies on their application as bioherbicides to combat the weeds that reduce crop productivity [3]. **Objective:** In this work, we investigated the mode of action of a microalga from the genus Klebsormidium that was isolated from Portuguese soil with the aim of being able to be used in agriculture. **Methods:** Seeds of monocotyledonous and dicotyledonous plants were germinated in petri dishes with solid nutrient medium, inoculated with microalga exudates and the biometric parameters were evaluated. **Results:** The results showed a selective herbicide effect of Klebsormidium sp, inhibiting the growth of dicotyledonous plants: Arabidopsis and Nicotiana, and having no effect on monocotyledonous species: Lollium, barley and maize. Plants of species with different sensibilities were chosen to grow in pots and some biochemical parameters were assessed, such as the enzymatic and non-enzymatic antioxidant system, the activity of the nitrogen metabolism and plant growth-related parameters such as the chlorophyll, proteins, sugar and starch contents. The metabolism of the monocotyledonous plants was not significantly affected by Klebsormidium sp., whereas the most sensitive species, dicotyledonous, showed chlorosis and necrosis due to cellular damage from stress. Sensitive plants relied on non-enzymatic antioxidant defenses, which were insufficient to cope with the consequences caused by the microalga, while the most resistant plants were able to activate the enzymatic antioxidant system to mitigate the algae effects. **Conclusions:** This study allows for a better understanding of the mode of action of Klebsormidium sp on plants, contributing to a better comprehension of the molecular mechanisms underlying the interactions between photoautotrophic microorganisms in soil and plants opening new avenues for the development of sustainable alternatives to chemicals currently used in agriculture and in the recovery of damaged soils.

Keywords: Klebsormidium sp; stress; sustainable agriculture

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PC63 Unfit for Consumption: Microbiological Risks of Natural Water Sources in Rural Areas

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ABSTRACT

Background: Access to clean and safe water is vital for public health [1], especially in rural areas, where many households depend on untreated natural water sources [2]. **Objective:** This cross-sectional study combines an epidemiological survey with microbiological analysis to assess the risks of untreated water consumption and identify populations vulnerable to waterborne diseases. **Methods:** Drinking water samples were collected from 31 wells (on private properties) and 9 natural streams (fountains) across two parishes (Chaves municipality). Samples were analyzed according to Decreto-Lei 69/2023, following ISO standards for total heterotrophic microorganisms, total coliforms, *Escherichia coli, Enterococcus* spp., and *Clostridium perfringens*. A survey was conducted to understand water usage patterns and identify at-risk populations. Epidemiological data on age, water usage, preferences, and contamination sources were collected from 28 households (58 individuals). **Results:** Microbiological analysis showed that 93% (37 out of 40) of samples were unsafe for consumption. Well water was heavily contaminated, with coliform bacteria (90%), *Escherichia coli* (55%), *Enterococcus* spp. (55%), and *Clostridium perfringens* (52%). Only two well water samples and one natural stream sample were in conformity with the legislation. The majority of individuals involved in the study (61%) belonged to age groups at higher risk (<15 years – 2% and >65 years – 59%), therefore more susceptible to waterborne diseases. Among households, 93% used well water, primarily for irrigation (48%), hygiene (17%), cooking and laundry (15% each), and drinking (5%). Twenty-nine percent drank water from natural streams. When asked about drinking preferences, 32% favored bottled water, 22% chose mains water, 18% preferred stream water, and 7% well water. Regarding contamination sources, 86% of households had small horticultural operations, 64% kept farm animals, and although most households (89%) had public sanitation, 11% still relied on septic tanks. **Conclusions:** This study suggests that untreated water sources,

such as wells and natural streams, may pose health risks in rural areas. Many households rely on these sources without full awareness of contamination risks, increasing their vulnerability to waterborne diseases. The study also has a citizen science component, as results will be shared with the local population to raise awareness and promote safe water usage practices.

