



UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II



SETAC

13
14 October
2025

Italian Language Branch

4th Workshop | Naples



*Book of
Abstracts*



A cura di Giovanni Libralato, Federica Cacciatore, Silvia Franzellitti, Claudia Vaj e Stefania Marcheggiani

Federico II University Press



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Università degli Studi di Napoli Federico II

Book of Abstracts

Fourth Workshop SETAC ITALIAN LANGUAGE BRANCH
Naples, 13-14 October 2025.

University of Naples Federico II, Complesso dei SS. Marcellino e Festo (Naples).

Federico II University Press



fedOA Press

Giovanni Libralato, Federica Cacciatore, Silvia Franzellitti, Claudia Vaj e Stefania Marcheggiani (Eds.), 2026. Fourth Workshop SETAC ITALIAN LANGUAGE BRANCH Naples, 13-14 October 2025. University of Naples Federico II, Complesso dei SS. Marcellino e Festo (Naples). Book of Abstracts

Accesso alla versione elettronica: www.fedoabooks.unina.it

ISBN: 978-88-6887-428-5

DOI: 10.6093/978-88-6887-428-5

© 2024 FedOAPress - Federico II University Press
Università degli Studi di Napoli Federico II
Centro di Ateneo per le Biblioteche “Roberto Pettorino”
Piazza Bellini 59-60
80138 Napoli, Italy
<http://www.fedoapress.unina.it/>
Published in Italy
Prima edizione: 04/2026

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Program

13th OCTOBER

9.30-10.00 Registration

10.00-10.15 Welcome greetings **Prof. Susana Loureiro**, President of SETAC Europe.

Opening of the workshop

Dr. Stefania Marcheggiani, President of SETAC Italian Language Branch (ISS), and **Prof. Giovanni Libralato** (Univ of Naples Federico II, local committee and Vice President SETAC ILB).

10.15-12.15

SESSION A: Effects of plastics and additives

Chairs: **Giuseppe De Marco (UNIME)** + **Francesca Biandolino (CNR)**

10.15-10.30 **Teresa Balbi (UNIGE)**, Effects of bioplastic leachates on early larval stages and adult mussels: responses at cellular, biochemical and physiological levels.

10.30-10.45 **Laura Ciaralli (ISPRA)**, Tracing the plastic footprint: site-specific microplastic ingestion and phthalate exposure in *Parapenaeus longirostris*.

10.45-11.00 **Alexandra Dal Lago (CREA)**, Microplastic pollution and its interaction with wild bees: a case study.

11.00-11.15 **Ilaria Savino (CNR)**, Aquaculture plastic nets as a source of reproductive stress in *Mytilus galloprovincialis*.

11.15-11.30 **Antonietta Siciliano (UNINA)**, Exploring the dual faces of lanthanum and gadolinium toxicity in *Hydra vulgaris*.

11.30-11.45 **Angela Maione (UNINA)**, Polystyrene Microplastics promote fungal pathogenicity: an in vitro and in vivo assessment of *Candida albicans* infection.

11.45-12.00 **Sara Accardo (ENEA)**, Chronic exposure to polyethylene microplastics and pharmaceuticals can negatively impact freshwater ecosystems, particularly *Daphnia magna*.

12.00-12.15 **Annalisa Zaccaroni (UNIBO)**, Quantification of microplastics in stomach contents of sea swallow (*Hirundichthys rondeletii*, Valenciennes, 1847).

12.15-13.00

SESSION: Best Young Italian Scientist Award

Chair: **Claudia Vaj (Corteva Agriscience)** + **Marco Evangelista (UNINSUBRIA)**

Teresa Chianese (UNINA), How a short-term exposure to polystyrene microplastic beads impair gonadal condition in the mussel *Mytilus galloprovincialis*: histological and molecular evidence

Alessandra D'Arelli (UNITS), Optimization of the Neuro-2a assay to detect the marine toxin tetrodotoxin

Lorenzo Federico (UNIMIB), Linking behavioural bioassays and soil biodiversity as an integrated strategy for environmental quality assessment

Marianna Imparato (UNINA), The effect of post-biotics-enriched diet on the antioxidant capacity in *Mytilus galloprovincialis* exposed to Cu₂O

Laura Piredda (UNIBO), Trace elements in the brain of the Rabbitfish (*Chimaera monstrosa*, Linnaeus, 1758) from the Strait of Sicily

Marisa Spampinato (UNINA), Do environmental concentrations of rare earth elements pose a risk to mussel embryos?

Sara Trabucco (UNIVE), Implementing safe and sustainable-by-design strategy in the development of TiO₂@SS for cosmetic application

13.00-14.15 Lunch Time

14.15-16.15

SESSION B: Regulatory aspects of environmental pollution

Chairs: **Loredana Manfra (ISPRA)** + **Simona Schiavo (ENEA)**

14.15-14.30 **Arianna Sgariboldi (UnInsubria)**, Integration of *in silico* methods into life cycle and sustainability assessments.

14.30-14.45 **Ilaria Capitani (CNR)**, Multi-analytical strategy for PFAS-related chemical changes detection in *Cannabis sativa* L. during phytoremediation

14.45-15.00 **Alessio Castagnoli (ISPRA)**, Innovative remediation strategies for contaminated marine sediments in Mediterranean ports: Insights from the TREASURE project

15.00-15.15 **Veronica Vivani (UNIVPM)**, Interactive effects of AMPA exposure and salinity fluctuations on *Mytilus galloprovincialis*: an ecotoxicological assessment under climate change scenarios

15.15-15.30 **Aldo Mileo (UNINA)**, The role of bisphenol a in human prostate physiology

15.30-15.45 **Roberta Nugnes (CNR)**, Ecotoxicological Assessment of Silver Nanoparticles in the Marine Environment: A Multispecies Approach

15.45-16.00 **Chiara Russo (Unicampania)**, Imidacloprid complexed with β -Cyclodextrins: ecotoxic impact in key aquatic species

16.00-16.15 **Tiziano Sanvito (Eosinstruments)**, Calibration-free SPES/SPES2 quantification and multiparametric characterisation of single particles in heterogeneous biological and environmental liquids

16.15-16.30 Coffee Break

16.30-17.45

SESSION B2: Regulatory aspects of environmental pollution

Chairs: **Ester Papa (UNINSUBRIA) + Marco Guida (UNINA)**

16.30-16.45 **Simone Marzeddu (ISPRA)**, The new Science-Policy Panel on chemicals, waste, and pollution: road to establishment and key policy milestones

16.45-17.00 **Anna Zanfardino (UNINA)**, High density polyethylene (HDPE) biodegradation by *Cladosporium halotolerans*

17.00-17.15 **Federica Dragone (ARPAC)**, Marine Strategy Framework Directive: tracking anthropogenic contaminants in marine biota for ecosystem protection

17.15-17.30 **Flavia Manuguerra (ARPAC)**, Marine Strategy Framework Directive: analysis of PAHs in marine sediments

17.30-17.45 **Fortunata Barone (ISPRA)**, Regulatory developments and risk management for pollutants of concern: the case of hexavalent chromium under REACH Regulation

17.45-19.00 **POSTER SESSION – APERIPOSTER**

14th OCTOBER

9.00-11.30

SESSION C: Exposure and effects of emerging contaminants

Chairs: **Paolo Pastorino (IZSPLVN) + Teresa Balbi (UNIGE)**

9.00-9.15 **Elena Orlo (UNINA)**, Hidden threats of UV filters: ecotoxicity of ethylhexyltriazone and its chlorinated byproducts in aquatic species

9.15-9.30 **Luisa Albarano (UNINA)**, Behind the masks: the environmental mysteries of cosmetic masks.

9.30-9.45 **Tommaso Campani (UNISI)**, Assessing sub-lethal effects of oral exposure to different chemicals in *Osmia bicornis*: a multi-biomarker approach

9.45-10.00 **Giuseppe De Marco (UNIME)**, Assessment of gadolinium impact on the digestive glands of marine mussels

10.00-10.15 **Mariachiara Galati (UNIME)**, Gadolinium, a rare earth element (REE): A potential risk for the reproductive health of the Mediterranean mussel *Mytilus galloprovincialis*?

10.15-10.30 **Alessandra Narciso (CNR)**, Ecotoxicological tests for evaluating combined toxicity of antibiotics and copper on ecosystems

10.30-10.45 **Michela Panni (UNIVPM)**, Cellular effects of environmental pharmaceuticals:

mechanisms of toxicity in fish PLHC-1 and Rtgill-W1 cell lines.

10.45-11.00 **Marisa Spampinato (UNINA)**, Do environmental concentrations of rare earth elements pose a risk to mussel embryos?

11.00-11.15 **Maria Ludovica Saccà (CREA)**, Impact of polystyrene, polyethylene and polymethyl methacrylate microplastics on the survival and gut bacteria of *Apis mellifera ligustica*.

11.15-11.30 **Marika Mezzelani (UNIVPM)**, Biological responses to pharmaceuticals in a non-target marine organism: In vivo–ex vivo insights.

11.30-11.45 - Award ceremony for the Best Young Italian Scientist

11.45-13.00

SESSION D: New Challenges for Environment and Human Health

Chairs: **Ermelinda Prato (CNR)** + **Valeria Di Onofrio (Uniparthenope)**

11.45-12.00 **Roberta De Angelis (ISPRA)**, Bathing area management: first results of the aces project

12.00-12.15 **Roberta Pedrazzani (UNIBS)**, The role of bioassays in Water Safety Plans

12.15- 12.30 **Michela Carlin (UNITS)**, In vitro characterization of pro-inflammatory of potential algal toxin at the skin level

12.30-12.45 **Emanuela Pace (ISPRA)**, PFAS pesticides monitoring in waters

12.45-13.00 **Ifra Ferheen and Maria Rosa Loffredo (ISS)**, Climate change and the spread of vibrio species in transitional waters: a combined in-field and microcosm investigation

13.00-13.15 **Stefania Boccia (UNINA)**, Unraveling cocaine's impact on cb1 receptor function and intestinal integrity

13.15-13.30 **Annalisa Bonanno (UNINA)**, Evaluating Sphaeropsidin A as a novel disinfectant against *Candida auris*: antibiofilm and functional characterization

13.30-14.30 Free Lunch

14.30-17.00

SESSION D1: New Challenges for Environment and Human

Chairs: **Giulia Maisto (UNINA)** + **Valerio Zupo (SZN)**

14.30-14.45 **Chiara De Carolis (IRSA CNR)**, Effectiveness of Compost in mitigating drought stress in *Rosmarinus officinalis* and its impact on soil microbial communities

14.45-15.00 **Giovanni Cilia (CREA GOV)**, Exploring the role of *Apis mellifera* as monitoring tools for Antimicrobial Resistance Genes: A case study in Bozen (South Tyrol, Italy)

15.00-15.15 **Lorella Pascolo (UNITS)**, Tracing environmental pollutants: Synchrotron micro-XRF analysis of metals in plastics and human tissues

15.15-15.30 **Federica Cacciatore (ISPRA)**, Salinization in transitional ecosystems: potential impacts on macrozoobenthic biodiversity

15.30-15.45 **Lorenzo Saviano (UNINA)**, Biogenic selenium nanoparticles: new frontiers for food safety protection

15.45-16.00 **Claudio Fenizia (UNIMI)**, Micro- and Nanoplastics induce dysregulation in human monocytes: insights into autophagy and inflammation

16.00-16.15 **Edith Padilla Suarez (UNINA)**, Tiny crustaceans, big warnings: antibiotic stress in a changing climate

16.15-16.30 **Simone Marzeddu (ISPRA)**, Possible integrations of REACH regulation and life cycle assessment methodology

16.30-16.45 **Gianluca Maschio (ISPRA)**, Pesticides in surface and groundwater - Italian monitoring data 2021

16.45-17.00 CLOSING CEREMONY

Wrap up and closing remarks from Prof. **Giovanni Libralato** (University of Naples Federico II) and Dr. **Stefania Marcheggiani** (President of SETAC ILB, ISS).

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Introduction

The Italian Language Branch (ILB) is one of the regional branches of SETAC Europe, established approximately 20 years ago to promote and support the development of several topics at national level including environmental sustainability, ecosystem preservation, and ecosystem integrity, through a multidisciplinary approach aligned with SETAC's mission and values.

The ILB groups Italian-speaking members of SETAC Europe, based both in Italy and abroad, including biologists, chemists, engineers, policymakers, environmental scientists, and stakeholders. It serves as a dynamic platform for discussion, collaboration, and knowledge sharing on emerging and re-emerging topics related to environmental science and human health in a one-health perspective.

At the international level, ILB actively contributes to SETAC Europe Annual Meetings, sharing significant scientific and technological insights. At the national level, it fosters connections among academia, industry, research institutions, and authorities, promoting the application of scientific knowledge in environmental policymaking.

At the national level, ILB organizes and supports training courses, conferences, and workshops, with a particular focus on engaging students and early-career researchers - motivating them to share innovative ideas and contribute to sustainable solutions.

Comprising 123 members from universities, research bodies, and industries both in Italy and abroad, the ILB promotes numerous no-fee self-funded events at national and international levels.

These events reflect the collaborative and inclusive spirit of SETAC ILB with members actively involved in their planning and execution.

Since 2009, SETAC ILB has experienced substantial growth, beginning with a national workshop:

- 1st National Workshop – hosted by the University of Siena (2022)
- 2nd National Workshop – held at the National Research Council in Rome (2023)

- 3rd National Workshop – hosted by the University of Milan (2024)

The 4th National Workshop was hosted by the Department of Biology, University of Naples Federico II. The scientific program featured 47 oral presentations and 24 posters, covering a broad range of topics distributed across sessions addressing:

- Effects of plastics and additives;
- Regulatory aspects of environmental pollution;
- Exposure and effects of emerging contaminants;
- New challenges for environment and human health.

The opening session started with the remote welcome to the participants of Prof. Susana Loureiro (Aveiro University, Portugal) that is the 2025-2026 SETAC EU President of the Board of Directors, and Dr. Stefania Marcheggiani and Prof. Giovanni Libralato, President and Vice President SETAC ILB, respectively.

This edition brought together 123 in-person participants, including 61 from academia, 59 from national research centres (CNR, ENEA, and others), 3 from private companies, and 1 from national agencies and institutes.

During the workshop, the “Best Young Italian Scientist Award”, established for the first time in 2018, took place. The best scored young scientist was awarded with a free registration to the SETAC Europe 36th Annual Meeting 17 – 21 May 2026 Maastricht, The Netherland. Seven young researchers participated in the 2025 competition; they were evaluated based on the submitted extended abstract by a panel of reviewers according to SETAC Europe's objective criteria and a five-minute pitch presentation during the workshop.

For the first time, the “Best Poster” was established. Among the presented posters, a panel of reviewers assessed the scientific content, general layout, and presentation. The first three best scored posters were awarded with an award certificate.

During the workshop closing ceremony, the President and Vice President SETAC ILB warmly invited to attend the next SETAC Europe Conference (Maastricht, May 17-21, 2026) and announced the upcoming 5th ILB Workshop to be held in Chioggia (Venice) in 2026, hosted by the University of Padova.



Follow our updates on our web page (<https://italianbranch.setac.org>) and social networks!

Oral Presentations

Session A - Effects of plastics and additives

Effects of bioplastic leachates on early larval stages and adult mussels: responses at cellular, biochemical and physiological level

T. Balbi^{1,2}

¹Department of Earth, Environmental, and Life Sciences, University of Genoa, Italy. ²National Biodiversity Future Center, Palermo, Italy.

The potential environmental discharge and biological effects of chemicals released by conventional plastics and bioplastics remain largely overlooked. Recent attention has focused on the release of additives, i.e. compounds intentionally incorporated during polymer manufacturing to confer the final product specific requirements. Commonly used additives include plasticizers, flame retardants, stabilizers, antioxidants, pigments, biocides. These are polymer- and function-specific, and their concentrations vary across final products. Since most additives are not bound to the polymer structure, they can be released into seawater within days, with consequent risk of exposure for wildlife and humans. Bioplastics are promoted as safer alternatives to tackle the long-term persistence of conventional plastics. However, information on the potential release of additives and non-intentionally added substances (NIAS) from bioplastics into the environment, as well as on the biological impact of their leachates in aquatic species, remain limited. In this work, the effects of leachates at environmentally relevant concentrations from common bioplastics in seawater were evaluated using the marine invertebrate *Mytilus galloprovincialis*, by measuring sub-lethal responses in adults and larval stages. Firstly, short-term *in vitro* experiments (0-120', 1-10-50-100% in ASW) were performed as a screening for potential biological effects on mussel hemocytes, using leachates obtained by polylactic acid (PLA) plates, polybutylene succinate and polylactic acid (PBS/PLA) spoons and glasses, polypropylene and starch-based polymer (PP/PSM) forks, supermarket (Sbs) and waste (Hera bag) bags in starch and polybutylene adipate cotereftalate (A/PBAT). Only Sbs leachates induced lysosomal destabilization-LMS and enlargement, while

immune parameters were unaffected. Subsequently, with the same leachates used previously, fertilization and larval development at 48 h post fertilization were assessed. As a results, fertilization was minimally affected; however, all leachates impaired larval development. Then, an *in vivo* experiment (7 days, 0.6%) was performed. In adults, leachates exposure induced a decrease in haemocyte LMS, affecting lysosomal parameters, while digestive gland lysosomal and enzymatic biomarkers were unaffected, except for GST activity in gills induced by PLA plates leachate. Finally, since a significant effect on haemocytes was observed, a second *in vivo* experiment (4 days, 0.1%) was performed with other leachates, obtained by cellulose pulp (paper/LDPE) and paper plates, paper/PLA glasses, plastic (PE/PP)/foil coffee bags. Preliminary results showed a strong inhibition of immune response following leachates exposure. A chemical characterization of all the plastic materials used was carried out in parallel. Overall, this study enhances understanding of the potential biological effects of additives in commercial bioplastics on the model organisms *Mytilus galloprovincialis*. *This work was supported by PRIN2022 YHXCWP "Adverse impacts of plastic additive mixtures in the marine environment: from molecules to whole organism physiology"*.

Microplastic pollution and its interaction with wild bees: a case study

A. Dal Lago^{1,2}, R. Ranalli³, M. Galloni², F.A.M. del Carmen⁴, G. Cilia¹

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Microplastic (MP) contamination is derived from the improper disposal of plastic waste, leading to the formation of particles ranging from 1 μ m to 5 mm in size. These particles are of increasing concern due to their widespread distribution and persistence in the environment, particularly in aquatic systems. MPs have been detected in various environmental compartments and

matrices, including water, soil, food, and biological tissues of both animals and humans. Wild bees, which play a key role in maintaining biodiversity and ecosystem stability, are experiencing population declines due to multiple stressors, including exposure to MPs. Previous studies have shown that MPs can negatively affect vital and cognitive functions in *Apis mellifera* and *Bombus terrestris*, and that honeybee colonies may serve as bioindicators of MPs spread in the environment. This study aimed to evaluate the impact of MPs on wild bee health by identifying the location of MPs on their bodies and characterising their shape and chemical composition. Sampling was carried out monthly from May to August 2024 at six urban sites in Northern Italy, each representing different levels of pollution. A total of 677 wild bee individuals were collected, representing 26 genera. Each specimen was examined under a stereomicroscope, and MPs were carefully removed using tweezers and stored in sterile microtubes. For each particle, body location (head, thorax, abdomen, wings, or legs), colour, and shape were recorded. A total of 3116 MPs were detected, with an average of 4.6 particles per individual, showing diverse morphologies and distributions. The genera *Xylocopa*, *Anthidium*, and *Bombus* exhibited the highest number of MPs, while only 9% of individuals carried no MPs. The most frequently observed type was "white filaments" (69%), predominantly located on the legs (38%). Ongoing chemical analyses are focused on identifying the MP polymers. These findings offer valuable insights into the spread of MPs in urban environments and their potential impact on wild bee communities. Further research is needed to understand species-specific responses to MP exposure and to emphasise the urgency of implementing measures to mitigate MP pollution.