Keywords: Waterborne diseases; water contamination; rural water sources; well water; natural streams

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PC64 Evaluating Mediterranean Diet Adherence, Physical Activity and Weight Perception in Portuguese Adolescents

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ABSTRACT

Background: A balanced diet and regular exercise are essential components of good health, with the potential to reduce the risk of obesity, cardiovascular disease and other chronic illnesses. Portugal is facing a high prevalence of childhood obesity, and a growing concern regarding sedentary lifestyles, reflecting a wider European trend in which approximately 340 million young people are overweight or obese [1]. Projections indicate a continued increase in these trends, exacerbated by a decline in physical activity, which has an impact on physical and mental health [1]. The Mediterranean diet has been shown to offer significant health benefits, yet there is a decline in adherence to this diet among young people [2]. **Objective:** Amid global concerns regarding unhealthy lifestyles among youths, this study aims to evaluate the dietary habits, physical activity, and weight management strategies of 3rd cycle students in Portugal. Specifically, it assesses adherence to the Mediterranean diet, physical activity habits, and overall weight perceptions among students aged 12 to 18. **Methods:** This quantitative and descriptive study collected self-reported data from a sample of 232 students, utilizing the KIDMED and YRB questionnaires to investigate dietary patterns, physical activity engagement, weight perceptions, and weight management practices. **Results:** The findings indicate that adolescents engaged in varied diets, with 65.5% reporting recent physical activity participation. However, 25% of students experienced exercise-related injuries, suggesting safety concerns. Extreme weight management practices were rare, yet disparities in weight perception and weight loss desire were evident, hinting at the influence of societal and media factors. Minimal gender differences were observed in certain habits. **Conclusions:** Although students generally exhibited healthy habits, concerns remain regarding safety in physical activities and weight perceptions. Future interventions should focus on enhancing students' literacy and awareness of the Mediterranean diet and physical well-being [2,3].

Keywords: dietary habits; physical activity; weight management

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PC65 Effectiveness of MetWLPro, a Tailored Metabolic Weight Loss Program in Overweight and Obese Women: A 6-Month Study

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ABSTRACT

Background: Obesity is a progressive and relapsing chronic disease with serious health consequences and is recognized as a major global public health concern. In Europe, over half of the adult population is classified as overweight, with obesity rates around 17%—a figure consistent with data from Portugal. Achieving and maintaining weight loss (WL) remains a significant challenge. First-line interventions such as dietary modification, caloric restriction, and increased physical activity often result in limited long-term adherence and effectiveness. Therefore, innovative nutritional strategies and integrated WL programs are urgently needed to address the multifactorial nature of obesity and support sustainable weight management. **Objective:** This study aimed to investigate the effect of a newly developed Metabolic Weight Loss Program (MetWLPro) on weight loss among adult women with overweight and obesity over a 6-month period. **Methods:** MetWLPro was a 6-month clinical

weight loss study conducted in 2021 across a community pharmacy network. The program combined individualized nutritional counseling focused on a metabolic nutritype-based Mediterranean diet with targeted nutritional supplementation. Eligible participants were women aged 18 to 85 years with a body mass index (BMI) ≥ 25 kg/m^2. A total of 1,078 participants (mean BMI 31.2 ± 4.9 kg/m^2) completed the program, which included personalized consultations with a nutritionist every 2–3 weeks over 30 weeks. Primary outcomes included changes in body weight (kg) and fat mass (%), while secondary outcomes assessed changes in fat-free mass and waist circumference. **Results:** Among the 1,078 participants, the average weight loss after 6 months was −6.1 ± 13.7 kg ($p < 0.001$), with −4.6 ± 13.9 kg ($p < 0.001$) observed at the 3-month mark. Significant reductions were also recorded in fat mass (−3.5 ± 6.6 kg, $p < 0.001$) and waist circumference (−8.4 ± 11.9 cm, $p < 0.001$). Fat-free mass decreased slightly (−0.98 ± 7.3 kg), but the change was not statistically significant ($p > 0.05$). **Conclusions:** This study demonstrates that the pragmatic implementation of a structured and integrated anti-obesity program, featuring an individualized nutritional approach based on metabolic nutritypes aligned with the Mediterranean diet, and supported by targeted nutritional supplementation, can lead to significant reductions in total body weight and fat mass, while preserving fat-free mass.