Aquaculture plastic nets as a source of reproductive stress in *Mytilus galloprovincialis*

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Plastic marine litter is a growing environmental threat, with over 150 million tons currently polluting the world's oceans. In the Mediterranean Sea, polypropylene (PP) nets used in mussel aquaculture are a significant source of sea-based plastic waste. The widespread use of PP nets in the long-line mussel farming system contributes to microplastic (MP) pollution as these materials degrade and release particles and chemical additives into the marine environment. The *Mytilus galloprovincialis* is the primary mussel species cultivated across the Mediterranean and beyond and is particularly vulnerable due to its reliance on external fertilization. Although MP ingestion by mussels is well-documented, the toxicological effects of PP-derived MPs and their leachates on mussel reproduction remain largely unexplored. This study investigates the effects of PP-derived MPs and leachates, originating from aquaculture nets, on the quality of mussel gametes. PP nets were collected along the Ionian Sea coast, near a mussel farming site in the Apulia Region (Southern Italy). The nets were cut into smaller pieces and pulverized using a blender. The resulting fragments were sieved through stainless steel meshes to isolate PP-MPs smaller than 100 μm . The selected particles were used to assess both the potential physical impacts of aged PP-MPs and the chemical effects due to the release of additives or adsorbed contaminants, using the prepared leachate. Male and female gametes of *M. galloprovincialis* were exposed to PP-MPs at concentrations of 1, 10, and 100 $\mu\text{g/l}$, as well as to a PP leachate. After 1 and 2 h of exposure, physiological parameters, including viability, mitochondrial membrane potential, oxidative status, and sperm motility, were analyzed. The results demonstrated distinct effects of PP-MPs and their leachates, suggesting a distinct mechanism of toxicity. Chemical analysis of the leachates confirmed the presence of various contaminants, including plastic additives and likely adsorbed environmental pollutants. These findings underscore the potential of plastic pollution, particularly from aquaculture-derived materials, to disrupt critical reproductive processes in mussels,

with possible implications for population dynamics and seafood safety.

Exploring the dual faces of lanthanum and gadolinium toxicity in *Hydra vulgaris*.

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Rare earth elements (REEs) such as lanthanum (La) and gadolinium (Gd) are increasingly used in electronics, renewable energy, and medical technologies, raising concerns about their environmental release and ecotoxicological effects. Although naturally present at trace levels, anthropogenic inputs may elevate their concentrations in freshwater ecosystems, where they occur as soluble ionic salts and poorly soluble particulate oxides. The different solubility and bioavailability of these species can influence toxicity pathways and ecological risks. This study assessed and compared the acute and sublethal toxicity of La and Gd in their chloride (ionic) and oxide (particulate) forms using *Hydra vulgaris* as a freshwater bioindicator. *Hydra* polyps were exposed for 96 h under semi-static conditions to environmentally relevant and higher concentrations of LaCl_3 , GdCl_3 , La_2O_3 , and Gd_2O_3 . Morphological alterations (score 1–10), regenerative ability following head and foot amputation, feeding rate (prey capture within 30 min), and tentacle histology (toluidine blue staining) were evaluated. Statistical analyses were performed via ANOVA and Tukey's post hoc test ($p < 0.05$). Results revealed that chloride salts were significantly more toxic than oxides, reflecting their higher solubility and immediate bioavailability. Morphological endpoints demonstrated pronounced tentacle shortening, body contraction, and tissue disintegration in chloride-exposed hydras, with EC_{50} values markedly lower for GdCl_3 (24.05 mg/l) compared to Gd_2O_3 (4.57 mg/l). Feeding rates declined sharply under chloride exposures, indicating impaired neuromuscular functions and reduced fitness. Regeneration assays showed concentration-dependent delays in both upper and lower body regeneration, suggesting interference with developmental processes critical for survival and reproduction. Histological examination confirmed severe epithelial damage in chloride treatments,

whereas oxides induced subtler structural alterations, consistent with slower ion release and physical interactions of particulates. These findings underscore the importance of chemical speciation in REE toxicity: ionic forms exert rapid, intense effects, while particulate oxides, though less bioavailable, can still elicit toxicity through prolonged or alternative mechanisms. *Hydra vulgaris* proved highly sensitive and suitable for detecting both forms, highlighting its utility in freshwater ecotoxicology for emerging contaminants like REEs. The observed impacts at sublethal levels raise ecological concerns regarding chronic exposures and call for speciation-specific risk assessment frameworks in environmental regulation.

Polystyrene microplastics promote fungal pathogenicity: an *in vitro* and *in vivo* assessment of *Candida albicans* infection

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Microplastics (MPs), ubiquitous in the environment due to the degradation of plastic materials, represent an emerging threat to ecosystems and human health. Among them, polystyrene microplastics (PSMPs) are widely used in consumer products and can be ingested through contaminated food and water. Despite the increasing awareness of MPs' potential toxicity, their interaction with human pathogens remains underexplored. In this study, we investigated the effects of 1 μm PSMPs on the infection ability of *Candida albicans*, a major opportunistic fungal pathogen, using both human intestinal epithelial cells (HT29) and *Galleria mellonella* larvae as experimental models. PSMPs were found to reduce HT29 cell viability at high concentrations and to be internalized by the cells, increasing their morphological complexity. When combined with *C. albicans*, PSMPs significantly enhanced fungal adhesion, biofilm maturation, and cell invasion, particularly when the host cells were pre-exposed or co-exposed to PSMPs. These conditions also resulted in elevated levels of cellular damage and pro-inflammatory IL-8 cytokine release, along with

a reduction in anti-inflammatory IL-5 and G-CSF cytokines. In vivo experiments in *G. mellonella* confirmed the ability of PSMPs to exacerbate fungal infection. Larval survival decreased significantly under pre-exposure and co-exposure conditions, suggesting that PSMPs may impair innate immune responses. Gene expression analyses showed downregulation of antifungal peptides Gallerimycin and Galiomicin in PSMP-exposed larvae, further indicating immune suppression. Moreover, fungal virulence genes *ALS3* and *HWP1* exhibited altered expression patterns depending on the timing of PSMP exposure, implying a potential modulation of fungal pathogenic mechanisms. Overall, our findings highlight the synergistic effects of PSMPs and *C. albicans*, demonstrating that microplastic exposure can enhance fungal virulence, promote biofilm formation, and impair host immune defenses. These results underscore the need to consider microplastics not only as pollutants but also as facilitators of infection, raising concerns for public health in the context of increasing environmental contamination.

Chronic exposure to polyethylene microplastics and pharmaceuticals can negatively impact freshwater ecosystems, particularly *Daphnia magna*

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Anthropogenic activities such as industry, agriculture, and urban development release various contaminants into the environment. Among the emerging pollutants, pharmaceuticals and microplastics (MPs) may pose significant risks to aquatic ecosystems and ecological health, raising serious concerns for both environmental and public health. These substances can enter water bodies through urban and agricultural runoff, degrading water quality and disrupting ecosystem functions. MPs, due to their small size and high specific surface area, can adsorb and accumulate co-existing contaminants in aquatic environments, including pharmaceuticals. This property allows them to act as vectors, enhancing the transport, persistence, and bioavailability of such substances to aquatic organisms.

Furthermore, MPs can modulate the toxicity of pharmaceuticals by altering their interactions with biological systems. Depending on the type of polymer, the physicochemical properties of the pharmaceutical compounds, and the characteristics of the exposed organisms, this interaction may result in synergistic effects (increased toxicity) or antagonistic effects (reduced toxicity). These complex dynamics underscore the multifaceted role of MPs in influencing contaminant behaviour and ecotoxicological risk. This study aims to evaluate the ecotoxicological impact of leachates derived from virgin polyethylene (PE) and oxo-degradable polyethylene (PEadd), both individually and in combination with three commonly detected pharmaceuticals - ibuprofen (Ib), sulfamethoxazole (Sulf), and propranolol (Prop) - on various aquatic organisms. Leachates were chemically characterized and tested through a battery of ecotoxicological assays involving organisms from different trophic levels: the bacterium *Vibrio fischeri*, the alga *Raphidocelis subcapitata*, and the crustacean *Daphnia magna*. Additionally, a detailed sublethal toxicity assessment was conducted on *D. magna*, focusing on feeding activity, heartbeat rate, and behavioural responses. The observed effects suggest that microplastics may alter pharmaceutical toxicity in complex and variable ways, potentially influenced by multiple factors such as polymer composition, contaminant properties, and organism sensitivity. These findings highlight the need for further studies to better understand the mechanisms underlying these interactions and their implications for aquatic ecosystem health.

Quantification of microplastics in stomach contents of sea swallow (*Hirundichthys rondelietii*, Valenciennes, 1847)

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Microplastic pollution is currently one of the most pressing and emerging environmental issues, posing a significant global problem, primarily due to its impact on marine fauna. The objective of the present work is to examine the stomach contents of some specimens of the Sea Swallow (*Hirundichthys rondelietii*) to assess the presence of

microplastics in them and, if the sample is positive, to quantify them. Furthermore, it is well understood how the presence of microplastics (MPs) can impact the growth of animals. The purpose of this work is, therefore, to evaluate a possible correlation between the presence of microplastics and the reduced growth rate of the specimens examined. To do this, 23 specimens of Sea Swallow sampled in July and August of 2020 and in the first month of 2021 in the area between the Canary Islands and the Gulf of Guinea were examined as part of the SACET project. The stomach was collected from each animal, and its content was analysed following digestion for the concentration of microplastics inside. The MPs were identified by staining with Nile Red and observed under a fluorescence microscope equipped with three types of filters (red, green, and blue). Using the ImageJ program, the area and perimeter of the particles, as well as the length of the identified fibres, were calculated. To determine a possible correlation between the presence and numerosity of microplastics and the

size of fish, fork length, as a proxy of age, was considered in the statistical analysis. The analyses revealed that most MPs were represented by particles, and an increasing trend was observed in the concentration of MPs across sampling areas. Statistical analysis using the Product-Moment and Partial Correlations showed that there is no correlation between the presence and numerosity of microplastics and the size of animals. Nevertheless, an increasing trend in the number of MPs with larger sizes can be observed. This aspect, when evaluated and considered from the perspective of feeding habits, may depend on the fact that a larger animal consumes a greater amount than a smaller one, thus increasing the risk of also ingesting microplastics. The present research confirms that MPs are now considered a ubiquitous contaminant, impacting all environments on a global scale, with a particular focus and concentration in the marine environment.

Session B - Regulatory aspects of environmental pollution

Integration of *in silico* methods into life cycle and sustainability assessments

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Climate change concerns any substantial variation in climate indicators, such as temperature and precipitation, which persists over decades or longer. While such changes were historically driven by natural factors, it is now widely recognized that human activities are the main cause of the ongoing transformations. The intensification of the greenhouse effect and the depletion of the stratospheric ozone are among the most significant side effects, and are commonly quantified through specific indicators: the Global Warming Potential (GWP) and the Ozone Depletion Potential (ODP), respectively. GWP measures the impact of a substance released into the atmosphere, based on its Radiative Forcing (RF) or Radiative Efficiency (RE), and on its atmospheric lifetime. ODP evaluates the ozone-depleting effect of a chemical compound in the stratosphere. The assessment of these indicators is essential for environmental impact studies and sustainable development strategies, particularly within Life Cycle Impact Assessment (LCIA) frameworks. However, GWP and ODP measures are usually not available for all chemicals, especially for novel or experimentally uncharacterized compounds.

In this presentation, we propose the application of *in silico* models based on Quantitative Structure–Activity Relationships (QSARs) to predict key climate-related indicators, including GWP, ODP, and RE. The dataset used to develop the models was derived from the Scientific Assessment of Ozone Depletion (World Meteorological Organization, 2022) and includes experimental data for over 600 substances. These substances are categorised into 20 different chemical classes, with the majority being hydrochlorofluorocarbons

(HCFCs), hydrofluorocarbons (HFCs), and halogenated ethers. Following data collection, a pre-treatment phase was carried out to handle missing values, duplicates, and to check names and identifiers available for the studied compounds. Mordred and ChemoFP software were used to calculate the molecular descriptors from Simplified Molecular Input Line Entry System (SMILES) representations of the compounds in the dataset. The descriptors were curated in a preliminary step and then selected using a Genetic Algorithm (GA). The selected descriptors were used to develop QSAR models based on Multiple Linear Regression (MLR) by means of Ordinary Least Squares (OLS) method. The resulting models were evaluated using various statistical metrics to assess their goodness-of-fit, internal robustness, and external predictive ability. The models, developed in accordance with OECD principles for QSAR modeling, can be applied to support preliminary screening of the climate impact of existing or emerging chemicals and for the identification of more sustainable alternatives to potentially hazardous compounds.

Multi-analytical strategy for PFAS-related chemical changes detection in *Cannabis sativa* L. during phytoremediation

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Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are highly persistent environmental pollutants commonly found in soils and water due to their widespread industrial use and chemical stability. Their accumulation raises serious concerns about ecosystem health and food safety. This study explores the use of *Cannabis sativa* L. (hemp) for phytoremediation of PFAS-contaminated environments. Plants were grown

under controlled conditions and exposed to two common PFAS compounds (PFOA and PFOS). After exposure, different plant tissues were collected and analyzed to detect chemical changes caused by PFAS uptake. Two analytical techniques were compared: FTIR in attenuated total reflectance (ATR) mode helped identify specific chemical group variation between untreated samples and those treated with PFOA and PFOS, while HSI in reflectance mode within the short-wave infrared (SWIR) range (1000–2500 nm) provided a rapid, non-destructive method to detect differences in plant tissues. Furthermore, HSI enabled the acquisition of spatially resolved data by generating an image with a full spectrum for each pixel, allowing for the assessment of PFAS distribution and the evaluation of sample homogeneity or heterogeneity. The comparison showed that both FTIR and HSI are effective tools for detecting PFAS-induced changes, each offering complementary information. This work highlights the potential of these methods to support environmental monitoring and phytoremediation strategies, and to provide a preliminary qualitative assessment of PFAS presence in plant tissues.

Innovative remediation strategies for contaminated marine sediments in Mediterranean ports: Insights from the TREASURE project

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The TREASURE project (Interreg Euro-MED 2021–2027) aims to improve environmental quality in Mediterranean ports by mitigating and reducing sediment and water pollution. It comprises governance framework development (WP1), a common monitoring approach (WP2), defining and testing of novel remediation techniques promoting circular-economy principles (WP3), and mainstreaming of results (WP4). ISPRA leads WP2 and supports WP3, applying the Weight of Evidence approach already included in IT France Maritime's GEREMIA project and Italy's Environment Ministry Decree n. 173/2016 on sediment handling and management. In WP2, ISPRA sampled sediments in three Livorno port

areas, performing chemical analyses, ecotoxicological bioassays and benthic community assessments, to establish a transnational Impact Evaluation and Hazard Management index. WP3 aims at testing sourcing and experimentation of novel techniques for environmental remediation in port areas starting from a catalogue of identified solutions. Within this framework, a specific area in the port of Livorno has been identified as suitable for bio-electrokinetic remediation (BIO-EK) feasibility studies. Granulometric characterization indicated lower permeability compared to previously treated sediments, suggesting prolonged treatment durations. Chemical analyses revealed contaminant concentrations of approximately Cd 3.7 mg/kg, Cu 350 mg/kg, Hg 2.26 mg/kg, Pb 246 mg/kg, Zn 542 mg/kg, hydrocarbons (C>12) 5000 mg/kg, and PCBs 284.89 µg/kg, confirming the area's suitability for BIO-EK trials. The experimental system comprises three 2 l sediment-lined electrokinetic cells, each connected to dual electrolyte reservoirs equipped with titanium-coated iridium-oxide mesh electrodes, in which two Bio-EK configurations will be benchmarked against conventional electrokinetic control. In the initial bioremediation phase, lasting until organic degradation is presumed complete (approximately 30 days), the electrode polarity will be reversed periodically to suppress acid-front propagation and enhance microbial breakdown of organic contaminants; upon completion of this stage, a standard electrokinetic regimen with fixed polarity will be maintained for an additional 90 days to mobilize and remove heavy-metal species. Organic contaminant levels will be quantified at the conclusion of the bioremediation phase (day 30) and again at the end of the full 120-day experiment, while heavy-metal concentrations will be measured at days 30, 60, 90, and 120 and, at termination, by subdividing each sediment liner into five equal segments and defining electromigratory patterns. Ecotoxicological assays performed on day 0, post-bioremediation (day 30), and at the EK conclusion (day 120) will provide additional insights on sediment recovery potential and ecological risk reduction, providing a robust framework for optimizing Bio-EK methodologies for sustainable sediment management in Mediterranean port environments.

Interactive effects of AMPA exposure and salinity fluctuations on *Mytilus galloprovincialis*: an ecotoxicological assessment under climate change scenarios

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Climate changes may exacerbate extreme weather events contributing to severe salinity fluctuations in coastal waters. These perturbations may enhance the mobility and bioavailability of persistent pollutants such as aminomethylphosphonic acid (AMPA), the main breakdown product of glyphosate. While the individual effects of altered salinity and AMPA exposure are relatively well characterized, their combined impact on marine organisms remains poorly investigated. This study aimed to assess, for the first time, the effects of environmentally realistic concentrations of AMPA (0.5 µg/l) at different salinity conditions (35, 25, 15 PSU) in *Mytilus galloprovincialis*, a recognized model species in marine ecotoxicology. After 21 days of exposure, mussel tissues were collected to analyze a wide panel of physiological, biochemical, and cellular biomarkers, including immunocyte responses, antioxidant defenses and oxidative damage, cholinergic function, detoxification mechanisms, peroxisomal proliferation and lipid metabolism modulation, metabolic capacity, energy reserve content, and cellular redox homeostasis. A Weight of Evidence (WOE) approach, was applied to integrate biomarker responses based on weighted criteria and provide quantitative and synthetic hazard indices for each treatment, considering the number, magnitude, and toxicological relevance of observed effects. Both AMPA exposure and salinity shifts induced multiple biological effects in mussels, but the harsher alterations were observed in organisms co-exposed to AMPA and severe hyposalinity. Such alterations regarded immune parameters, including reduced phagocytosis rate and shifts in haemocyte subpopulation compositions; also, neurotoxic effects were highlighted, according to a significant inhibition of acetylcholinesterase activity in gills, as well as the inhibition of carboxylesterase activity in the digestive gland,

indicating possible impairment in xenobiotic detoxification pathways. Antioxidant enzymes activity and total oxyradical scavenging capacity showed limited but consistent modulation across treatments. Lipid metabolism was strongly affected, as evidenced by enhanced Acyl-CoA oxidase activity, lipofuscin accumulation, and decrease of neutral lipid content. Furthermore, AMPA exposure caused strong stimulation of the electron transport system, alongside a reduction in glycogen reserves and increased proline content in gills, suggesting altered energy dynamics and osmotic stress responses modulation. Overall, the results demonstrate that AMPA, across all tested salinity conditions, triggered a broad spectrum of biological responses in mussels. However, reduced salinity, particularly at 15 PSU, significantly amplified organismal stress. This was reflected by more pronounced immune and neurotoxic responses, disruption of lipid and energy metabolism, and signs of osmotic imbalance. These findings underscore the importance of considering climate-related abiotic stressors in combination with chemical pollutants in realistic ecotoxicological risk assessments, particularly under future climate change scenarios.