Keywords: weight loss; obesity; personalized intervention program

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PC66 Ecotoxicity effects of 3-chloromethcathinone (3-CMC) on the swimming behaviour of Daphnia magna: Preliminary data

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ABSTRACT

Background: The continuous release of various compounds can have potentially harmful effects on non-target organisms, raising concerns about the ecosystem and human health [1,2]. 3-chloromethcathinone (3-CMC) is a chiral synthetic cathinone, classified as a new psychoactive substance (NPS) with similar effects to amphetamines and posing potential toxicological risks [3]. After consumption, 3-CMC and its metabolites are excreted in urine and reach surface water via sewage, due to inefficient removal in wastewater treatment plants. [4]. **Objectives**: This study aimed to evaluate the ecotoxicity of racemate 3-CMC on the swimming behaviour parameters using the freshwater microcrustacean, *Daphnia magna*, as an aquatic model. **Methods**: Neonates were used in a total of 20 daphnids per replicate, in a total of 5 replicates per group. The organisms were exposed to 260, 325 and 520 µg/L nominal sublethal concentrations of racemate 3-CMC for 9 days based on a previous 48 h immobilization assay. Daphniids were kept in moderately hard reconstituted water (MHRW) at 20 °C ± 2 °C, with a cycle of 16:8 h (light/dark) and fed every 48 h with *Raphidocelis subcapitata* suspension. Swimming behaviour evaluated considering swimming speed, active time and total distance travelled. **Results:** No significant differences were observed in both active time and swimming speed. However, a significant decrease was observed in the total distance travelled at the highest concentration (520 µg/L). **Conclusions:** These results suggest that 3-CMC has a limited impact in the swimming behaviour of *D. magna*. However, additional physiological parameters are under evaluation to better understand the global effects of this environmental contaminant.

Keywords: Environmental contaminants; synthetic cathinone; Environmental risk assessment

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PC67 Self-reported prevalence of diseases commonly linked to poor environmental sanitation and water-borne diseases among residents of Anil, Rio de Janeiro - Cross-sectional study

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ABSTRACT

Background: Understanding the epidemiological profile of diseases linked to environmental sanitation enables the development of preventive public health measures tailored to specific geographic and socio-environmental contexts. Poor sanitation is a key factor contributing to the spread of water-borne diseases. Environmental sanitation practices prevent diseases and ensure greater social hygiene [1]. **Objective**: This study aimed to assess and compare the self-reported prevalence of water-borne and sanitation-related diseases, as well as access to water and sanitation infrastructure, between residents of the Canal do Anil community ("favela") and the central area of the Anil neighborhood in Rio de Janeiro. **Methods**: The analytical observational study was approved by the CEP/CONEP system (Opinion No: 2.620.525, CAAE 74415017.2.0000.5282, approved on 25 April 2018). This cross-sectional analytical study involved a non-probabilistic sample of 494 residents from the Canal do Anil area. A face-to-face questionnaire was administered covering sociodemographic data, health status, and sanitation conditions. Data were described using counts and percentages. Prevalence confidence intervals were calculated using the exact and Wald methods. Comparisons between groups were performed using Chi-square tests. **Results**: Residents of the Canal do Anil community reported significantly fewer cases of zika (1.6% vs. 5.2%) and chikungunya (2.1% vs. 6.4%; p=0.002) compared to residents of the central neighborhood. They also reported fewer skin diseases (8.5% vs. 13.8%; p<0.001). In terms of sanitation, a lower percentage reported having filtered water at home (83.6% vs. 93.5%; p<0.001), while more residents noted unpleasant smell in the water (12.2% vs. 5.2%; p=0.003). The use of canal water for domestic activities (9.9% vs. 0%; p<0.001) and sightings of children playing in the canal (75.2% vs. 48%; p<0.001) were also more common **Conclusions**: Despite poorer sanitation conditions and self-perception of water quality, residents of the Canal do Anil community reported fewer cases of some diseases. This finding may reflect differences in disease awareness, access to healthcare, or underreporting, warranting further investigation.

Keywords: Water-borne diseases; sanitation; environmental health; Epidemiology; urban health inequities

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PC68 Effects in apoptosis and neurotransmitters after exposure to 3-chloromethcathinone during embryonic development of zebrafish: preliminary results

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ABSTRACT

Background: Synthetic cathinones are a class of new psychoactive substances (NPS) widely consumed worldwide. Their large-scale production and constant structural modifications lead to the emergence of new NPS every year. 3-Chloromethcathinone (3-CMC) was first identified on the European market in 2014 and is a halogenated and N-alkylated derivative of cathinone [1]. 3-CMC has a chiral centre, giving rise to two enantiomers: (*R*)-3-CMC and (*S*)-3-CMC. It shares structural similarities with methcathinone and 4-chloromethcathinone (4-CMC, clephedrone). Like other cathinones, 3-CMC interacts with the monoamine transporter system, acting as a psychostimulant by increasing the release of dopamine, norepinephrine, and serotonin [2]. The presence of NPS in wastewater and surface waters has been growing, underscoring the importance of investigating the potential toxic effects that these substances may have on aquatic organisms [3]. **Objective:** This study aimed to evaluate the effects induced by 3-CMC on neurotransmitter levels (dopamine, serotonin, and their metabolites) and apoptosis in zebrafish (*Danio rerio*) larvae. **Methods:** Embryos, ≈ 3 hours post-fertilization were exposed for 96 hours to different concentrations of 3-CMC (0.02 to 200 µg/L, 3 replicates). After exposure, 10 larvae were randomly collected and immersed in acridine orange dye. Apoptosis levels were then measured through fluorescence (excitation/emission: 535/590 nm). Additionally, 20 larvae were randomly collected for neurotransmitter assessment. Serotonin, 3,4-dihydroxyphenylacetic acid (DOPAC, a dopamine metabolite), and 5-hydroxyindolacetic acid (5-HIAA, a serotonin metabolite) were measured by liquid chromatography coupled to an UV detector at 210 nm while dopamine was assessed at 280 nm. **Results:** After 96 hours of exposure, no significant changes were detected in dopamine, serotonin, or their metabolite levels. Similarly, 3-CMC did not induce apoptosis in exposed larvae at any tested concentration compared to the control. **Conclusions:** These findings suggest that the tested concentrations of 3-CMC during zebrafish embryonic development may not induce effects on neurotransmitters or apoptosis. However, these results are

preliminary, and since NPS are expected to increase in the aquatic environment further research is needed to understand their impact (including 3-CMC) on other biomarkers, improving the accuracy of environmental risk assessment namely to assess significance and develop mitigation measures.

Keywords: Psychoactive substances; Environmental risk assessment; environmental contaminants

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PC69 Preliminary data on the ecotoxicity effects of the novel synthetic cathinone 3,4-methylenedioxypyrovalerone (MDPV) on Danio rerio during early developmental stages

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ABSTRACT

Background: The use of synthetic cathinones (SC) for recreational purposes has become increasingly common among young individuals in recent years [1]. As a result, SC have been detected in aquatic environments even at low concentrations (between ng L^{-1} to µg L^{-1}) [2] that can negatively impact freshwater vertebrates [3]. Since SC are designed to affect the nervous system, they could potentially cause unpredictable harmful effects on nontarget organisms [1]. Several SC, including 3,4-methylenedioxypyrovalerone (MDPV) were frequently detected in wastewater and aquatic environments [2]. Given the limited research on the ecotoxicity of MDPV, it is essential to evaluate its potentially harmful effects on aquatic organisms. **Objective:** This work aimed to assess the adverse effects of racemic MDPV on the mortality and embryonic development of zebrafish (*Danio rerio*) after 96 hours of exposure. **Methods:** Zebrafish embryos (≈ 3-hours post-fertilization (hpf)) were exposed to different nominal concentrations of MDPV (0.18, 0.35, 0.70, 1.4, and 2.8 µg L^{-1}) for 4 days at 28 °C, using 50 animals per concentration and control (5 replicates). Mortality data was recorded every day until day 4. Embryonic development data, namely the first spontaneous movements were evaluated at 24-hpf in a random subsample of 10 individuals per concentration and replicate, and hatching rate at 48- and 72-hpf in all alive organisms per concentration and each replicate. **Results:** MDPV did not cause significant effects on either mortality or embryonic development parameters. Despite that, it should be noted that organisms exposed to MDPV showed a slight increase in the 48-hpf hatching rate (at all concentrations tested) compared to the control. **Conclusions:** The present study shows that MDPV exposure seems not to impair *D. rerio* development in the early stages, however, more studies should be performed to verify and clarify the observed effects, as well as the assessment of potential teratogenic effects.