The role of Bisphenol A in human prostate physiology

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Bisphenol A (BPA) is a synthetic compound widely used to make materials in polycarbonate plastic and epoxy resins as well as plastic bottle, food packaging, household appliances, food cans, and metal jar lids. In the last decades, BPA massive use gained attention, becoming a concern issue for the human health, especially for the reproduction system, due to chronic exposure at low dosage and its capability to Androgen receptor (AR), inducing an antagonistic effect. Prostate is an accessory gland in male reproductive system, under the control of steroid hormones, like testosterone or Dihydrotestosterone, which are essential for prostate development and maintenance of prostatic physiology. Chronic BPA exposure could

lead an inappropriate activation of AR pathways in prostate gland during all the life, affecting negatively its homeostasis. In this work, we showed our preliminary data about the comparison between BPA and Dihydrotestosterone (DHT) on 2D and 3D non tumoral human prostate cultures. Our results highlighted a non-monotonic BPA effect in both systems, which induced inhibition of cell proliferation at low dosage. In 2D cell culture condition, DHT proliferative effect decreased when the cells were treated with DHT+BPA. Moreover, the latter could dislocate AR localization, affecting cell responsiveness of hormones stimuli. In 3D cell condition, we confirmed the key role of DHT during prostate organoid (PO) development: it acted in a specific temporal window during the PO grow (between day 4 and 6) to allow the complete organoid cystic formation. Our preliminary data showed that when POs were treated with BPA in absence of DHT, any complete PO development was detected, demonstrating that BPA replace the natural hormone. In the presence of DHT, BPA treatment may interfere with DHT inducing a formation of smaller complete POs. To sum up, BPA was able to interfere with DHT function, altering cell behaviour, interacting weakly with AR, acting as antiandrogenic compound, affecting the normal prostate development.

Ecotoxicological assessment of silver nanoparticles in the marine environment: a multispecies approach

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Silver nanoparticles (AgNPs) are used in a wide range of consumer and industrial products due to their broad-spectrum antimicrobial properties. However, their growing application raises concern regarding their potential impact on aquatic ecosystems. One goal of the INTEGRANO project is to propose and evaluate design strategies in line with the European Safe and Sustainable by design

(SSbD) framework. On top of the process there is the assessment of the potential hazards of nanomaterials at different stages of their life cycle. In this regard, the present study investigates the ecotoxicological effects of functionalized AgNPs – modified with curcumin (AgCur) and hydroxyethylcellulose (AgHEC and AgHEC6.4), compared with Ag reference (namely AgNaked). Marine species from different trophic levels were selected to provide a comprehensive toxicity assessment, including bacteria (*Aliivibrio fischeri*), phytoplankton (*Dunaliella tertiolecta*, *Phaeodactylum tricornutum*, *Skeletonema pseudocostatum*), and zooplankton (*Amphibalanus amphitrite*, *Artemia franciscana*, *Tigriopus fulvus*, *Brachionus plicatilis*, *Paracentrotus lividus*, and *Aurelia* sp.). The experimental set-up combined standard and innovative endpoints, including bioluminescence, growth inhibition, mortality, developmental and behavioral alterations. Preliminary results showed that functionalized AgNPs caused stronger and earlier toxic effects than AgNaked. These effects were particularly evident in algal growth inhibition and sea urchin embryo development. *S. pseudocostatum* and *P. lividus* were the most sensitive species. Notably, behavioural endpoints such as swimming speed alteration and pulsation frequency, appeared to be sensitive indicators of sublethal stress, often detectable at concentrations lower than those inducing lethality or growth inhibition. The functionalisation process did not achieve the desired mitigation of toxicological potential, confirming that surface chemistry has a strong impact on particles reactivity. Nevertheless, this is not necessarily a negative result, as the ultimate goal is to develop solutions that make the particles more toxic to microorganisms but safer for human biological targets. Therefore, if this increased reactivity is accompanied by greater selectivity or by the use and release of a smaller amount of material, the goal can be considered achieved. Overall, the proposed experimental models proved to be very effective in assessing the toxicological potential, which will be correlated with the physicochemical properties and the desired selectivity.

Imidacloprid complexed with β -cyclodextrins: ecotoxic impact in key aquatic species

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Imidacloprid (IMI), a widely used neonicotinoid insecticide in agriculture, has raised increasing environmental concerns due to its persistence in aquatic ecosystems and its toxicity toward non-target organisms. This study aimed to evaluate the potential of β -cyclodextrin (β -CD) to modulate the ecotoxicological profile of IMI by characterizing its inclusion complexes at 1:1 and 1:2 molar ratios. Biological effects were investigated on two model aquatic species representing different trophic levels: the green alga *Raphidocelis subcapitata* as producer, and the rotifer *Brachionus calyciflorus* as primary consumer. Acute and chronic toxicity assays were performed following standardized guidelines. Acute toxicity was assessed in rotifers as mortality after 24 h of exposure (ASTM E1440-91, 2004), while chronic toxicity was assessed by inhibition of reproduction in rotifers after 48 hours of exposure (ISO 20666, 2008) and inhibition of algal growth in *R. subcapitata* after 72 h of exposure (OECD 201, 2011). Acute exposure to IMI alone induced 50% lethality (LC₅₀) in *B. calyciflorus* at thousands of $\mu\text{mol/l}$. However, when IMI was complexed at 1:1 and 1:2 molar ratios with β -cyclodextrin (β -CD), toxicity significantly increased, with LC₅₀ values decreasing to 53.69 μM and 76.89 μM , respectively. This marked enhancement of toxicity suggests increased bioavailability and biological effects, likely due to improved membrane permeation facilitated by β -CD. Chronic toxicity data revealed a different pattern, especially at environmentally relevant concentrations. Rotifers showed higher sensitivity than algae, with IMI alone causing a 50% reproductive inhibition at 8.99 μM (*B. calyciflorus*) and 442.38 μM (*R. subcapitata*). Inclusion complexes with β -CD further amplified toxicity in chronic tests, lowering EC₅₀ values below 1.5 μM in rotifers. However, when endpoints as EC₂₀ were considered, particularly at realistic environmental concentrations (in the range of tenths to hundredths of μM), β -CD complexation significantly reduced toxicity compared to free IMI,

with adverse effects decreasing by up to 98%. These findings indicate that while β -CD complexes at relevant chronic exposure concentrations may mitigate the ecological impact of IMI by substantially lowering its toxicity, highlighting a potential advantage of β -CD complexation for environmental safety.

Calibration-free SPES/SPES² quantification and multiparametric characterization of single particles in heterogeneous biological and environmental liquids

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Complex particles and mixtures are common in various environmental liquids and products. Whether these particles are of natural or anthropogenic origin, their reliable analysis and counting are often hindered by the complexity and heterogeneity of the samples. This complexity arises from the presence of particles with diverse optical properties, such as varying refractive indices, internal structures (e.g., core-shell, mesoporous), shapes (e.g., rods, plates), and the presence of impurities or synthesis residues. In this presentation, we introduce novel analytical technologies called Single Particle Extinction and Scatterings (SPES+SPES²) for detecting and studying particles in heterogeneous liquids. This innovative approach leverages calibration-free optical parameters, such as particle extinction cross-section, particle polarizability, and F90, derived from single particles detected in a liquid. These parameters enable the retrieval of particle size distribution for individual populations, absolute and relative numerical concentrations, particle stability, and detailed information on optical particle structure in the liquid. The SPES+SPES² methods offer significant advancements in the field of particle analysis by providing a comprehensive and precise characterization of complex particles and mixtures. This methodology is particularly effective in overcoming the challenges posed by the heterogeneity and diverse optical properties of particles in environmental and consumer systems. We will present practical examples of the SPES+SPES² methods applied to: (1) the analysis of mixtures of organic particles, such as microplastics of different sizes and carbon black particles, and

inorganic particles, such as mineral particles and oxides, as well as cells in the same liquid. (2) the detection and characterization of submicron plastic particles released from plastic containers used for food storage and during the sterilization process of early childhood devices. These examples will demonstrate the versatility and robustness of the SPES+SPES² methods in providing detailed and accurate particle analysis in various contexts of environmental interest and for the assessment of particle hazards for the environment and on human safety of products.

The new Science-Policy Panel on chemicals, waste, and pollution: road to establishment and key policy milestones

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Reducing pollution deriving from the unsound management of chemicals and waste is one of the most sensitive global challenges the world is facing due to its impact on human health and the environment. The UNEA Resolution 5/8 represents a pivotal endeavour to strengthen the science-policy interface on this topic at the global level, through the decision that a Science-Policy Panel (SPP) should have been established to contribute further to the sound management of chemicals and waste and prevent pollution. This initiative aims to complement existing bodies such as the Intergovernmental Panel on Climate Change (IPCC) and the Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES), by addressing the third pillar of the triple planetary crisis, climate, biodiversity and pollution, through a dedicated mechanism to contribute further to the sound management of chemicals and waste and to prevent pollution. The need for this panel arises from the fact that the global goal of minimizing adverse impacts from chemicals and waste by 2020 was not achieved, and waste management costs

are projected to double over the next 25 years. The Open-Ended Working Group (OEWG) was tasked in 2022 with developing the foundational elements by 2024. Despite significant progress, even the last session (OEWG 3.1, June 2024) proved to be inconclusive on the Foundational Documents, with particular regard to the Panel's scope, governance arrangements, and decision-making procedures. A resumed third session (OEWG 3.2) was held from June 15-18, 2025, in Punta del Este, Uruguay, with the aim of finalizing the Panel's Foundational Document and Rules of Procedure. This was paramount for the subsequent Intergovernmental Meeting (IM) (June 19-20, 2025) to formally assign the name of the SPP and adopt its core structures. This presentation aims to explore the negotiation dynamics, and the expected design of the SPP, emphasizing its future role in informing global action on chemicals, waste, and pollution.

High density polyethylene (HDPE) biodegradation by *Cladosporium halotolerans*

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Polyethylene (PE) is high molecular weight synthetic polymer, very hydrophobic and hardly biodegradable. To increase polyethylene biodegradability it is very important to find microorganisms that improve the PE hydrophilic level and/or reduce the length of its polymeric chain by oxidation. In this study, we isolated *Cladosporium halotolerans*, a fungal species, from the gastric system of *Galleria mellonella* larvae. Here, we show that *C. halotolerans* grows in the presence of PE polymer, it is able to interact with plastic material through its hyphae. Formation of erosion, defects and holes observed in SEM micrographs suggest that this fungus adheres to the surface where the degradation process occurs, and carbonaceous compounds are released in the medium. The *C. halotolerans* supernatant protein pattern analysis revealed some differences associated with polyethylene presence, the most significative is the presence of FAD binding_3 domain containing enzyme, a protein that binds FAD as prosthetic group used by various

oxidoreductase enzymes probably involved in PE degradation.

Marine Strategy Framework Directive: tracking anthropogenic contaminants in marine biota for ecosystem protection

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The marine environment represents a crucial resource that requires protection, preservation, and safeguarding to maintain its biodiversity, ecological integrity, and productivity. In response to anthropogenic pressures on marine ecosystems, the European Parliament and the Council of the European Union enacted the Marine Strategy Framework Directive (MSFD-2008/56/EC) on June 17, 2008, which Italy transposed through Legislative Decree No. 190 of October 13, 2010. The directive aims to safeguard marine ecosystems and ensure the sustainability of human activities. Assessment is based on 11 qualitative descriptors, with Descriptor 8 focusing on the quantification of pollutants in water, sediment, and biota matrices and their effects on the marine environment. Monitoring aims to maintain Good Environmental Status (GES), ensuring that pollutant concentrations remain within regulatory limits and biological processes are not significantly impacted. For biota, the mandatory parameters for chemical analysis are specified in Table 1/A of Legislative Decree 172/2015. Annual sampling of fish and shellfish is conducted at stations both within and beyond 12 nautical miles to ensure adequate GES assessment. Specifically, the target species for monitoring include *Mullus barbatus*, *Merluccius merluccius*, and *Mytilus galloprovincialis*. In the case of mussels, concentrations of Polycyclic Aromatic Hydrocarbons (PAHs) are measured in comparison with the Environmental Quality Standards (EQS), following the SNPA Guideline 20/2019. Key PAH compounds monitored include benzo(a)pyrene (as a marker for other PAHs) and fluoranthene, with anthracene serving as an indicator for long-term accumulation. For PAH determination, wet samples are treated with sodium sulfate, extracted via ultrasonic hot saponification using an alkaline alcohol solution, followed by liquid-liquid back-

extraction in an apolar solvent. The extracts are then purified on silica gel using a 1:1 hexane/dichloromethane mixture and analyzed using gas chromatography-mass spectrometry (GC-MS). PAH concentrations are reported as a function of wet weight. During the three-year period 2021-2023, PAH concentrations were consistent with the statutory EQS.

Marine Strategy Framework Directive: analysis of PAHs in marine sediments

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Our seas are characterized by a great variety of habitats, in which there is a very rich marine biodiversity; they are a source of support for a multiplicity of services, from food supply to tourism activities, to the regulation of fragile ecosystem and climate balances. The need to reduce anthropogenic impacts on marine waters, and to preserve the ecological diversity and vitality of clean, healthy led the European Union to issue the Marine Strategy Framework Directive 2008/56/EC, transposed in Italy by the Legislative Decree No. 190 of October 13, 2010. The Directive, based on an integrated approach through the study of 11 descriptors, sets as a goal for member states the achievement of good environmental status for their marine waters and identifies, within each European marine region and based on hydrological, oceanographic and biogeographic factors, sub-regions within which member states must carry out monitoring and identify appropriate programs of measures. Among all of them, the focus is on Descriptor 8. The objective of this descriptor is to assess the concentration of contaminants (metals, PAHs, PCBs, pesticides, VOCs) associated with human activities conducted at sea, particularly in areas impacted by maritime traffic and industrial plants, within marine sediments. Official methods for the analytical determination of PAHs in marine sediments involve sample drying (at 30-35°C), followed by sieving (< 2mm). The sieved fraction undergoes an initial extraction phase using a sonicator, in accordance with the EPA 3550C (2007) method. This is followed by purification using silica gel, as outlined in the EPA 3630C (1996) method. For instrumental

determination, GC-MS is employed following the EPA 8270E (2018) method for acquisition and analysis. The results did not indicate any exceedances of the levels established by legislation. For PAHs in sediments, the mandatory parameters for chemical analysis and corresponding limits are those specified in Table 2/A of Legislative Decree 172/2015.

Regulatory developments and risk management for pollutants of concern: the case of hexavalent chromium under REACH Regulation

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This work addresses the critical environmental and human health concern associated with hexavalent chromium Cr(VI), a relevant contaminant and established human carcinogen, mutagen, and reproductive toxicant. Direct and indirect environmental exposure to Cr(VI) significantly impact human health of general population. Its persistence and mobility in the environment result in widespread contamination of soil and aquatic systems, which primarily stems from industrial discharges associated with surface treatment processes, including coating, passivation, anodising, stainless steel production, and leather tanning. The European Union (EU) has implemented a stringent regulatory framework, through the authorisation process under the Regulation EU No. 1907/2007-REACH (*Restriction, Evaluation, Authorization of Chemicals*), to mitigate the inherent risks. This framework ensures highly controlled uses of Cr(VI) substances while actively promoting its substitution with less hazardous alternatives where technically and economically available. The authorisation process

critically evaluates the efficacy of Risk Management Measures (RMMs) and Operating Conditions (OCs) to minimise human exposure and environmental emissions across various compartments (water, air and soil) and waste management. Cr(VI) is listed in the European Chemical Agency (ECHA) database under REACH, with estimated production and imported volumes within the European area ranging between 106 and 107 tonnes/year. Its inclusion in REACH Annex XIV (Regulation EU no. 348/2013) due to its non-threshold hazardous properties, implies that industries must demonstrate health and environmental risks control to the lowest technically achievable level. In September 2023, the European Commission formally entrusted ECHA with the mandate to prepare an REACH Annex XV restriction proposal targeting use of Cr(VI) substances in the formulation of mixtures, electroplating on plastic and metal substrates, the use of primers and other slurries, various surface treatment processes, as well as functional additives and process aids. This proposed restriction under REACH regulation aims to prevent the release of at least 17 t/y of Cr(VI) and to reduce the health effects for both workers and general population via environment. It also seeks to streamline regulatory oversight by reducing the substantial number of individual authorisation applications (i.e., 526 until February 2025), thereby accelerating the transition to safer alternatives. This comprehensive approach, encompassing detailed risk assessments and alternative analyses, offers a more efficient and effective strategy for the management of Cr(VI) related issues compared to individual authorisations. Furthermore, this work provides an analysis of the main implications of current and evolving EU legislation, focusing on regulatory advancements and the practical challenges of implementing control measures across relevant industrial sectors.

Session C - Exposure and effects of emerging contaminants

Hidden threats of UV filters: ecotoxicity of Ethylhexyl triazone and its chlorinated byproducts in aquatic species

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Ethylhexyl triazone (EHT) is a hydrophobic UVB filter commonly used in sunscreens due to its high photostability and UV absorbance. However, its environmental persistence and transformation during water disinfection raise concerns about ecotoxicological risks. This study evaluated the acute and chronic toxicity of EHT and its chlorination byproducts (DBP1–DBP4), formed under simulated swimming pool chlorination conditions, using a multi-species freshwater test battery. Toxicity assays were conducted across four freshwater organisms belonging to two trophic levels: the alga *Raphidocelis subcapitata*, the rotifer *Brachionus calyciflorus*, the anostracan *Thamnocephalus platyurus*, and the ostracod *Heterocypris incongruens*. EHT exhibited acute toxicity toward *B. calyciflorus* (LC50 = 12.86 mg/l), classifying it as “harmful to aquatic organisms” (EU Directive 93/67/EEC). EHT caused 37.5% mortality in *T. platyurus* at 15 mg/l, and 42.5% mortality in *H. incongruens* at only 6 mg/l. DBP1, DBP3, and DBP4 induced 30–40% mortality in rotifers and ostracods at concentrations ≤ 10 mg/l. Under chronic exposure, no significant effects were observed on algal growth up to 15 mg/l. Moving up the trophic chain, *B. calyciflorus* showed reproductive inhibition, with EC50 values ranging from hundreds to thousands of $\mu\text{g/L}$. *H. incongruens* emerged as the most sensitive species: EHT inhibited ostracod growth at EC50 ≈ 140 $\mu\text{g/l}$, while DBP1 and DBP4 were significantly more toxic (EC50 = 5 and 8 $\mu\text{g/l}$, respectively). DBP3 induced moderate toxicity (EC50 ≈ 65 $\mu\text{g/l}$), and DBP2 was the least toxic (EC50 > 200 $\mu\text{g/l}$). Sub-chronic endpoints for *H. incongruens* were particularly informative: EHT

determined EC20 values in the tens of $\mu\text{g/l}$, while DBP1 and DBP4 triggered EC20s at low $\mu\text{g/l}$ levels. A sediment risk quotient (RQ) assessment based on EC10 values and environmental concentrations of EHT (up to 2 ng/g) yielded RQ > 1 , suggesting potential risk to benthic invertebrates.