Keywords: chiral psychoactive drugs; aquatic toxicity; zebrafish

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PC70 Environmental exposure to metal(loid)s: An emerging risk factor for osteoporosis in postmenopausal women?

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ABSTRACT

Background: Osteoporosis is a major public health concern, particularly in the aging population, due to its association with increased fracture risk and reduced quality of life [1]. Although well-established risk factors contribute to bone mineral density (BMD) loss [2], environmental exposure to metals and metalloids is an emerging but underexplored determinant. **Objective:** To evaluate the association between exposure to metals and metalloids and osteoporosis in postmenopausal women aged 50 and older. **Methods:** This cross-sectional study included 380 postmenopausal women, aged 50–70 years, residing in Cascavel, Paraná, Brazil. BMD was measured using dual-energy X-ray absorptiometry. Urinary concentrations of 20 metals and metalloids ─ aluminium (Al), barium (Ba), cadmium (Cd), cobalt (Co), cesium (Cs), copper (Cu), mercury (Hg), lithium (Li), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), rubidium (Rb), antimony (Sb), selenium (Se), tin (Sn), strontium (Sr), thallium (Tl), uranium (U), and zinc (Zn) ─ were quantified using inductively coupled plasma mass spectrometry, and corrected for creatinine levels. Univariate and multiple regression analyses were used to investigate the association between metal(loid) exposure and BMD or osteoporosis risk. **Results:** A total of 73 women (19%) were diagnosed with osteoporosis. Univariate analysis revealed that urinary levels of Cd, Mn, Pb, Sb, Sn, and Zn were significantly increased in the osteoporosis group after adjustment for confounders ($p < 0.05$). Additionally, significant negative correlations were found between BMD and urinary levels of Al, Cd, Hg, Mn, Sb, and U after adjustment for confounders ($p < 0.05$). Higher urinary levels of Cd (OR = 1.495, 95% CI: 1.048; 2.131, $p = 0.026$), Mn (OR = 1.014, 95% CI: 1.001; 1.028, $p = 0.040$), Pb (OR = 1.016, 95% CI: 1.000; 1.033, $p = 0.048$) and Sb (OR = 2.059, 95% CI: 1.073; 3.950, $p = 0.030$) were independently and significantly associated with increased odds of osteoporosis. **Conclusions:** Our findings highlight a significant and clinically relevant association between environmental exposure to Cd or Sb and an increased risk of osteoporosis in postmenopausal women. Further longitudinal studies are essential to better understand the long-term effects of metal(loid) exposure on bone health, which could guide the development of targeted prevention strategies for osteoporosis.

Keywords: metals; metalloids; bone mineral density; women; aging; osteoporosis

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PC71 Exploring the toxicity effects of 3-chloromethcathinone (3-CMC) on the morphophysiological parameters of *Daphnia magna*

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ABSTRACT

Background: The constant discharge of several potentially harmful compounds to the environment raises significant concerns about the risk of toxicity to non-target organisms and human health [1,2]. The 3-chloromethcathinone (3-CMC) is a chiral synthetic cathinone belonging to the group of new psychoactive substances (NPS) [3]. After consumption, this substance and/or its metabolites are excreted in urine, reaching the surface water through the sewage systems due to the inefficient removal in the wastewater treatment plants [4]. **Objectives**: This work aimed to evaluate the ecotoxicity of racemate 3-CMC on the morphophysiological parameters using the freshwater microcrustacean *Daphnia magna* as an aquatic model. **Methods**: Neonates under 24 hours old were used and exposed to 260, 325 and 520 µg/L of the racemate 3-CMC nominal sublethal concentrations for 9 days. Twenty daphniids were used per replicate, for five replicates per group. They were kept in moderately hard reconstituted water (MHRW), at 20 °C ± 2 °C, with a cycle of 16:8 h (light/dark) and fed every 48 h with a suspension of *Raphidocelis subcapitata*. Data were analysed with Jamovi using general linear models, unifactorial design (significance level of 0.05). **Results**: An increase in body size was observed at all concentrations. An increase in heart size was also observed, although only at the lowest concentration. No significant differences were observed in heart rate for all exposure concentrations. An increased mortality of daphnia was observed at high concentrations. **Conclusions:** These findings demonstrate that exposure to sublethal concentrations of racemate 3-CMC can significantly affect the morphophysiological development of *D. magna*. A significant impact on body size was observed at all concentrations, suggesting a potential interference at the lipid metabolic pathway. However, an increase in heart size was observed only at the lowest concentration (260 µg/L), which may suggest an early adaptive response that diminishes with higher exposures. These results highlight the need for further investigation into the long-term impacts and mechanisms of action of 3-CMC in aquatic ecosystems.