Behind the masks: the environmental mysteries of cosmetic masks

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During the COVID-19 pandemic, the global market for beauty and cosmetic masks has experienced unprecedented growth. From a valuation of \$5.7 billion in 2016, the market increased to \$6.3 billion in 2017 and was projected to reach \$8.8 billion by 2021, with a compound annual growth rate (CAGR) of 9.1%. While cosmetic face masks are widely marketed for skincare and personal wellness, their composition, often based on single-use synthetic materials such as polypropylene, polyurethane, and nitrile, raises significant environmental concerns. After use, these products are typically discarded as mixed municipal waste, with limited recovery or recycling, increasing the risk of their accumulation in marine ecosystems. This study investigates the environmental impacts of discarded cosmetic face masks through a time-resolved artificial weathering experiment in synthetic seawater. Mask fragments were incubated for various durations (1 to 180 days) under controlled laboratory conditions to simulate environmental aging. Samples were analyzed using Raman spectroscopy, Total Organic Carbon (TOC), and Total Carbon (TC) analysis to quantify the leaching of organic additives and polymer degradation products. Results revealed significant release of organic contaminants over time, particularly plasticizers, UV stabilizers, and other

polymeric additives commonly found in cosmetic mask matrices. Raman analysis confirmed structural degradation of polypropylene- and nitrile-based materials, while TOC/TC data indicated increasing concentrations of leached organics correlated with exposure time. To evaluate biological effects, acute ecotoxicological assays were conducted on the marine invertebrate model *Artemia franciscana*. Exposures to leachates from weathered masks induced elevated mortality in both juvenile and metanaupliar stages, particularly after 30 to 90 days of leaching. In addition, molecular biomarkers of stress were assessed using RT-qPCR targeting genes related to the defense system. Significant up- and down-regulation of genes such as hsp26, hsp60, hsp70, COXI, and COXIII was observed, indicating mitochondrial dysfunction and cellular stress pathways triggered by chronic exposure to leachate components. This study highlights cosmetic masks as an emerging vector for marine contamination, not only as microplastic debris but also as a diffuse source of bioactive chemical pollutants. The findings call for further attention to the environmental footprint of cosmetic and personal care products, and for the development of safer, biodegradable alternatives and improved waste management strategies.

Assessing sub-lethal effects of oral exposure to different chemicals in *Osmia bicornis*: a multi-biomarker approach

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Pollinators are experiencing alarming declines, with chemical pollution recognized as a key driver. While ecotoxicological studies often focus on insecticides, pollinators in agricultural environments are also exposed to mixtures of other compounds such as fungicides, herbicides, heavy metals and veterinary drugs. The combined ecotoxicological effects of these pollutants remain poorly understood, despite their potential to induce biochemical and cellular alterations in

pollinators. This study is part of the PRIN 2022 project “An interdisciplinary approach to unravel the effects of combined exposure to chemical pollutants on insect pollinators (POLYPOLL)”. The present study aims to test the sub-lethal effects of oral exposure to different chemicals in *Osmia bicornis*, using a multi-biomarker approach that includes biochemical and cellular endpoints. Females were exposed to a heavy metal (copper) and a commercial fungicide (boscalid), alone and in combination. Four additional groups were treated with an herbicide (glyphosate) and a veterinary drug (ivermectin), alone and combined. From each insect, haemolymph, gut and head were collected for biomarker analysis. Immune system function, detoxification and metabolic responses, neurotoxicity and genotoxicity biomarkers were evaluated. An induction of detoxification pathways was observed after exposure to copper and its mixture with boscalid. Ivermectin, alone and with glyphosate, induced metabolic alterations. An inhibition of esterase activities emerged with boscalid exposure and no significant changes were observed in immune markers across treatments. The analysis of all biomarker responses proved to be a useful and integrated tool for better understanding the sub-lethal effects of contaminants on the health status of these insects.

Assessment of gadolinium impact on the digestive glands of marine mussels

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Among emerging contaminants, chemicals used for therapeutic and diagnostic purposes are prominent. In the clinical imaging, gadolinium (Gd)-based contrast agents (GBCAs) are widely used in the magnetic resonance imaging and then excreted in urine. Because GBCAs are difficult to remove by usual sewage treatment systems, Gd reaches aquatic environments as a micropollutant posing an hazard for aquatic biota. The aim of the present study was to assess the biological impact of Gd on the bivalve filter feeders *Mytilus galloprovincialis*. Mussel specimens were exposed for 28 days to two forms of Gd (Gd oxide

nanoparticles, Gd₂O₃; Gd chloride, GdCl₃) at two realistic concentrations (1 µg/l; 10 µg/l). Samples of digestive glands, chosen for their recognized detoxifying activity against xenobiotics, were taken at 7 (T7), 15 (T15), and 28 (T28) days of exposure and used for histological, biochemical, and molecular analyses. The results obtained showed a strong hemocyte infiltration at T7, especially in samples exposed to 10 µg/l of GdCl₃. The antioxidant system, evaluated by molecular and biochemical assays, showed an increase in the activity of catalase (CAT), glutathione S-transferase (GST), and superoxide dismutase (SOD) enzymes, counterbalanced by a downregulation of the related genes (Cu/Zn SOD, Mn SOD, CAT, and GST) and an upregulation of the gene for ovothiol (OvoA) synthesis. The inhibition of lactate dehydrogenase, on the other hand, suggests the triggering of gluconeogenic processes to mitigate the systemic energy deficit. These results confirm the high sensitivity of mussels towards this kind emerging micropollutant, highlighting the biological impact of Gd for non-target species. *This work was supported by PRIN PNRR 2022 - CUP B53D23024710001: "GADolinium (Gd), an emergent contaminant, is a new threat to the living beings: a comparative study to assess its biological TOXcity in animal models (GADOTOX)".*

Gadolinium, a Rare Earth Element (REE): A potential risk for the reproductive health of the Mediterranean mussel *Mytilus galloprovincialis*?

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Gadolinium (Gd) is a lanthanide classified as a rare earth element (REE), as it possesses unique properties, such as paramagnetism. Its wide versatility in the technological field for production of visual devices, and in the health field, as a contrast agent to improve the yield of magnetic resonance images, have encouraged its exploitation. At the same time, the non-performing capacity of urban waste treatment plants has determined its distribution in different environmental contexts, among which the most affected are marine ecosystems close to the most

industrialized centres. Therefore, the aim of this work was to evaluate the potential toxic effect of two known forms of gadolinium (GdCl₃, Gd₂O₃) on the reproductive health of the Mediterranean mussel *Mytilus galloprovincialis*, following a chronic exposure of 28 days, considering the temporal course of biological responses (T0, T7, T15, T28) at 1 and 10 µg/l in male and female gonads. Histological analyses performed by the H/E method confirmed the impact of Gd, showing the activation of innate immunity, with the presence of hemocytes distributed on the connective tissue surrounding the follicles of both sexes. Histochemical analyses performed by the modified dPAS/PAS method revealed the impact of both forms of Gd highlighting in the gonadal tissues the energy reserves of glycogen, a crucial polysaccharide during the maturation process, such as gametogenesis. Metabolomic (¹H-NMR) results gave the possibility to understand the induced biological response at the level of energetic (glucose, glycogen, BCAAs, etc.) and osmoregulatory (betaines, etc.) pathways. Overall, these findings further support the role of Gd as an emerging micropollutant of high ecological impact, with the potential to represent a real threat to non-target biota of marine ecosystems. *This work was supported by PRIN PNRR 2022 - CUP B53D23024710001: "GADolinium (Gd), an emergent contaminant, is a new threat to the living beings: a comparative study to assess its biological TOXcity in animal models (GADOTOX)".*

Ecotoxicological tests for evaluating combined toxicity of antibiotics and copper on ecosystems

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Agricultural and animal husbandry practices can lead to co-presence of antibiotics (AB) and heavy metal contamination in aquatic and terrestrial environments. Indeed, household or livestock wastes (such as biosolids and manure) applied as organic fertilizers may contain residues of antibiotics previously administered to humans or livestock. At the same time copper is commonly used to control plant diseases as a fungicide and its residues are present in soils. The combined effects

of antibiotics and copper are still poorly understood, although they are biocides and can have detrimental effects for ecosystem and human health. This study aimed at investigating the acute toxicity of selected ABs among the most found in environment (sulfamethoxazole - SMX, ciprofloxacin - CIP and chlortetracycline - CTC) in different combinations with Cu using standardized ecotoxicological assays across multiple trophic levels and environmental compartments. In particular, acute bioassays were conducted with the *Aliivibrio fischeri* (ISO 11348-3:2019) bacterium, the *Lepidium sativum* plant (APAT 2004) and the freshwater crustacean *Daphnia magna* (ISO 6341:2013). Moreover, soil water extracts from a real co-contaminated soil were analysed in terms of ecotoxicity with the same three test organisms. Results demonstrated that combined exposures frequently resulted in synergistic or additive toxicity, particularly at environmentally relevant concentrations, but also antagonistic ones were detected, even in cases of combination between highly intrinsic toxic compounds (CTC and Cu). These phenomena should be studied in a more specific way to better understand the contaminant interaction mechanisms. In this case, the interaction patterns were assessed using concentration addition (CA) model, revealing that traditional single-compound toxicity assessments may underestimate ecological risk of contaminants. On the contrary, no effects were displayed by soil water extracts suggesting a buffering effect provided by the soil matrix. In fact, comparing the soil contaminant concentrations (7 mg/kg for ABs and 30 mg/kg for Cu, in line with environmental ones in agroecosystems), ABs and Cu were found in water extracts in residual amounts, lower than their effective concentrations. However, the increase of the recurrence of extreme weather events cannot exclude an enhanced bioavailability of soil contaminants and can pose an additional risk to natural soil functioning. These findings highlight the usefulness of acute ecotoxicological tests as rapid, cost-effective tools for identifying potentially hazardous pollutant interactions and emphasise the need for integrated risk assessment frameworks that address chemical mixtures in ecosystems.

Cellular effects of environmental pharmaceuticals: mechanisms of toxicity in fish PLHC-1 and RTgill-W1 cell lines.

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Active pharmaceutical ingredients (APIs) are emerging contaminants of particular concern for their ubiquitous presence in aquatic ecosystems and their potential impact on non-target species. In natural field conditions, organisms are commonly exposed to mixtures of several co-occurring drugs, which may interact and lead to unexpected effects in terms of toxicity or metabolic changes. The present study aimed to evaluate the ecotoxicological potential of environmentally relevant concentrations of pharmaceuticals from major therapeutic classes frequently detected in aquatic ecosystems, including antidepressants, antiepileptics, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), antihypertensives, antidiabetics, and lipid-lowering agents. The effects of environmental pharmaceuticals and their mixtures were assessed by measuring cytotoxicity, reactive oxygen species (ROS) production, and mitochondrial membrane potential alterations in fish hepatic (PLHC-1) and gill (RTgill-W1) cell lines, selected for the critical role of these tissues in drug uptake and metabolism in aquatic species. The results revealed a lower sensitivity of PLHC-1 cell line compared to RTgill-W1, suggesting that hepatic cells may have a greater capacity to counteract the chemical stress due to their key role in detoxification processes. Among the therapeutic classes, psychiatric drugs exhibited the highest reactivity. Paroxetine, naproxen, and atenolol were identified as the most toxic compounds within antidepressants, NSAIDs and cardiovascular drugs, respectively. Results from mixture treatments indicated that toxicity was not related to the number of combined drugs, but rather on their mechanistic interactions, evidenced by the notably higher toxicity of binary mixtures compared to those containing a greater number of compounds.

Individual pharmaceuticals induced more pronounced effects than their mixtures, where antagonistic interactions between antidepressants and NSAIDs were observed in PLHC-1 cell line. The integration of data using the Weight of Evidence model provided quantitative insights into the toxicity of several pharmaceuticals and their mixtures, enabling easier comparisons between different molecules, biological tissues and exposure conditions. This approach can be particularly useful for future prioritization and risk assessment of these environmental pollutants on non-target aquatic species.

Impact of polystyrene, polyethylene and polymethyl methacrylate microplastics on the survival and gut bacteria of *Apis mellifera ligustica*

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Microplastics (MPs) are a growing environmental concern with far-reaching ecological implications. Due to their constant interaction with air, water, soil and plant surfaces, honeybees can serve as effective sentinel organisms for assessing environmental pollution. This study investigated the effects of three types of microplastic (MP), polystyrene (PS), polyethylene (PE) and polymethyl methacrylate (PMMA), on honeybee mortality and the abundance of key bacterial taxa associated with beneficial gut functions, using quantitative polymerase chain reaction (qPCR). Laboratory assays involved feeding the bees these MPs either individually or in combination. Exposure to all the tested MPs significantly reduced the bees' survival rates, with the combined treatment having the most severe impact, which suggests an additive toxic effect. Microbial analysis revealed that both time and MP treatment influenced the structure of gut bacteria, with PE inducing the most significant changes, including chronic enrichment of *Lactobacillus* Firm5 and *Bifidobacterium asteroides*. Although these taxa are typically associated with beneficial gut functions, they may indicate a dysbiotic response under prolonged MP exposure. The data support the hypothesis that honeybee gut bacteria might interact with MP particles through the formation of biofilms,

warranting further research into microbe-plastic dynamics. Although no acute microbial effects were detected from combined MP exposure due to early bee mortality, this study highlights the need for further research into the long-term, sublethal impacts. These findings emphasise the multifaceted threats posed by MPs to pollinator health and environmental microbiomes.

Biological responses to pharmaceuticals in a non-target marine organism: *in vivo* – *ex vivo* insights

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The widespread occurrence of pharmaceuticals in aquatic environments is an emerging concern due to their potential toxic effects on non-target marine species, even at low concentrations. The need to develop and validate sustainable effect-based methodologies, aligned with the 3Rs principle and capable of detecting early cellular and biochemical alterations, has been increasingly highlighted. In this context, the *ex vivo* approach based on precision-cut tissue slices (PCTS) has been extensively applied in mammalian research, while its use in aquatic toxicology is still in its early stages. This method combines the main advantages of *in vitro* systems while preserving the native physiological structure and multicellular interactions of the tissue. In this study, the traditional *in vivo* exposure approach was complemented with the *ex vivo* PCTS technique, using the Mediterranean mussel *Mytilus galloprovincialis* as a model organism to evaluate the effects of environmental pharmaceuticals that are frequently detected in coastal waters. Slices of the digestive gland were exposed to a range of concentrations of selected drugs, including the lipid-regulating agent gemfibrozil, the antidiabetic compounds metformin and rosiglitazone, the selective serotonin reuptake inhibitor (SSRI) fluoxetine, and the non-steroidal anti-inflammatory drug (NSAID) diclofenac, some of which are included in the more recent update of the Watch List (EU 2025/439) under the Water Framework Directive. A wide set of cellular and biochemical responses was investigated to detect alterations in key physiological processes, such as immune response, biotransformation pathways,

lipid metabolism, and redox homeostasis. These integrated biomarkers provided a comprehensive overview of the toxicological impact of the tested compounds on non-target species. Overall, results obtained by integrating in vivo and ex vivo tests increased our understanding of the main metabolic pathways affected by the selected pharmaceuticals and revealed specific mechanisms of action that may underline sub-lethal effects in marine organisms. The combination of traditional and innovative

approaches strengthened the reliability of the findings and demonstrated the feasibility of applying the PCTS method in aquatic ecotoxicology. Overall, the PCTS technique proved to be a powerful and sustainable tool for elucidating the modes of action of pharmaceuticals, offering valuable information for better understanding their potential environmental impacts.

Session D - New Challenges for Environment and Human Health

Bathing area management: first results of the ACeS project

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The global recreational use of water in coastal areas, often associated with various leisure activities, underscores the increasing need to maintain these areas in an ever-improving quality status. The Directive 2006/7/EC regulates the management of bathing water quality through preserving, protecting, and improving the quality of the environment, and safeguarding human health by complementing the Directive 2000/60/EC, which aims to preserve water quality and manage the water resource. In recent years, risk analysis has also been applied to water protection, since it represents a dynamic model that can be exported to the hygienic-sanitary safety of different types of waters. In this context, the "ACeS - Water, Climate, and Health: from the Environmental Protection of Resources to Access to Water, to Safe Use" project aims to reduce knowledge gaps on the presence and spread of potentially harmful pollutants, like emerging pathogens (including antibiotic-resistant ones) and of some chemicals contaminants (heavy metals, polycyclic aromatic hydrocarbons - PAHs - and polychlorinated biphenyls - PCBs - among others) in bathing areas exposed to anthropogenic pressures (industrial, urban, and agricultural discharges), thus integrating health skills with environmental ones. In particular, the preliminary results from the chemical, ecotoxicological, and microbiological characterization of three environmental matrices (water, sediment, and

biota), conducted on two recreational coastal sites of the Tyrrhenian and Adriatic Sea, will be illustrated. In the Adriatic, clam samples are generally compliant with the legal limits, but some stations showed high values of *Escherichia coli* and positivity for *Vibrio parahaemolyticus* and norovirus. In the Tyrrhenian Sea, *Donax sp.* exceeded the limits for *E. coli*, with detection of *Vibrio cholerae* in one sample. Water and sediment samples are compliant with heavy metals, dioxins, PCBs, and PAHs, even if Cr exceeded the EQS threshold of 50 mg/kg in sediments of both areas, falling within the ranges known in the literature. Regarding ecotoxicological analyses, sediment samples are generally non-toxic, with biostimulation effects attributed to the high presence of nutrients. Some samples from the Tyrrhenian Sea showed a slight toxicity for *Tigriopus fulvus*. In general, the study areas present a good environmental quality, with some isolated critical issues. Ongoing analyses (antibiotics, hepatitis E) could provide further details to complete the assessment. An integrated "One Health" methodology is proposed to assess human and ecosystem health, with simplified tools for chemical, ecotoxicological, and microbiological risk management.

The role of bioassays in Water Safety Plans

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The adoption of Water Safety Plans (WSPs) represents a fundamental strategy for the protection of drinking water quality and public health. WSPs have usually relied on chemical monitoring and conventional microbiological indicators to assess water safety. However, this approach cannot assess the actual biological risks posed by complex mixtures of pollutants, especially emerging micropollutants such as pharmaceuticals, personal care products,

pesticides, and by-products from treatment processes. These substances can interact, producing synergistic, additive, or antagonistic effects that are not measured by standard chemical analyses. In this context, the integration of bioassays into WSPs allows to improve the assessment of water quality and possible related risks. Bioassays enable the detection of the biological effects of both known and unknown substances in complex mixtures, providing a direct measure of toxicity. This is particularly relevant when pollutants are present at very low concentrations, below regulatory limits or instrumental detection thresholds, but still capable of affecting human health and aquatic ecosystems (according to the so called “something from nothing phenomenon”). The use of bioassays is consistent with the One Health approach, which links human, animal, and environmental health. Several types of bioassays can be integrated into WSPs, including *in vitro* tests (e.g., for endocrine disruption, genotoxicity, or baseline toxicity) and *in vivo* or *ex vivo* models targeting specific organisms or biological functions. Selecting appropriate test batteries is essential to cover different modes of action and to capture effects across various biological levels, from cellular to organism and community scales. Recent developments in effect-based trigger values (EBTs) also offer a promising tool for exploiting bioassay results within a regulatory context. The inclusion of bioassays in WSPs does not replace chemical analyses but integrate them, providing a more holistic understanding of water quality. This combined strategy strengthens the capacity to identify potential hazards, prioritise interventions, and communicate risks. It also supports the transition towards effect-based monitoring frameworks, as encouraged by the European policies. Future efforts, however, should focus on harmonising bioassay selection, defining robust EBTs, and integrating bioassay data into risk assessment models and decision-support tools. After reviewing the main bioassays available in technical literature, some case studies are presented.

***In vitro* characterization of the pro-inflammatory potential of the algal toxin ovatoxin-a at the skin level**

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Ovatoxin-a (OVTX-a) is the major palytoxin (PLTX) analogue identified in the benthic dinoflagellate *Ostreopsis cf. ovata* from the Mediterranean Sea. In this area, the risk for human health is mainly associated with inhalation of marine aerosol and/or skin contact with seawater during dinoflagellates blooms. Although the hazard posed by PLTX has been extensively studied, very few data are currently available for OVTX-a. Hence, the present work is aimed at characterizing *in vitro* the hazard posed by OVTX-a at the cutaneous level. In particular, a comparative approach with respect to the reference toxin PLTX was followed to study OVTX-a pro-inflammatory potential. Initially, spontaneously immortalized HaCaT cells were selected as a model for the screening of OVTX-a cytotoxic effects in skin keratinocytes. Short-term exposure (4 h) to OVTX-a (1.0×10^{-16} M - 1.0×10^{-7} M) caused a concentration-dependent cell viability reduction and cell necrosis increase, with a potency lower than that induced by PLTX. Subsequently, a sub-cytotoxic concentration (1.0×10^{-11} M) was used to analyze the pro-inflammatory potential of OVTX-a in comparison to PLTX. Different exposure conditions were considered: a short one of 4 h, a long exposure of 24 h and a recovery condition consisting of 4 h treatment with each toxin followed by 20 h without them. The release of a large panel of cytokines and chemokines (IL-1 α , IL-1 β , IL-6, IL-7, IL-8, IL-18, IL-33, TNF- α , IFN- γ , MCP-1, RANTES, GM-CSF, MIF, ECF-CCL11, PGE2) was analyzed and, in general, only a few (IL-6, IL-8, TNF- α and PGE2) were significantly released by HaCaT keratinocytes treated with OVTX-a, albeit with a lower potency with respect to PLTX. Clustering analysis demonstrated two primary clusters at high similarity: one between short-term exposure to OVTX-a and negative control and one between long exposure to PLTX and positive control, suggesting a lower pro-inflammatory potential for OVTX-a. Finally, the possible involvement of the immune system was analyzed setting up a co-culture model composed of toxin-treated HaCaT keratinocytes and THP-1 monocytes. The inflammatory response was evaluated by means of monocytes activation, measuring monocyte

chemotaxis and the expression of selected markers of differentiation. OVTX-a induced a markedly lower monocytes migration compared to PLTX, confirming a lower potency than that of PLTX. These results provided a contribution to the characterization of the toxic effects of OVTX-a at the cutaneous level, providing an important basis for outlining its pro-inflammatory profile.

PFAS pesticides monitoring in waters

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Per- and polyfluoroalkyl substances (PFAS) are characterised by a stable, unreactive fluoro-carbon segment, which gives them unique properties such that they can be used in a variety of products including cosmetics, textiles, paints, pharmaceuticals and pesticides. PFAS group encompasses more than 10000 chemicals and their ubiquitous detection in the environment and living organisms, including humans, is a cause for growing concern. PFAS chemicals exhibit a range of concerning properties, such as carcinogenicity, adverse effects even at low concentrations on liver, immune system and endocrine systems; moreover, they show high mobility and bioaccumulation potential and may persist in water and sediments, resulting in long-term exposure of aquatic organisms. Pesticides are used to protect plants and crops against weeds, diseases and pests, usually applied directly on outdoor crops and therefore direct emission to the environment takes place. PFAS active substances are characterised by molecular stability and lipophilicity, which can alter membrane permeability and binding to target proteins. Therefore, PFAS pesticides use in agriculture has been increasing over the last decades. Although the legislation on the authorization of pesticides (Reg. (EC) 1107/2009) establishes law limits also based on persistence, it is possible to find PFAS pesticides and their transformation products in the environment. In particular, due to their properties, residues of PFAS pesticides can accumulate in environmental matrices and persist even after their use has ceased. About 47 PFAS active substances have been identified, 37 of these are still approved for use in pesticides in the EU.

Monitoring of PFAS pesticide in surface and ground waters can help to understand the extent of PFAS contamination and the implications this could have for human and environmental health. The analysis of the occurrence of PFAS pesticides in surface and groundwater is presented, within the framework of the national monitoring of pesticides in waters.

Climate Change and the spread of *Vibrio* species in transitional waters: a combined in-field and microcosm Investigation

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The current study is a part of work conducted within the framework of the European Union BlueAdapt project (<https://blueadapt.eu/>), investigating the impact of climatic changes on the spread of pathogenic bacteria and antimicrobial resistance in coastal waters. Determining the potential spread and occurrence of *Vibrio* spp. in transitional waters is crucial from the One-Health perspective, as majority of vibriosis cases result from recreational exposure to contaminated bathing water that can be potentially life-threatening. This work brings insights from in-field and microcosm experiments on investigating how climate-related stressors such as temperature, salinity, and pH can affect the survival of *Vibrio* spp. in transitional water. Sampling was performed at three sites along the Arrone River mouth during the 2023 bathing water season using artificial plastic substrates (APSS) to sample targeted pathogens and at the time of collection environmental parameters were also collected. *Vibrio* strains were recovered from APSS, which were submerged for one month in each site, using a combination of microbiological, biochemical, and molecular techniques. Simultaneously, a microcosm experiment tested the survival of two *Vibrio cholerae* strain (PV785470) inoculum concentrations (10^2 c.f.u/ml and 10^5 c.f.u/ml) isolated from our lab against a salinity gradient ranging from 2 to 32 PSU and under two temperature conditions (25°C and 37°C). The experiment was evaluated using a time-killing kinetic method. In total, 195 *Vibrio* strains were obtained, and biochemical and molecular identification results revealed the presence of three *Vibrio* species (*V. parahaemolyticus* n=22, *V.*

cholerae n= 27, *V. vulnificus* n=16). These strains demonstrated significant resistance to all tested antibiotics with the positive detection of genes like *sul1*, *tetA*, *tetB*, *blaCTX-M*, and *cmIA1*. These results indicate higher prevalence of different *Vibrio* spp. in hot season which positively correlates with temperature shifts and intensive recreational activities during this period. While the microcosm experiment results revealed that the survival of *V. cholera* was not significantly affected by variations in salinity or temperature. The lack of significant temperature or salinity effects on *V. cholerae* survival in controlled conditions suggests that other factors, such as nutrient availability or microbial interactions, may play a prominent role in pathogen persistence. In a nutshell, this study highlights the significant impact of climate-driven environmental changes on the prevalence of *Vibrio* spp. in transitional waters and underscores the need for its tracking, which is crucial for implementing effective One-Health strategies to prevent and reduce risks to human health.

Unravelling cocaine's impact on CB1 receptor function and intestinal integrity

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Among illicit substances, cocaine (COC) is one of the most widely abused psychostimulants, with global production reaching approximately 1,982 metric tons in 2020 (UNODC), making it the second most-used narcotic worldwide. This makes drug addiction one of the most critical public health challenges. Cocaine exerts its potent central nervous system (CNS) effects primarily by blocking the dopamine transporter (DAT), leading to increased extracellular dopamine levels and intense euphoria, which contribute to its high addictive potential. Recent advances in neuropharmacology have highlighted the significant role of the endocannabinoid system (eCB) in modulating the physiological and behavioral effects of addictive substances, including cocaine. Central to this system is

cannabinoid receptor type 1 (CB1), a G-protein-coupled receptor predominantly expressed in the CNS but also found throughout peripheral tissues. While substantial evidence supports CB1's involvement in the CNS effects of cocaine, much less is known about how cocaine affects the eCB system outside the brain. Notably, CB1 is also expressed in various peripheral organs, including the gastrointestinal (GI) tract, where it modulates functions such as gut motility, secretion, and inflammation. The gut-brain axis, a bidirectional communication network, may serve as a critical but underexplored interface in cocaine addiction. Our study aimed to determine whether COC is able to affect the expression of the CB1 receptor in a human colorectal adenocarcinoma cell line (CACO2). CACO2 cells were exposed to COC at environmentally relevant concentrations ranging from 330 nM to 0 nM, highlighting the COC non-monotonic effect: COC positively affected the metabolic activity of the CACO2 cell line at a concentration of 1 nM, as demonstrated by the MTT assay. Moreover, exposure to COC for 24 hours downregulated CB1, which could also lead to a relationship between the CB1-activated pathways and the expression and localization of junction proteins. Although these data are preliminary and require further studies, they demonstrate, for the first time, that COC influences CB1 expression as well as cell metabolic activity in CACO2 cells. These phenomena may play a key role in the process known as "leaky gut," which is considered one of the triggering or aggravating causes of several intestinal diseases.

Evaluating Sphaeropsidin A as a novel disinfectant against *Candida auris*: antibiofilm and functional characterization

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Candida auris is an emerging fungal pathogen of global concern, characterized by multidrug resistance, high transmissibility in healthcare settings, and strong biofilm-forming capacity. Its recent emergence has been linked to environmental and climatic changes that may have facilitated the evolution and selection of thermotolerant, resilient fungal strains. These factors, combined with limited antifungal

treatment options, underscore the urgent need for innovative antifungal and disinfection strategies. In this study, we investigated the antifungal activity of Sphaeropsidin A (SpA), a diterpene compound derived from the phytopathogenic fungus *Diplodia*, and its liposomal formulation (SpA-L), against *C. auris*. Both compounds demonstrated significant antifungal activity in vitro, with SpA-L showing enhanced efficacy in disrupting biofilm formation and integrity. In vitro assays revealed strong inhibition of fungal growth, particularly under biofilm conditions, which are notoriously resistant to conventional antifungals. Our investigation into the antifungal mechanism of this compound revealed notable alterations in reactive oxygen species (ROS) production and disruption of the fungal cell cycle. Furthermore, SpA-L impaired the ability of *C. auris* to adhere to and invade epithelial cell monolayers, a key step in host colonization and persistence. Importantly, SpA-L exhibited no cytotoxic effects on mammalian cells, suggesting a favorable safety profile. The antifungal activity of both formulations was further confirmed in a *Caenorhabditis elegans* infection model, where treatment with SpA and SpA-L significantly reduced fungal burden and improved host survival. These findings demonstrate not only the therapeutic potential of these compounds but also their capacity to interfere with key virulence mechanisms of *C. auris*. Given the increasing environmental resilience and healthcare-associated spread of *C. auris*, the strong antibiofilm and antifungal properties of Sphaeropsidin A and its liposomal formulation support their development as novel disinfectant agents. These compounds may represent a valuable addition to infection control measures aimed at preventing *C. auris* colonization and persistence on medical surfaces and devices.

Effectiveness of Compost in mitigating drought stress in *Rosmarinus officinalis* and its impact on soil microbial communities

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Drought is a major abiotic stressor in Mediterranean agroecosystems, negatively impacting plant productivity and soil microbial functioning. Although *Rosmarinus officinalis* (rosemary), is well-adapted to semi-arid environments, it may become sensitive to prolonged or recurrent water deficits. Organic amendments such as compost are known to enhance soil structure, porosity, water retention and carbon storage, favouring soil microbiota, that can support plant when stressed. Compost role in supporting plant resilience and microbial dynamics under drought conditions is not well known. However, compost can contain different contaminants of anthropogenic origin e.g. pharmaceuticals and antibiotic resistance genes (ARGs). This study has assessed the effectiveness of four composts with various origin (mixed urban and agricultural wastes: CA and SO; agricultural waste: UP and TO) in mitigating drought stress during a one-month microcosm greenhouse experiment, using rosemary as a model plant. Rosemary performance was evaluated in terms of biomass production. Microbial abundance (DAPI count) and microbial activity (dehydrogenase) were assessed to evaluate soil microbial responses to different conditions. Moreover, the relative abundance of antibiotic resistance genes (ARGs) was quantified by qPCR to assess the potential risk of resistance spread into the agroecosystems and the potential increase in drought stress conditions. To evaluate environmental safety and potential leaching, compost ecotoxicity was also assessed on water extracts of differently amended soils using standardized bioassays: *Aliivibrio fischeri*, *Lepidium sativum*, and *Daphnia magna*. At the start of the experiment, all compost-amended soils showed higher microbial abundance compared to the control. At the end of the experiment, microbial abundance remained higher in well-watered control soils; however, under drought conditions, the two mixed urban composts (CA, SO) promoted microbial activity compared to the unamended treatment. Notably, SO maintained high dehydrogenase activity at both the initial and final sampling time, indicating a buffering effect on microbial activity under drought stress. At the end of the experiment, CA enhanced rosemary biomass under drought stress, suggesting increased plant

tolerance. Soil water extracts were not toxic for *A. fischeri*. While some composts exhibited toxicity for *L. sativum* (the two from agricultural waste and CA) and *D. magna* (at the initial time the two from agricultural waste), which reduced over time. Further analyses are in progress to quantify ARGs in soils. This work contributes to clarify the long-term implications for soil quality, plant-microbiome systems and safe integration of organic amendments in drought-prone agroecosystems and shows the potential of specific compost formulations to improve plant resilience versus abiotic stresses.

Exploring the role of *Apis mellifera* as monitoring tools for antimicrobial resistance genes: a case study in Bozen (South Tyrol, Italy)

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Antimicrobial resistance (AMR) is a growing global concern with far-reaching consequences for human, animal, and environmental health. While most AMR surveillance focuses on clinical and livestock settings, increasing attention is being paid to environmental reservoirs. Honeybees (*Apis mellifera*), due to their wide foraging range and direct contact with air, water, plants, and soil, are increasingly recognised as effective bioindicators of environmental AMR. This study aimed to assess the presence of antimicrobial resistance genes (ARGs) in honeybee colonies from seven apiaries in South Tyrol, sampled three times during the active season. DNA was extracted from pools of 10 honeybees per sample, and a total of 32 ARGs—linked to six main antibiotic families (β -lactams, fluoroquinolones, aminoglycosides, sulfonamides, tetracyclines, and colistin)—were targeted using seven multiplex PCR assays. Overall, 13 different ARGs were detected across 40.6% of the investigated colonies. The highest proportion of detected genes belonged to the tetracycline resistance group, with *tetA*, *tetM*, *tetL*, and *tetW* collectively accounting for 30.7% of the positive hits. This antimicrobial group was followed by β -lactam resistance genes, including *bla_{TEM}*, *bla_{SHV}*,

bla_{CMY2}, and *bla_{OXA48}*, representing 23.1% of detected genes. Resistance to fluoroquinolones and aminoglycosides was confirmed by the presence of *qnrB* and *qnrS*, and *strA* and *aacA1* genes for 15.4%, respectively. Additionally, the *sul1* gene associated with sulfonamide resistance and *mcr-1* linked to colistin resistance were each detected, together making up 15.4% of the ARGs found. In several samples, the co-presence of multiple ARGs belonging to different antibiotic classes—such as β -lactams, tetracyclines, and fluoroquinolones—was observed, suggesting a potential for multi-resistance traits and possible horizontal gene transfer events in the environmental microbiota associated with honeybee exposure. In conclusion, this study highlights the potential of using *A. mellifera* in AMR surveillance strategies and supports their integration into One-Health approach. Further studies should explore the seasonal dynamics of ARG presence in honeybee populations and their relationship with environmental and anthropogenic factors. This work contributes to clarify the long-term implications for soil quality, plant-microbiome systems and safe integration of organic amendments in drought-prone agroecosystems and shows the potential of specific compost formulations to improve plant resilience versus abiotic stresses.

Tracing environmental pollutants: synchrotron micro-XRF analysis of metals in plastics and human tissues

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In our study, we use synchrotron-based X-ray fluorescence (XRF) microscopy to analyze and map the distribution of trace metals in environmental plastic debris and tissues from animals and humans. The use of synchrotron light offers exceptional sensitivity and spatial resolution, enabling the detection of metals at trace levels and offering detailed knowledge of their localisation

within complex matrices. Our dual effort (environmental plastics and mammals) emphasises the interconnected relationship between environmental contamination and human exposure. The global pollutant microplastics, both first and second generation, can easily enter the food chain. Among them, the resin pellets, used in manufacturing, pose significant risks. Their densities (PE and PP) allow them to float in the ocean's microlayer, where they absorb pollutants at concentrations up to 500 times higher than surrounding water. In the environmental context, we examined selected pellets belonging to the Italian Pellets Watch (ItPW), the first national survey of the Italian coastline, collected in different environmental contexts (Sardinia, Abruzzo and Tuscany). By XRF measures at Puma beamline (Soleil synchrotron, France), we assessed how metals such as lead (Pb), iron (Fe), chromium (Cr), nickel (Ni), strontium (Sr), manganese (Mn), bromide (Br) and others associate with degraded polymer surfaces and cores. By characterizing these interactions, we aim to better understand the role of plastics not only as persistent waste but also as vectors for toxic elements. In parallel, we investigated the presence of similar metals in a Jackal gold kidney (through autopsy) and human placenta tissues obtained at delivery (at the Hospital), as examples of organs involved in detoxification and accumulation. In both types of tissue, chemically fixed and paraffin-embedded, we detected significant traces of Pb, Cr, Br, Ni, Sr, and Mn, which variably colocalised among themselves or with Fe. The detection by XRF of trace metal contaminants in jackal kidneys (and other organs) can be valuable when employing the gold Jackal as a sentinel species for environmental pollutants (as in Northeast Italy). In contrast, finding trace metals in human placenta indicates hazardous environmental exposure of the foetus that needs further investigation, while providing potential biomarkers for assessing the distribution of multiple pollutants at this level. We expect that the comparison of metal signatures between environmental samples, animals and human tissues will allow us to trace possible pathways of pollutant transfer, assessing the health implications of chronic exposure to contaminated environments. Overall, our findings also demonstrate the powerful application of

synchrotron XRF in environmental and biomedical research.

Salinization in transitional ecosystems: potential impacts on macrozoobenthic biodiversity

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Salinization is an increasingly critical environmental issue affecting freshwater, transitional, and coastal ecosystems. Although salinity is rising globally, it is still often perceived as a natural feature of aquatic systems, with limited impact on biodiversity and ecosystem functioning. However, when combined with other stressors, such as chemical pollutants, salinity can amplify ecotoxicological effects and compromise the resilience of aquatic organisms. Physiological responses to salinity are among the earliest and most sensitive indicators of environmental stress, including reduced survival and growth, altered metabolic rates, and increased osmolyte concentrations. These organism-level effects can scale up to affect community composition and disrupt ecological processes and services. Transitional environments, such as coastal lagoons, are particularly vulnerable due to their shallow depth and the dynamic interaction between freshwater and seawater. These systems exhibit high variability in physicochemical parameters, especially salinity. Key drivers include climate change, sea level rise, reduced freshwater inflow, tidal dynamics, evaporation, and anthropogenic alterations, such as dredging and canalization. Northern Adriatic lagoons, including Venice lagoon, Marano-Grado lagoon, and Po Delta lagoons, are currently experiencing rising salinity levels, as reported by the National Climate Change Adaptation Platform. We investigated the correlations between water parameters and macrozoobenthic biodiversity in Venice lagoon using data from the long-term monitoring activities established under the Water Framework Directive, conducted annually by ARPAV in collaboration with

ISPRA since 2011. Macrozoobenthos refers to the assemblage of benthic invertebrates inhabiting soft sediments. These predominantly sedentary organisms respond sensitively to environmental conditions and integrate disturbance over time. Their distribution is influenced by abiotic factors such as salinity, sediment type and hydrodynamics, as well as biotic interactions like competition and predation. Due to their ecological roles and varying tolerance to stress, macrozoobenthos communities are widely used as bioindicators to assess the ecological quality of transitional and coastal waters. Trend analyses of key water parameters, including salinity, temperature, nutrients, and carbon compounds, revealed a significant increase in salinity and a concurrent decline in nutrient concentrations. Biological data showed a marked reduction in both species' richness and abundance of macrozoobenthos, indicating a progressive degradation of community structure. A temporal decline in the differentiation of macrozoobenthic assemblages along salinity gradients was also observed, suggesting a trend toward community homogenization. These findings underscore the importance of expanding continuous monitoring networks and advancing research across biological levels, as is already occurring in freshwater systems, in order to better understand and mitigate the complex impacts of salinization.