Keywords: psychoactive drugs; ecotoxicity; environmental risk assessment

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PC72 From pollutant to product: the bioactive potential of fungal biomass obtained through bioremediation processes

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ABSTRACT

Background: Microplastics are widespread environmental pollutants, and recent attention has turned to the potential of bio-based approaches for their removal and mitigation in various environmental settings. Among these biotechnological methods, fungi have emerged as key players due to their resilience, ease of use, and minimal health risks. While the effectiveness of fungal bioremediation in removing microplastics has been demonstrated, the fate of the fungal biomass after treatment remains largely unexplored [1–3]. **Objective:** This study aims to investigate the antioxidant activity of extracts derived from *Penicillium brevicompactum*, used in the biological removal of low-density polyethylene (LDPE) microplastics. **Methods:** Bioremediation tests were conducted to assess the ability of *Penicillium brevicompactum* to remove LDPE microplastics. The resulting fungal biomass underwent high-pressure extraction, and the obtained extracts were then tested for antioxidant activity. For that, two different assays were used: free radical scavenging activity (DDPH assay) and total antioxidant capacity (ABTS assay). **Results:** Bioremediation assays demonstrated that *Penicillium brevicompactum* was able to remove approximately 40% of the LDPE microplastics over a 21-day period. The extracted fungal biomass showed antioxidant activity, suggesting that the extracts could have potential applications in various industries. **Conclusions:** The study highlights the potential of fungal biomass as a valuable resource, with possible applications in reducing environmental waste and producing bioactive compounds for industries such as cosmetics, pharmaceuticals, and food additives. Although the bioactivity of the extracts was limited and may not immediately be amenable to commercial applications, it highlights the potential applications of such bioremediation strategies within a context of circular economy. However, there is the need for further research to fully realize the benefits of this innovative approach.

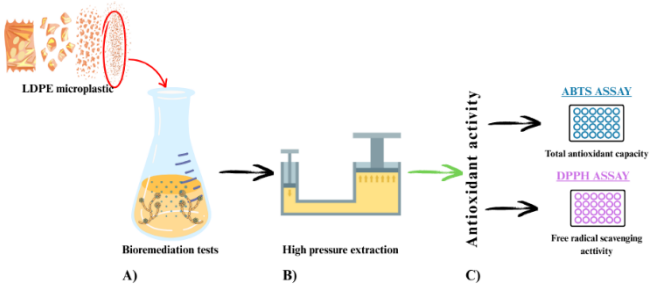

Figure 1. Schematic representation of the methodology: a) bioremediation assay of LDPE microplastics by *Penicillium brevicompactum*; b) fungal biomass obtained underwent high-pressure extraction; c) resulting extracts tested for antioxidant activity using two assays: DPPH and ABTS.

Keywords: microplastics; antioxidant activity; bioremediation

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PC73 Evaluation of P-glycoprotein activity mediated by fiscalin derivatives at the rat intestinal barrier

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ABSTRACT

Background: P-glycoprotein (P-gp) is an active efflux pump that reduces xenobiotics' accumulation inside cells, and which activity can be modulated by inhibitors, inducers and activators [1,2]. The first ones have been used to counteract the multidrug resistance (MDR) phenomena and the inducers and activators have been proposed as a therapeutic approach in intoxication scenarios. In fact, P-gp binds several unrelated hydrophobic drugs and its activity can be changed in order to increase or decrease drugs intracellular accumulation. Previous *in vitro* studies showed that fiscalins, marine-derived compounds, are able to alter P-gp transport activity in differentiated neuronal SH-SY5Y cells. Interestingly, some fiscalin derivatives showed to act as inhibitors, whether others were activators of P-gp expressed in SH-SY5Y cells [3]. **Objectives:** Considering the fact that P-gp is a major efflux pump expressed in barrier tissues, influencing xenobiotics' pharmacokinetics and bioavailability, the main purpose of this study is to investigate the modulatory effects of two fiscalin derivatives, FISC 1 and FISC 2, on P-gp activity in the rat intestinal barrier, using *ex vivo* approaches. The study was performed at the distal portion of the rat ileum, using everted intestinal sacs as an *ex vivo* model, aiming to evaluate the potential immediate effects of the fiscalin derivatives on P-gp transport activity, as a result of a direct inhibition or activation of this pump. P-gp activity was evaluated in rat everted intestinal sacs after a direct and short contact of the tested fiscalin derivatives (5 µM), in the presence and absence of ZOS (5 µM). A fluorescent P-gp substrate widely used in these assays is rhodamine 123 (RHO 123), which allows for the direct evaluation of P-gp activity by measuring RHO 123 fluorescence in samples of mucosal medium, determined by spectrofluorometry. **Results and Conclusions:** The findings revealed that FISC 1 is able to activate P-gp activity at rat intestinal barrier, highlighting the pharmaco(toxico)kinetic relevance of this efflux protein.

Keywords: fiscalin derivatives, P-glycoprotein, inhibition, activation, ex vivo, rat intestine

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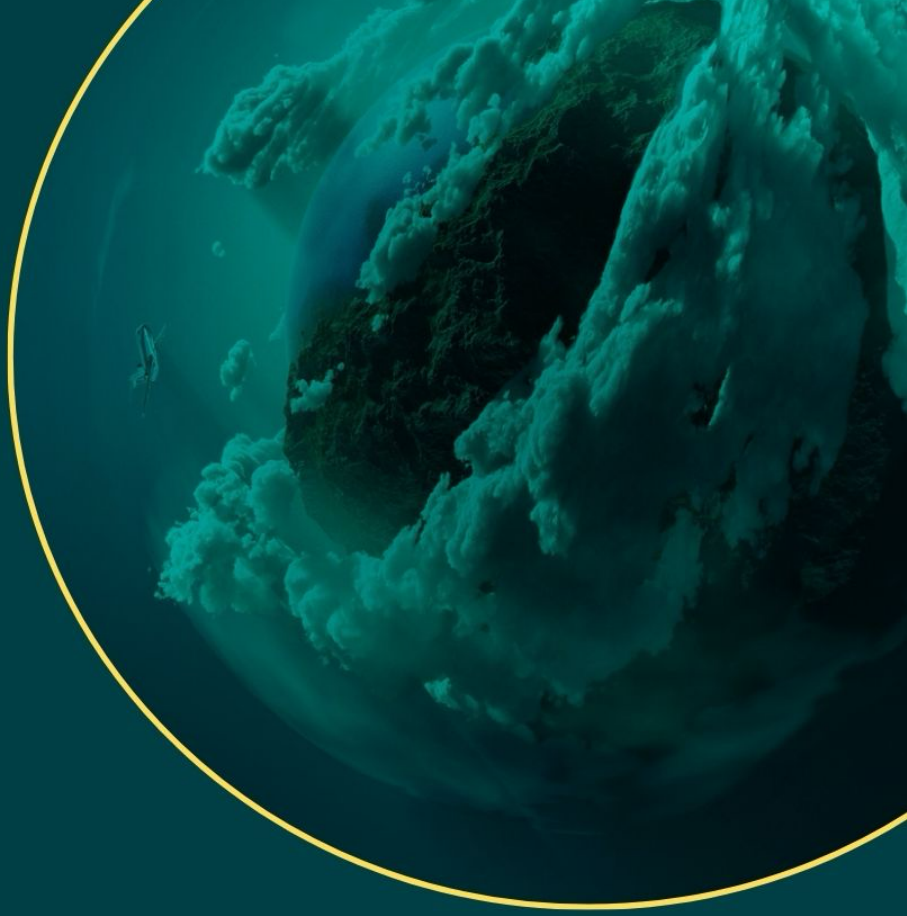

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