Biogenic selenium nanoparticles: new frontiers for food safety protection

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Biosynthesized Selenium Nanoparticles (bSeNPs) possess unique characteristics that make them excellent candidates for future agricultural applications that are under study within the "Biogenic nano-selenium fortified tomatoes: quality and safety in a green perspective" (Se4SAFE) project funded by PRIN PNRR 2022 (CUP: E53D23010580006), even though concerns about food safety and environmental impact must be carefully explored. Inorganic forms of selenium, such as selenite and selenate, can be reduced in acidic environments to SeO_3^{2-} and SeO_4^{2-} ,

respectively, or to elemental (zero-valent) selenium (Se) under more strongly reducing conditions. In recent years, several biological systems capable of bioconverting metalloids into less harmful forms have been tested. Among these, bacterial strains are among the most studied due to their metabolic versatility and environmental persistence. Some strains can produce bSeNPs via the bioconversion of toxic selenium oxyanions (like selenite and selenate) in an eco-friendly "green synthesis" process. This process not only reduces selenium toxicity but also produces nanoparticles with an organic coating, making them attractive for agro-nanotechnological applications. Organic selenium found in biofortified plants is more bioavailable to humans than its inorganic counterpart. Therefore, agronomic biofortification through fertilization with organic selenium is considered an effective method to produce selenium-rich crops, thereby improving selenium intake in target populations, especially when fresh products are consumed. However, data on bSeNPs remain scarce regarding both aquatic and terrestrial ecosystems, as well as human health. The simultaneous use of *Bacillus mycoides* SeITE01 and *Stenotrophomonas maltophilia* SeITE02 in a dedicated bioreactor enabled microbial bioconversion of SeO_3^{2-} into Se^0 . This transformation was confirmed by a color change in the culture medium from pale yellow to red, typical of colloidal elemental selenium. A toxicity comparison was conducted between Na_2SeO_4 and bSeNPs, using *Daphnia magna* and *Raphidocelis subcapitata* as test organisms. EC50 values were only measurable for sodium selenate treatment. The toxicity assessment of sodium selenate revealed EC50 values of 0.92 mg/l for *D. magna* (after 48 h) and 0.25 mg/l for *R. subcapitata* (after 72 h). In contrast, bSeNPs showed no toxicity in the same bioindicators, although they did induce morphological changes in the microcrustaceans and altered algal spatial distribution, warranting further investigation. Microbial bioconversion reduces selenium toxicity, supporting its use in bioremediation. Furthermore, bSeNPs provide agro-environmental benefits, including selenium biofortification in crops, reduced health and environmental risks, enhanced plant stress resistance, and improved post-harvest shelf life.

Micro- and Nanoplastics induce dysregulation in human monocytes: insights into autophagy and inflammation

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The presence of micro- and nanoplastics (MNPs) in the environment has become a growing environmental and health concern due to their widespread presence and potential biological impact. Chronic exposure to MNPs in humans occurs via ingestion, inhalation, and dermal contact, and there is a growing body of evidence linking it to inflammation, immune dysregulation, and an increased risk of chronic and autoimmune diseases. However, the molecular and cellular mechanisms underlying these effects remain to be fully elucidated. In this study, we evaluated the impact of both polystyrene (PS) MNPs and UV-aged PS-MNPs of different sizes (0.5, 1, 5 μm) on freshly isolated human monocytes. Overall, the focus was to examine their effects on key immunological and cellular pathways, including inflammation, endocytosis, mitochondrial function, and the autophagy-lysosomal system. To define appropriate experimental concentrations, IC₅₀ values were calculated based on viability assays. Subsequent functional assays revealed a size- and dose-dependent reduction in cell viability and an increase in apoptosis following MNPs exposure. MNPs internalization in human monocytes was assessed using transmission electron microscopy (TEM), flow cytometry, and confocal microscopy. Our results demonstrated that monocytes internalize MNPs via endocytosis, resulting in their accumulation within lysosomes. This internalization process led to the formation of a multilamellar morphology, accompanied by a substantial accumulation of autophagosome. Gene and protein expression related to the autophagy-lysosomal pathway, mitochondrial damage, and inflammation were examined using RT-qPCR and western blot, respectively. Our results highlighted upregulation of key markers involved in autophagy (LC3), lysosomal function (LAMP1), cellular activation (CD80, HLA-DR11), inflammation (TNF α ,

IL-8) and altered expression of genes involved in mitochondrial fission and recycling. Furthermore, we observed a concomitant downregulation of HLA-A, NLRP3, and CASP1, suggesting a complex modulation of antigen presentation and inflammasome activity. Notably, UV-aged MNPs triggered more intense inflammatory and cytotoxic responses than pristine particles. Our findings provide novel insights into the immunological and intracellular impact of MNPs on human monocytes. MNPs impair immune cell function by disrupting mitochondrial and lysosomal integrity, inducing autophagy, and activating inflammatory pathways. Notably, UV-aged MNPs exert more pronounced cytotoxic and pro-inflammatory effects compared to pristine particles, suggesting that environmental degradation enhances their immunotoxic potential. These findings highlight the potential health risks associated with environmental MNPs, especially aged forms, and stress the importance of further research into their long-term effects on human health.

Tiny crustaceans, big warnings: antibiotic stress in a changing climate

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Pharmaceuticals, particularly antibiotics, are increasingly recognized as emerging contaminants in aquatic ecosystems. While their acute toxicity is well-documented, chronic sublethal effects at environmentally relevant concentrations remain poorly understood. Moreover, climate change may intensify the ecological risks posed by these compounds by altering their fate, bioavailability, and toxicity. This study investigates the chronic effects of two antibiotics, chloramphenicol (CAP) and vancomycin (VAN), on the freshwater cladoceran *Daphnia magna* under different temperature regimes. A 21-day chronic toxicity assay was conducted using *D. magna* exposed to environmentally relevant (ER) and acute EC₁₀ concentrations of CAP and VAN at two temperatures: 20 °C (standard) and 25 °C (simulating moderate warming). Acute 48-hour EC₁₀ values were determined as 56.99 mg/l for CAP and 41.92 mg/l for VAN. ER concentrations used were 6.00E-5 mg/l for CAP and 4.00E-4 mg/l for VAN. Test organisms were monitored for survival, reproduction (total neonates, time to first brood),

and growth (body length at days 7, 14, and 21). Preliminary results for CAP indicate temperature-dependent sublethal effects. At 20°C, ER exposure slightly increased reproduction and body size compared to controls, suggesting a possible hormetic response. However, EC₁₀ exposure reduced maturity rates while maintaining moderate neonate output. At 25°C, reproductive output and body size declined in controls, while ER exposure sustained reproduction and increased body size. Interestingly, EC₁₀-exposed individuals showed higher maturity but did not display enhanced reproduction or growth. These findings suggest that thermal stress may interact with low-dose antibiotic exposure to alter energy allocation strategies in *D. magna*, potentially reflecting compensatory or hormetic effects. Although survival remained largely unaffected, the observed changes in reproduction and growth underscore the need to consider temperature as a key factor in chronic ecotoxicological assessments. These results emphasize that warming can amplify or modulate the impacts of contaminants that may otherwise appear low-risk under standard testing conditions. Further analysis, including data for vancomycin, is ongoing to better understand the joint effects of temperature and pharmaceutical exposure. This study contributes to bridging knowledge gaps in chronic ecotoxicology and supports more realistic environmental risk assessments under global change scenarios.

Possible Integrations of REACH Regulation and Life Cycle Assessment Methodology

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The European Union's Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation and the Life Cycle Assessment (LCA) framework, despite their distinct primary objectives and regulatory statuses, offer significant potential for integration. Both systems fundamentally aim to minimize negative environmental impacts and protect human health from chemicals. A key avenue for integration involves leveraging the data generated through REACH for use in LCA. REACH implementation is anticipated to greatly increase the availability of

chemical data, including physicochemical properties, environmental fate, and experimental (eco)toxicity data. This data is crucial for strengthening (eco)toxicity assessments within the LCA framework, particularly in Life Cycle Impact Assessment (LCIA), where data availability and quality have been persistent challenges. Methodological guidelines and databases developed under REACH can potentially support data collection and help address data gaps in LCA studies. Furthermore, the underlying theory and calculation methods for (eco)toxic impacts show considerable alignment, facilitating data exchange. Conversely, LCA tools and life cycle thinking can inform and enhance the integration of environmental considerations within REACH processes. LCA tools can be used to enhance the environmental assessments required by REACH. Although less explored, existing ECHA guidance for Authorisation mentions LCA as a potential assessment tool, suggesting possibilities for using LCA-based arguments to support authorisation applications. LCA's ability to provide a holistic view across the entire life cycle, including broader environmental consequences of alternatives or product-level changes, can complement the substance-centric focus of REACH. Furthermore, specific approaches are being developed to integrate REACH-related aspects into the LCA methodology. Exploiting these mutual dependencies and potential synergies is essential for developing more comprehensive environmental assessment strategies and promoting sustainable chemical management and product development. More details regarding both methodological approaches, synergies and future perspectives of the topic will be presented at the conference.

Pesticides in surface and groundwater - Italian monitoring data 2021

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The 2021 monitoring of pesticides in waters involved 4,864 monitoring points and 16,273 samples. Pesticides were found in surface waters in 55.6% of the 1,997 monitoring points, in groundwater in 23.2% of the 2,867 points. 412 substances were searched and 208 were found.

Monitoring of pesticide provides information on water quality and environmental risks that were not foreseen in the authorization phase. Although the EU has one of the most comprehensive regulatory frameworks on pesticides, the overall result indicates a widespread presence of pesticides. The 2021 monitoring results are presented in terms of the detection frequency and concentrations distribution, where the mean measured concentrations are compared with the Environmental Quality Standards fixed by European and national legislations to obtain levels of contamination. The frequency of pesticides overall increased in the last ten years of the survey, in a manner related to the extension of the monitoring points and the number of searched substances. The interpretation of the monitoring data is in fact strongly influenced by the effectiveness with which the monitoring was performed, bringing out a contamination not detected in the past. The increase of the detection frequency was more pronounced for surface water than groundwater where hydrological dynamics are very slow. The measured concentrations were generally fractions of $\mu\text{g/l}$ (parts per billion), but the harmful effects of substances can also occur at very low concentrations. Moreover, pesticide residues were detected in deep aquifers, which are less exposed to percolation. ISPRA has developed the information system for pesticide monitoring (SIMP). The system stems from the need for a useful tool for the acquisition, processing and dissemination of information on national pesticide monitoring in inland waters, supplied annually by the Regions/Autonomous Provinces. The Regions/Autonomous Provinces enter the results of their monitoring activities for the previous year in a data sheet (in Excel format). The sheet contains information on the master data of the monitoring stations and information on the analytical determinations carried out. ISPRA processes and evaluates monitoring data. The SIMP operates through the following phases: checking incoming data and filing them in the database; consultation, modification and updating of data in the database; georeferencing of the monitoring points; production of statistical outputs (tables, graphs) and contamination maps; publication of processing results on the Institute's Pesticide Portal. The Pesticide Portal is an open tool for the public to consult information on the national monitoring of

pesticides in surface and groundwater:
<https://sinacloud.isprambiente.it/portal/apps/sites/#/portalepesticidi>

Best Young Italian Scientist Award



1st classified

Linking behavioural bioassays and soil biodiversity as an integrated strategy for environmental quality assessment

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Soil health represents a key component for achieving the European Union's climate neutrality and biodiversity restoration goals. With over 60% of European soils degraded, effective monitoring and prioritisation are essential to meet EU climate and biodiversity targets. Changes in edaphic communities provide an early indication of soil health and ecosystem functioning. However, biodiversity analyses are primarily diagnostic and demand substantial time and costs. Therefore, it is crucial to implement rapid and cost-effective prognostic strategies to effectively support these studies. Behavioural bioassays of soil organisms offer ecologically relevant early-warning indicators of altered soil conditions. In particular, avoidance responses and changes in gregarious behaviour in social edaphic species can signal population decline and habitat fragmentation. Avoidance responses are expressed as active migration from stressed soils, while disaggregation effects reflect a fragmentation of the gregarious species, suggesting an infochemical disruption. Consequently, soils that elicit pronounced avoidance or altered social behaviours are likely to exhibit reduced local diversity, since sensitive species will disperse away from stressed soils. As recently highlighted by the European Commission, metrics of biodiversity loss remain not aligned with laboratory-based ecotoxicological endpoints. To address this gap, this pilot study evaluates the potential of behavioural bioassays as screening tools to assess the biodiversity of invertebrate and bacterial communities in three urban soils subjected to different levels of anthropogenic pressure. Multispecies bioassays were conducted using model organisms with contrasting morpho-ecological traits, including soft-bodied organisms (*Eisenia fetida*) and hard-bodied organisms (*Folsomia candida* and *Porcellionides pruinosus*). Behavioural test outcomes were compared with ecological indices on pedofaunal and microbial biodiversity to verify the consistency between behavioural responses and the structure and functionality of biological communities. The behavioural responses of the model organisms showed a consistent association with reductions in soil invertebrate biodiversity. Soils inducing avoidance or social disaggregation, particularly in terrestrial isopods, were characterised by lower taxonomic richness and communities dominated by generalist and tolerant species. In contrast, soils that did not elicit behavioural alterations exhibited higher edaphic diversity and a greater presence of taxa sensitive to environmental changes. Conversely, analyses of bacterial communities did not reveal significant differences in alpha-diversity indices among sites, suggesting that microbial diversity may respond differently or be less sensitive than edaphic fauna to local-scale functional habitat alterations.

These findings support the use of behavioural bioassays, alongside faunal diversity analyses, as an effective first-tier tool for soil health assessment. This multilevel approach bridges ecotoxicology and biodiversity, improving the efficiency of soil monitoring programs and informing targeted management in urban, peri-urban, agricultural, and other anthropogenically impacted landscapes.

Original article: <https://doi.org/10.1007/s11252-026-01908-6>



2nd classified

Optimization of the Neuro-2a assay to detect the marine toxin tetrodotoxin

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Tetrodotoxin (TTX) is a potent marine neurotoxin involved in cases of foodborne fish poisoning, characterized by severe neurological symptoms that can even lead to death. Originally identified as a natural contaminant of pufferfish from the Tetraodontidae family, TTX and its analogs have since been detected in other edible marine organisms, including some Pacific bivalve molluscs and, in recent years, also in the Adriatic Sea and along the coasts of other Mediterranean countries. The presence of the toxin in a wide variety of organisms from several European countries has raised concerns about food safety due to the lack of regulations on the maximum allowable levels in marine foods within the European Union (EU) and, consequently, the absence of an adequate monitoring program. For these reasons, there is a growing need for rapid and sensitive methods for detecting these toxins. Therefore, the aim of this study is to optimize the functional Neuro-2a assay, an *in vitro* assay on mouse neuroblastoma cell line Neuro-2a, for the detection of TTX in molluscs, based on the toxin's mechanism of action: the blockade of voltage-gated sodium channels (VGSC). The assay allows the detection of the toxin due to its ability to inhibit the Na-dependent cytotoxicity induced by the combination of veratridine, a VGSC activator, and ouabain, Na⁺/K⁺ ATPase inhibitor. The assay was optimized considering the following experimental parameters: (i) cell seeding density; (ii) type of medium culture and time of culture; (iii) exposure of cells to toxin; (iii) optimal ratio between concentration of ouabain and veratridine; (iv) cell viability assessment (MTT test). Once the assay procedure was optimized to obtain a better response to TTX, the assay was characterized according to Eurachem guidelines. In particular, the linearity range of the calibration curve (0.41-33 ng/ml), the limit of detection (LOD) of 0.134 ng/ml and the limit of quantification (LOQ) of 0.447 ng/ml were defined with good test repeatability, intra- and inter-day. The assay was also able to detect saxitoxin, which shares the mechanism of action with TTX, while sensitivity to the structural analogue 4,9-anhydrous-TTX was lower. In conclusion, the results of this study represent a first step toward the standardization of a functional assay, useful as a screening method for quantifying TTX in molluscs intended for food use.



3rd classified

Do environmental concentrations of rare earth elements pose a risk to mussel embryos?

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Introduction

Over the years, ecotoxicology has paid significant attention to emerging contaminants (ECs), such as rare earth elements (REEs), due to their potential risks to human and ecosystem health. However, currently these pollutants are not fully monitored and regulated in environmental and public health programs [1].

Among the 17 REEs, lanthanum (La), gadolinium (Gd) and lutetium (Lu) are especially important due to their wide use in technology and medicine [2]. La is used in the hybrid components for vehicles production, Gd serves as a contrast agent in magnetic resonance imaging and Lu is used in scintillators for advanced medical imaging.

Even as these applications are considered essential for human health and modern technologies, they also contribute to the release of REEs into aquatic environments. Once in the marine environment, these elements can stay for a long time and come into contact with living organisms. But it still knows little about their environmental behavior, bioaccumulation, and toxicity, particularly when they occur in mixtures [3].

Materials and methods

Embryotoxicity tests were performed on *Mytilus galloprovincialis* embryos following ASTM E724-21 guidelines. Embryos were exposed under static conditions for 48 hours in filtered seawater.

La, Gd, and Lu were tested individually, in all possible binary combinations, and as a ternary mixture. Three nominal concentrations were selected based on literature data: environmental (La: 20 ng/l, Gd: 2 ng/l, Lu: 0.2 ng/l), and 10- and 100-fold higher, to simulate both low-impact and high-risk scenarios.

After the exposure, embryos were fixed and examined using light microscopy. The percentage of abnormal larvae was recorded, and

developmental malformations were categorized into specific types based on morphological criteria, allowing for a qualitative and quantitative assessment of toxic effects.

Results and Discussion

Embryotoxicity

All the elements individually induced a considerable toxic effect, especially at the lowest tested concentrations (Fig. 1a). Specifically, La and Lu at 1x caused the highest incidence of abnormalities, affecting approximately 90% of embryos. Gd also induced a high percentage of malformations at 1x (76%), whereas its toxicity decreased markedly at 100x, suggesting a non-linear dose-response relationship.

Binary mixtures (Fig. 1B) showed lower toxicity than the individual elements, assuming potential antagonistic interactions. However, the La+Lu mix at 100x led to 100% malformed embryos, pointing to a possible synergistic effect at high doses.

Notably, the La+Gd mix showed a clear dose-response trend, going from 0% malformed embryos at 1x to nearly 90% at 100x.

Ternary mixture (Fig. 1C) had an interesting pattern: the 10x concentration caused the most damage (almost 100%), while 1x and 100x had lower effects (39% and 46%), again suggesting a non-linear response.

Embriotoxicity malformations

When looking at the types of malformations, no specific type was clearly linked to a particular element or concentration. However, type F malformations, where development stops early, were the most common, especially under the most toxic conditions, such as La 1x, Lu 1x, and the La+Lu 100x mix (Fig. 1A–B).

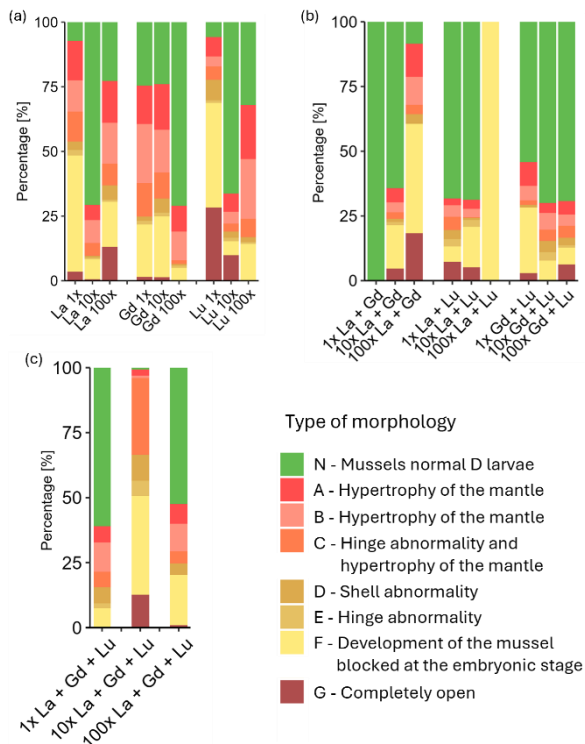


Figure 1. Embryotoxicity and malformation profiles in *M. galloprovincialis* embryos after 48-hour exposure to La, Gd, and Lu, at 1x (environmental), 10x, and 100x concentrations, individually (a) and in binary (b) and ternary (c) mixtures. The bar charts show the percentage of normal (green) and malformed embryos, with malformation types indicated by colour and described in the legend (A–G).

One striking anomaly appeared in Lu 1x: about 30% of embryos were completely open, a severe and unusual effect. This was also seen occasionally in other treatments, but not in a regular pattern. In the ternary mixture at 10x (Fig. 1C), many embryos showed both type F and type A malformations, the latter being mantle hypertrophy. This could be due to rare earth elements acting like calcium and interfering with calcium-related developmental processes. This might happen because rare earth elements can behave like calcium and disrupt the processes that depend on it during development [3,4].

These results show that even very low levels of REEs can strongly affect embryonic development and cause a range of malformations. The complex effects of mixtures also show how important it is to study how these elements interact in order to better understand their potential ecological risks.

Conclusions

This study shows that rare earth elements, even in very small amounts, can strongly affect the early development of marine invertebrates. The toxicity seen in mixtures highlights how important it is to study their combined effects. Future research should focus on understanding how these elements work and help develop rules to limit their release into aquatic environments.

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How a short-term exposure to polystyrene microplastic beads impair gonadal condition in the mussel *Mytilus galloprovincialis*: histological and molecular evidence

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Introduction

A growing environmental concern is the excessive use of plastics, whose degradation leads to the creation of microscopic particles known as microplastics (MPs) [1]. The small size (<1mm) facilitates their accumulation, and now they are found in sea and fresh water, soil and even the air we breathe. In aquatic environments, they can be ingested by a wide range of organisms, from plankton to large marine mammals, thus entering the food chain and potentially reaching humans. The effects of MPs can be effectively studied using the mussel *Mytilus galloprovincialis*, an excellent bioindicator of marine pollution. This sessile filter feeder plays a crucial role in the trophic chain.

Materials and methods

Mussels, after a 15-day acclimation period, were exposed for 48 h to 5 µm polystyrene MPs at concentrations of 0.5 µg/ml or 1 µg/ml. Then, the animals were opened, and the gills and mantles were collected and processed for subsequent analyses. Mallory Trichrome staining was used to assess the general tissue morphology. PAS and Picrosirius Red stains were also applied to detect any changes in mucopolysaccharides and collagen fiber deposition, respectively [2]. Biochemical assays to estimate the redox state and Western blot to determine the level of HSP70 protein were also performed on the mantles.

Results and discussion

Histological analyses

In the gills of treated mussels, polarized light microscopy revealed the presence of microplastics, precisely where mechanical damage to the gill epithelium was detected (Fig. 1A). In mantles, male or female gonads were identified; both gonads exhibited morphological disorganization (Fig. 1B,C). In particular, the presence of empty spaces between germ cells increased proportionally to the exposure dose,

accompanied by hemolymphatic infiltrates in the connective tissue. In male gonads, a dose-dependent germ cell disorganization in sperm cysts was observed (Fig. 1B). In contrast, female gonads showed an increasing number of degenerated vitellogenic oocytes, correlated with the highest MPs dose (Fig. 1C). PAS-positive cells, lipofuscin granules and collagen deposition, index of fibrosis, were abundant in MPs-exposed gonads.

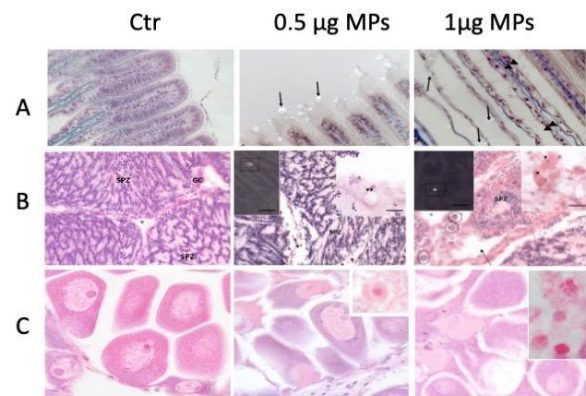


Figure 1. A) Gills. Interruption or disruption of the thickened gill epithelium is evident in the areas of contact with the MPs. Colourless MPs aggregates are visible (arrows). B) Testis. Control, intact spermatic cysts, rich in germ cells (GC) and spermatozoa (SPZ), are visible; in MP-treated animals, empty areas between the germinal cells (arrow), disorganized spermatic cysts, and haemolymph cells (arrowheads) are evident. MPs aggregates are visible under polarized light (insets). C) Ovary. Control, follicles at various stages of differentiation are observed; in MP-treated animals, disorganized follicles and degenerated vitellogenic oocytes are visible, along with infiltrates of haemolymph cells (insets).

Oxidative stress

MPs exposure induced an increase in lipid peroxidation (LPO), a hallmark of early oxidative damage, and the upregulation of antioxidant proteins. Catalase activity significantly increased at both MPs exposures; Glutathione S-transferase (GST) activity showed an increased trend at the highest MPs concentration. These results suggest increased detoxification of hydrogen peroxide and an enhanced cellular defence mechanism against oxidative stress. Interestingly, neither of the of

polystyrene MPs concentrations affected the total glutathione (TG) content (GSH + GSSG) (Fig. 2).

HSP70 protein levels

Western blot analysis showed a significantly higher protein level in MPs-exposed mantles; the increase in HSP70 protein was dose-dependent (Fig. 3).

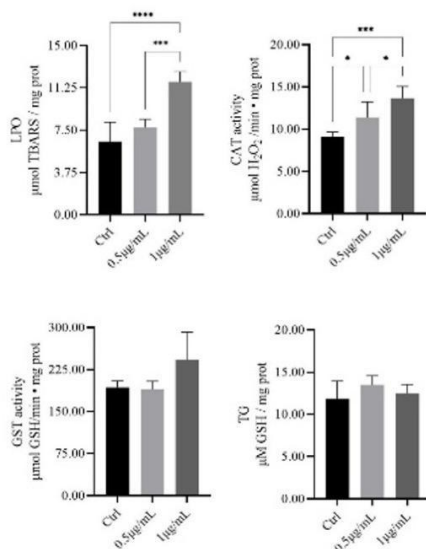


Figure 2. Redox state of *M. galloprovincialis* mantles. Each value is represented as mean \pm SEM (n=6). * $p < 0.05$; *** $p < 0.001$; **** $p < 0.0001$

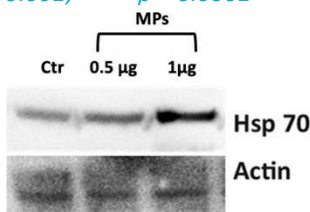


Figure 3. Western blot on mantle protein extracts, stained with anti-HSP70 and anti-actin. Densitometric analysis showed one-fold (0.5 $\mu\text{g}/\text{ml}$) and five-fold increase (1 $\mu\text{g}/\text{ml}$) in HSP70 in MP-treated animals.

Conclusions

The findings presented in this study demonstrate that 0.5 μm MPs can penetrate the gills of *M. galloprovincialis* and reach internal organs, such as the mantles, which contain the reproductive tissues. Disorganization of gonads and germ cells, induction of cell stress, as well as oxidative stress, and a general pro-inflammatory state have been observed. Microplastic pollution has a profound impact on marine ecosystems, contributing to a widespread loss of biodiversity. These results further support the validity of *M. galloprovincialis* as a valuable model organism for evaluating the effects of exposure to marine pollutants and

assessing reproductive risk. Reduced reproductive capacity in individuals, caused by a non-physiological arrangement of germ cells, can threaten the survival of the species.

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The effect of post-biotics-enriched diet on the antioxidant capacity of *Mytilus galloprovincialis* exposed to Cu₂O

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Introduction

Marine organisms are increasingly exposed to pollutants like heavy metals, which cause oxidative stress by generating reactive oxygen species (ROS) [1; 2]. Copper, although essential, is toxic in excess and often enters marine environments via agricultural and industrial runoff [3]. *Mytilus galloprovincialis* are effective bioindicators due to their filter-feeding and pollutant-accumulating abilities [4].

Postbiotics, non-living microbial byproducts, have demonstrated benefits in gut health, immunity, and antioxidant defence [5; 6]. However, their role in reducing heavy metal induced stress in marine bivalves remains

understudied. This research explores whether a diet enriched with postbiotics from homemade kefir can mitigate Cu₂O toxicity in mussels.

Materials and methods

Cu₂O nanoparticles (~80 nm) were characterized via DLS and TEM and shown to agglomerate in seawater, releasing negligible ionic Cu²⁺. A cell-free supernatant (CFS) was prepared from co-cultures of *L. lactis subsp. hordniae*, *L. lactis subsp. lactis*, and *P. kudriavzevii*, and demonstrated moderate antioxidant activity in DPPH and ABTS assays [7; 8]. Mussels were divided into four groups:

- G1⁻: algae only, unexposed
- G1⁺: algae only, Cu₂O-exposed
- G2⁻: algae + CFS, unexposed
- G2⁺: algae + CFS, Cu₂O-exposed

After 15 days of feeding and 3 days of Cu₂O exposure, various physiological, biochemical, and molecular markers were analyzed, including antioxidant enzyme activities and gene expression (CAT, SOD, GST, HSP70, HSP90).

Results and discussion

Nanoparticle Characterization

Cu₂O formed aggregates in seawater and remained largely in particulate form, releasing only trace amounts of ionic Cu [9].

Antioxidant Activity

CFS showed DPPH scavenging = 34.5% and ABTS⁺ = 52.2%, confirming antioxidant potential.

Biometrics and Condition Index (CI)

No significant changes in shell dimensions. CI improved in CFS-fed mussels (G2⁻). Cu₂O exposure decreased CI in G1⁺ but not in G2⁺, indicating a protective effect of postbiotics (Fig.1 A).

Haemocyte Count and Viability

Cu₂O exposure increased THC and reduced haemocyte viability, especially in G1⁺. G2⁺ showed milder immune activation and less cytotoxicity [10].

Enzyme Activities (CAT, GR, GST)

CAT increased in G1⁺, not in G2⁺ (suggesting reduced stress in G2⁺).

GR decreased in G1⁺ but remained stable in G2⁺. GST decreased in both G1⁺ and G2⁺, but less in G2⁺ (Fig. 1 B-C-D)

Gene Expression

CAT, SOD, GST: Upregulated in G1⁺; stable or downregulated in G2⁺.

HSP70, HSP90: Strongly upregulated in G1⁺; mildly increased in G2⁺.

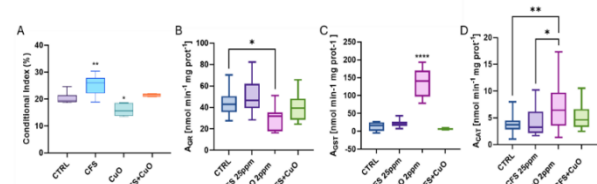


Fig. 1 Conditional Index (A), GR Activity (B), GST Activity (C), CAT Activity (D) in *M. galloprovincialis* exposed to CuO that received or not CFS-MIX as supplement at diet.

Conclusions

The results confirm that Cu₂O exposure induces oxidative stress and immune activation in mussels. Postbiotic supplementation reduced oxidative damage and immune overactivation. This is attributed to the antioxidant and

immunomodulatory properties of microbial metabolites such as SCFAs, proteins, and cell wall fragments [11; 6]

The modulation of key antioxidant enzymes (CAT, GR, GST) and heat shock proteins supports the hypothesis that postbiotics enhance cellular resilience under metal stress [12].

This study adds to the growing evidence supporting postbiotics as promising feed additives in aquaculture for improving stress tolerance and overall health in shellfish [13]. Kefir-derived postbiotics enhanced antioxidant defences and reduced Cu₂O-induced stress in *M. galloprovincialis*. Their inclusion in aquaculture diets could help mitigate environmental pollutant effects and support mussel health.

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Trace elements in the brain of the Rabbitfish (*Chimaera monstrosa*, Linnaeus, 1758) from the Strait of Sicily

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Trace elements (TEs) represent a problem affecting deep-sea marine ecosystems, which are considered the final sink for TEs. Evaluating the accumulation, distribution, and trophic transfer of TEs from natural and anthropogenic sources in this region is essential. Among the organisms living in a deep-sea environment, less attention has been paid to elasmobranchs despite their biological and ecological importance. This study monitored levels of TEs (As, Co, Cr, Cu, Fe, Ni, Pb, Hg, Se, Zn, Cd) in 21 rabbitfish (*Chimaera monstrosa*, Linnaeus, 1758) brains. In 2023, brains were collected through the MEDITS survey in the Sicily Channel area and analysed for TEs quantification with the ICP-OES technique. Sex, weight, and length were considered for statistical analysis, highlighting sex-related differences for Fe, Ni, Hg, and Se levels, with Fe and Se being higher in males and Ni and Hg in females. Also, total length is negatively correlated with Co, Cr, Ni, Pb, Hg, and Cd. To our knowledge, this is the first study evaluating TEs in the brain of rabbitfish. Our results highlight what seems to be a sex difference in TEs accumulation in the species and an apparent protective mechanism in the brain toward TEs (and Hg in particular) cerebral toxicity. These findings underscore the need for further research, particularly in understanding and potentially replicating this protective mechanism in other species, which could have significant implications for public health. Additional laboratory studies are necessary to evaluate protective biochemical mechanisms, if any, in the brains of elasmobranchs following exposure to TEs.

Implementing safe and sustainable-by-design strategy in the development of TiO₂@SS for cosmetic application

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Introduction

The safety and sustainability aspects of advanced materials (AdMa) are critical considerations throughout their lifecycle, from development to end-of-life. As engineered nanomaterials (NMs), AdMa are intentionally produced to possess unique properties that enable innovations across various industries, such as electronics, healthcare, construction, and energy. However, the innovative nature of AdMa underscores the necessity of conducting risk assessment even in the early stages. In this context, the European Commission "Safe and Sustainable by Design" (SSbD) framework [1] for chemicals and materials provides a valuable approach to guide the development of AdMa throughout the value chain, aiming to minimize their potential risks to human health and the environment.

Specifically, the first three steps of the SSbD framework address different safety aspects: I) hazard assessment based on intrinsic properties; II) occupational health and safety (including exposure) during the production and processing phases; III) exposure during the final application phase. Together, these steps form the Safety Dimension and allow to obtain valuable information for AdMa risk assessment at very early stages of the innovation process. This will primarily involve extrapolating all available information from the literature concerning similar materials and/or their individual components to facilitate a preliminary safety evaluation of specific AdMa. In this context, a representative advanced multi-component nanomaterial was selected as a case study within the framework of the EU-funded SUNRISE project. The aim was to support the implementation of the first three safety steps [2] of the SSbD framework by applying different characterization procedures.

Materials and methods

The case study examined consists of TiO₂ nanoparticles (P25) functionalized with sodium surfactin (SS), a cyclic heptapeptide obtained through natural fermentation of agribusiness by products. This AdMa could be used as inorganic UV filter in sunscreens due to its nano-sized properties and high surface area, which is reported to improve the photocatalytic activity as well as its capability of absorbing UV rays, thus preventing skin damage. As part of the SSbD strategy, the surface functionalization of P25 with a natural, non-toxic macromolecule was performed in order to mitigate its potential risks related to inhalation exposure (production phase) and toxicity to the marine ecosystem (use phase) [3].

To support the implementation of the Safety Dimension steps, analytical techniques such as Attenuated Total Reflectance - Fourier Transform Infrared Spectroscopy (ATR-FTIR), Brunauer–Emmett–Teller Surface Area Analysis (BET), Dynamic Light Scattering (DLS), Electrophoretic Light Scattering (ELS), and Centrifugal Separation Analysis technique (CSA) were selected to evaluate structural integrity, surface area, colloidal stability, and sedimentation behaviour in different aqueous media. Ecotoxicological assessments were performed using OECD TG 201 (freshwater algae test) and OECD TG 236 (zebrafish embryo toxicity test). Photoprotective efficacy was evaluated through in vitro SPF analysis following ISO 24443:2012 using spectrophotometric measurements and a solar simulator.

Results and discussion

Physicochemical characterization confirmed the structural stability of the functionalized nanoparticles post-sonication, showing no signs of chemical degradation. Specifically, ATR-FTIR

analysis revealed that sodium surfactin (SS) remained stably attached to the P25 surface after 5 minutes of probe sonication at 100 W in ultrapure water (UPW) (Fig. 1). It is well known that a high surface area of advanced materials (AdMa) can influence their (eco)toxicity, due to the increased number of potentially reactive sites available for interactions with cells. BET analysis showed moderate specific surface areas ($48.358 \pm 0.718 \text{ m}^2/\text{g}$) and pore volumes ($0.094 \text{ cm}^3/\text{g}$) across the samples.

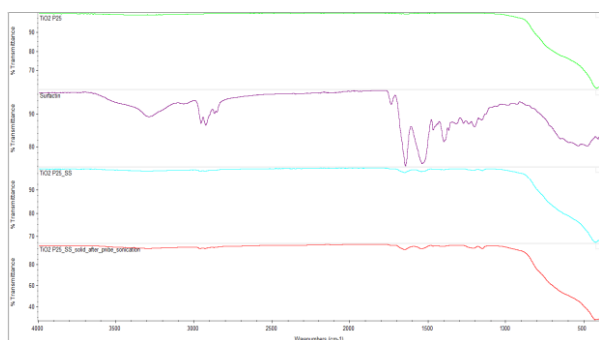


Figure 1 – ATR-FTIR spectra of P25, sodium surfactin, TiO₂@SS pristine, solid after probe sonication and supernatant after probe sonication

DLS, ELS, and CSA analyses indicated a decrease in colloidal stability in artificial freshwater (AFW) and artificial marine water (AMW), as expected due to the increasing ionic strength. Specifically, higher hydrodynamic diameters were observed when moving from ultrapure water (UPW) to AMW, with values ranging from 429 nm in UPW to 2023 nm in AMW. A reduction in the absolute value of the zeta potential was also observed, suggesting a compression of the electric double layer caused by the higher ionic strength of the media.

Sedimentation velocity measurements confirmed this trend, showing increased particle destabilization with increasing ionic strength: $0.03 \mu\text{m}/\text{s}$ in UPW and $0.27 \mu\text{m}/\text{s}$ in AMW.

As far as the ecotoxicity results is concerned, P25@SS showed significantly reduced toxicity toward algae (EC₅₀ increased from 1.4 mg/l to 18 mg/l), indicating successful toxicity mitigation through functionalization. Zebrafish embryo assays revealed no acute toxicity for either functionalized or unmodified P25 at tested concentrations. Photoprotection studies demonstrated enhanced SPF for the functionalized P25 (SPF = 8.52) compared to pristine P25 (SPF = 6.45), suggesting that the SS coating improved the dispersion of the material in the sun cream and its efficacy, without

compromising safety. These findings underline the effectiveness of the SSbD approach in improving material performance while reducing environmental impact.

Conclusions

Through this stepwise approach, our study contributes to provide useful information on the AdMa risk profile already in the early design stage and to bridge existing knowledge gaps for the implementation of regulatory policies on AdMa.

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Posters

1st classified



Hazards of microplastics to the marine ecosystem: BIOPLAST4SAFE project remarks

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The project BIOPLAST4SAFE, titled "Biomonitoring of biodegradable micro and nanoplastics: from the environment to humans in a one health perspective", promotes the study of characteristics, fate and potential (eco)toxicological effects of plastics, with a specific focus on biodegradable polymers as a potential EU strategy for mitigating plastic pollution. Within this project the feedback from different marine organisms to the plastic threat, mainly microplastics, has been explored. These synthetic organic polymer particles differing in size, shape, surface texture, chemical composition (polymers and additives) and specific density may affect the exposed biota in a variety of ways (e.g., reduced food uptake, lower energy reserves, affected physiological functions, effects at organism and population levels). In addition, microplastics affect the surrounding environment, being vectors of chemical and biological agents transferring pollutants, invasive species and pathogens in the environment. In this contest, biological effects of microplastics obtained by fragmentation of biodegradable (e.g., polylactic acid - PLA) and non-biodegradable (e.g., polypropylene - PP) products were studied using a battery of marine species, from different taxonomic and trophic levels. Here we review and compare the first results related to the ecotoxicological effects of PP and PLA, calculating Effect Concentrations (ECs), while Predicted No Effect Concentrations (PNECs) values were derived from the EC50s, according to the ECHA guideline (2008). Some sublethal and lethal effects were observed in representative organisms of the food web as producers, consumers and decomposers, in acute and chronic exposures.

Furthermore, PLA and PP showed similar ecotoxicity, speculating about the effectiveness of biodegradable polymers in mitigating plastic pollution. In conclusion, further microplastic ecotoxicity assessment are needed, preferably including at least three trophic levels, to support an Environmental Risk Assessment (ERA) procedure of microplastics.

2nd classified



Integrating molecular, histopathological and immunohistochemical responses of the sentinel organism *Mullus barbatus* to evaluate the environmental status

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The monitoring of environmental quality is deemed a priority. For a proper risk assessment, the type of contaminants and the possible effects associated with them need to be considered. The Mediterranean Sea is a highly polluted ecosystem with organic and inorganic contamination originating from anthropogenic activities. To safeguard the environmental status of the sea, the European Union has implemented the Water Framework Directive (WFD; 2000/60/EC) and the Marine Strategy Framework Directive (MSFD; 2008/56/EC) legislations which include the use of biological tools to detect the quality of aquatic systems. Many environmental risk assessment studies in coastal ecosystems and the Guidance of IMAP (Integrated Monitoring and Assessment Programme) of UNEP (United Nation Environment Programme) recommended the use of benthic fish species as sentinels, due to their ecological and economic importance, sensitivity and habitat close contact to sediment floors. In the context of the Italian Monitoring activity (2019-23) sensu MSFD, *Mullus barbatus* was selected as sentinel species, and a panel of well-established molecular biomarkers, such as micronuclei frequency in erythrocytes (MN), metallothioneins levels (MT), EROD assay and lysosomal membranes stability (LMS-HEXO), was used. In this study these analyses were integrated with histopathology and immunohistochemistry (MT and Cyp1 α). In October and November 2023 specimens of red mullet were sampled in four coastal sites (Roccella

Ionica, Crotone, Corigliano Calabro and S. Maria di Leuca) in the Ionian Sea. Molecular, histopathological and immunohistochemical analyses were performed on separate individuals within the same population/site. Preliminary results showed the presence of parasitosis in all sampled sites, and some alterations both at molecular (i.e. DNA damage and activation of the biotransformation system) and histopathological level in some sites. Immunohistochemistry of Cyp1 α showed an increased expression in livers of fish from contaminated sites respect to the control ones, while MT expression appeared to be not specific. These preliminary data suggested the suitability to use histopathological and immunohistochemical markers to integrate the molecular markers in the environmental quality assessment, despite the complexity of relationship between them.

3rd classified



Microplastics and human monocytes: effects on lysosomes, mitochondria and autophagy

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Micro- and nanoplastics (MNPs) are an emerging environmental concern due to their ubiquity and potential impacts on human health. Humans are exposed to MNPs via ingestion, inhalation, and skin contact. Recent studies have shown that MNPs can induce cellular stress by altering the autophagy-lysosomal system, meanwhile triggering inflammatory and cytotoxic responses, ultimately leading to cell death. Indeed, MNP-induced alterations at the level of the autophagy-lysosomal functions poses significant risks to cellular health by exacerbating chronic inflammation and cellular stress, thus contributing to the development of diseases like neurodegeneration, fibrosis, and metabolic disorders. However, little is known about the mechanisms of MNPs internalization and their effects on human cells, especially upon intracellular organelle dynamics and functions.

Here, we evaluated the impact of polystyrene (PS) MNPs of different sizes, such as 0.5, 1, 5 μ m, on freshly isolated human monocytes, with a focus on autophagy, lysosomes, and mitochondrial function. Transmission electron microscopy (TEM) was used to examine ultrastructural MNP localization and organelles. Gene and protein expression related to the autophagy-lysosomal pathway and mitochondrial damage were examined using RT-qPCR and western blot, respectively. The evaluation of the autophagy process was performed with MNPs concentrations calculated with IC50 curve. Our results showed that MNPs are internalized by monocytes through endocytosis and accumulate within lysosomes highlighting a multilamellar morphology, which went along with massive autophagosome accumulation. TEM imaging also revealed alterations in mitochondrial morphology, while oxygraphy indicated changes in the respiratory chain. Consistent with these observations, genes and proteins associated with autophagy (e.g., LC3) and lysosome formation (e.g., LAMP1) were upregulated. Furthermore, genes involved in mitochondrial fission and recycle were altered, suggesting changes in cellular respiration. In conclusion, this study provides novel insights into the cellular and molecular mechanisms of MNP internalization in human monocytes and highlights the potential damage caused by MNP exposure to immune cells. These findings contribute to understanding the long-term health risks associated with MNPs and underscore the need for further research on their impact on human health.

3rd classified



Can fishing method and time between death and tissue sample collection affect biomarker analysis? A case of study on fish *Mullus barbatus*

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Recently, biomarker analysis has been widely used in assessing environmental pollution effects. In carrying out biomarker analyses on cells or macromolecules such as proteins (enzymes) or DNA in marine organisms, it is necessary to consider the potential molecular degradation that may occur during the time between the organism

death and the tissue sample storage. Indeed, this temporary delay could significantly affect the integrity of macromolecules and, consequently, the reliability of biomarker analytical results. In such context, the UNEP (United Nation Environmental Programme) Guidelines on biomarker analyses suggest a specific protocol for fish capture and tissue collection: fish (*Mullus barbatus*) should be caught using a 15-minute trawl haul, performed at a slow towing speed (≤ 3 knots); organisms should be killed on board immediately after collection and rapidly dissected. However, field sampling often involves some practical challenges both in following specific fishing protocols and in performing rapid fish dissections on board. The main goals of the present study are 1) to assess the effect of different fishing protocols on biomarker results and 2) to define the maximum post-mortem time within which no significant alterations in biomarkers results are observed. For the first objective, biomarker analyses were performed on fish caught by trawl net using either the UNEP recommended sampling protocol or the routine commercial fishing one. The UNEP protocol involved a trawling duration of 15 minutes while the commercial one involved approximately 2 hours of trawling; both were performed at a towing speed of 3 knots, using the same gear. In both tested sampling protocols tissue samples collection was carried out immediately after fish death (within 1 hour from catch). For the second objective, fish dissection and tissue sample collection were performed at seven different post-mortem time intervals following the commercial fishing protocol: T0, T3, T6, T12, T18, T24 and T36 corresponding respectively to 1, 3, 6, 12, 18, 24 and 36 hours after the capture and death of the organisms. Biomarkers were those recommended by UNEP Guidelines, namely the acetylcholinesterase inhibition (AChE), the micronucleus assay (MN), the lysosomal membrane stability (LMS-ESO), the cytochrome p450 activity (EROD), and other routinary biomarkers used in environmental monitoring activities linked to oxidative stress. Each biomarker was statistically tested between the two different fishing protocols, and between T0 and the other post-mortem times at which fish dissection and tissue samples collection were performed.

Development of an integrated strategy for the ecotoxicological assessment of urban aquatic ecosystems affected by climate change

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The synergistic interaction between increasing urbanisation and climate change represents a critical driver of chemical pollution in urban aquatic ecosystems. Extreme weather events—particularly intense rainfall and droughts—promote the mobilisation and redistribution of contaminants, including emerging substances into surface waters. These dynamics compromise ecosystem integrity and exacerbate risks to public health. In the urban context of Rome, fish mortality events recorded in 2020 and 2021 following intense precipitation events along the Tiber River have highlighted the urgent need for structured assessment protocols. Previous ISS investigations detected multiple contaminants, e.g. carbamazepine, cypermethrin, PFOS, even at low concentrations, suggesting persistent and widespread contamination. This project aims to develop a comprehensive ecotoxicological assessment strategy for urban waterbodies subject to climatic extremes, using the Tiber River and its tributaries (Aniene and Almone) as a case study. The strategy integrates chemical, ecotoxicological, and meteorological data to assess contaminant distribution and toxicological effects under contrasting climatic conditions. Additionally, targeted bioaccumulation studies on native fish species will support trophic transfer assessments. Representative sites across northern, central, and southern Rome have been selected based on varying degrees of anthropogenic pressure. Seasonal sampling campaigns are planned during summer droughts (July–August) and post-storm periods (March, October), which are recognised as critical for contaminant mobilisation. Chemical analyses will target a broad suite of pollutants, including pesticides, pharmaceuticals, PFAS, phthalates and plasticizers. Ecotoxicological assays will employ species across trophic levels—freshwater algae, *Daphnia magna*, and *Danio rerio*—to evaluate

endpoints such as embryotoxicity, genotoxicity, neurotoxicity and teratogenicity. Behavioural endpoints (e.g., coiling activity tail and locomotor activity in zebrafish embryos) will be applied to detect early-life sublethal effects and mixture toxicity. Data integration will enable the development of predictive models for contaminant fate under varying climate scenarios. Ultimately, the project aims to provide science-based guidelines for the adaptive and sustainable management of urban aquatic ecosystems under climate pressure, with transferability to other European urban settings. In the urban context of Rome, future projections indicate a rise in average maximum temperatures, along with a doubling in the frequency of extreme weather events and significant alterations in monthly precipitation patterns. These forecasts underscore the necessity of integrating chemical and ecotoxicological monitoring with systematic analysis of climatic variables, in order to fully understand the interactions between climate change and environmental quality in urban aquatic ecosystems.

Monocyte response to Micro- and Nanoplastics: *in vitro* evaluation of inflammation, viability, and UV-induced photoaging

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The growing prevalence of micro- and nanoplastics (MNPs) in the environment raised concerns regarding their potential impact on human health. Chronic exposure to MNPs has been linked to inflammatory responses and immune system impairment, increasing the risk of chronic and autoimmune diseases. Previous *in vitro* and *in vivo* studies have demonstrated that MNPs can alter activity and gene expression immune cells. However, the exact mechanisms by which MNPs and aged MNPs modulate the immune system remain largely unexplored. The aim of this project is to analyze the *in vitro* effects of MNPs on human immune cells, focusing on the immunological pathways involved. Specifically, we evaluated MNPs cellular alterations in cell proliferation and

viability, changes in gene expression and inflammatory responses, and their impact on phagocytosis. The effects of different concentrations and sizes of MNPs were evaluated on primary human monocytes. Monocyte internalization of MNPs was assessed using confocal microscopy and flow cytometry. Flow cytometry was also used to monitor the expression of immune system activation markers, and gene expression was analyzed via qPCR, focusing on genes related to antigen presentation, inflammasome, and inflammation. The same experimental procedures were applied to determine the effects of photoaged MNPs by ultraviolet (UV) exposure. Monocytes rapidly internalized MNPs, causing reduced cell viability and increased apoptosis, showing a size and dose-response relationship with immune activation. We detected increased production of some inflammatory cytokines, such as TNF α and IL8. Additionally, MNPs influenced monocyte differentiation, increasing the expression of activation markers like CD80 and HLA-DR11 in classical monocytes. Gene expression analysis revealed a reduction in HLA-A expression, which may influence antigen presentation. Furthermore, levels of NLRP3 and CASP1 were reduced, suggesting decreased inflammasome activation. Notably, preliminary data also indicate that UV-aged MNPs induce a more pronounced inflammatory response compared to untreated MNPs, suggesting that photoaging may amplify the immunotoxic effects of MNPs. The findings suggest that MNPs significantly affect the innate immune response, with detrimental effects on cellular viability, inflammation, and immune function. However, more studies are needed to assess the long-term risks of MNPs exposure, focusing on *in vivo* models simulating chronic exposure conditions.

An integrated multi-biomarker approach for assessing the effectiveness of agricultural mitigation measures on pollinators

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Pollinator insects offer fundamental ecosystem services for the environment, agriculture and economy. However, wild pollinators have experienced a significant decline in abundance and diversity, mainly due to human activities. The EU launched the European Green Deal to counter this trend, including specific measures to safeguard wild pollinators and biodiversity. Nevertheless, the real benefits of these strategies on pollinator health remain unclear, making pollinators' health status monitoring crucial to evaluate their efficacy. This study is part of the PRIN-PNRR 2022 project "Assessing the effectiveness of mitigation measures on pollinator decline: an integrated multi-biomarker approach" (ÆM-POLLY). It aims to monitor and compare the health status of wild pollinators sampled in different sites (natural and agroecosystems with or without mitigation measures), using a multi-biomarker approach (to highlight changes in physiological responses) and to assess mitigation measures' effectiveness. We selected two crop types: vineyards in Tuscany and orchards in Emilia-Romagna, characterised by the presence (agroecological) or absence (conventional) of mitigation measures and adjacent natural areas. During late spring and summer, we sampled the most abundant pollinators: *Apis mellifera*, *Bombus terrestris* and hoverflies. On the specimens, we applied a set of biomarkers to evaluate immune responses, detoxification and metabolic processes, energy mobilization, neurotoxicity and genotoxicity effects. Honeybees from conventional Tuscany vineyards showed higher DNA damage than those from agroecological and natural sites. Honeybees from agroecological sites showed better health than those from conventional or natural areas, showing the positive impact of mitigation measures on pollinator well-being.

Non-lethal approaches in sea anemone as a new tool for ecotoxicological investigation

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Sea anemones are worldwide-distributed invertebrates that live in various environmental

conditions. Their adaptation has produced peculiar physiological traits that make them susceptible to environmental changes, and for these reasons, they represent suitable sentinel species. In this study, *Anemonia viridis* has been used as animal model for investigating environmental multiple stressors, and for set up non-lethal approaches using advanced in-vitro techniques for ecotoxicological investigations. In particular, we examined the single and combined effects of increased temperature and ibuprofen in the primary cell cultures and precision-cut tissue slices of *A. viridis*. Primary cell cultures obtained from tentacles and pedal disk without animal sacrifice were exposed for 48 h to different conditions; at the end of exposure, cells were collected and used for confocal microscopy and transcriptome analyses. In parallel pedal disk was sampled again without animal sacrifice and cut with a vibratome to obtain slices of 200 µm thickness, which were maintained in culture and exposed for 48h. At the end of exposure, slices were collected and biochemicals, transcriptome and confocal microscopy analyses were carried out. These approaches and the development of methodological protocols consent to use sea anemone tissue without animal sacrifice to obtain primary cell culture and organotypic culture for advanced *in vitro* ecotoxicological investigations and represent a new tool that can also be applied for modern-omics techniques.

Antimicrobial strategies to combat oral pathogens

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Oral bacterial diseases are among the most widespread chronic conditions, impacting both quality of life and the progression of other systemic and neurodegenerative disorders. The growing issue of antibiotic resistance (AMR) highlights the urgent need for alternative treatments beyond conventional antibiotics. In this context, host defence peptides (HDPs) are emerging as promising bio-inspired antimicrobial agents due to their ability to target bacterial membranes and disrupt biofilms. A potential strategy to enhance the effectiveness of HDPs against oral pathogens

involves their integration into 3D hybrid systems. By combining these peptides with nanomaterials and incorporating them into polymer-based scaffolds, their stability and bioavailability can be improved. In this study, we employed a bioinformatics approach to identify novel antimicrobial peptides, focusing on SQQ30, a peptide derived from the human SOGA1 protein. We evaluated its antimicrobial activity, interaction with bacterial lipopolysaccharides (LPS), and its anti-inflammatory and antioxidant properties. To further enhance its therapeutic potential, we propose incorporating SQQ30 into 3D polycaprolactone-chitosan scaffolds, offering an innovative approach to combat oral infections and reduce reliance on conventional antibiotics.

Contaminants impact on diatom resting phases: the case study of pharmaceutical products on *Phaeodactylum tricornutum*

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Resting phases occur naturally in phytoplankton life history. In aquatic environments where chemical-physical parameters are continuously fluctuating, cells may reduce their metabolic activity, halt their growth and enter a resting phase. Cells that underwent resting phase may settle in benthic environments where they can remain viable for long periods of times forming seed banks, reservoirs of cells that may germinate in the future and contribute to community assemblage and biodiversity maintenance. While impact of contaminants on phytoplankton vegetative growth was extensively studied, little is known on possible contaminants perturbation of phytoplankton resting phases. In the event of contaminant exposure, are phytoplankton sinking toward benthic environments entering a resting phase? Do contaminants alter the resting phase, reduce cell viability and their ability to germinate? The research activities of PhyCoRest project build on the concept that, in order to better understand the possible impact of anthropic pollution on phytoplankton dynamics, it is necessary to investigate all of the different phases of phytoplankton life history, including resting

phases. Experiments will be carried out with the model pennate diatom *Phaeodactylum tricornutum* and the spore-forming centric diatom *Chaetoceros socialis*. *P. tricornutum* cells were exposed to Diclofenac (DCF) and Ethinylestradiol (EE2) concentrations able to induce 50% growth inhibition (EC50) under standard laboratory conditions (72 hours incubation under constant light and agitation). They were then transferred into a vessel that allowed sinking behavior along a vertical light gradient toward dark conditions on the bottom. Growth, viability, photosynthetic activity and ability to accumulate reserve lipids were investigated before and after two months incubation in resting populations. The ability of viable cells to re-enter the vegetative growth phase once re-established light and nutrients conditions was assessed. The effect of DCF occurred during the vegetative growth phase (72h standard incubation) while resting population was not affected by DCF treatment and no significant differences were observed with respect to the control condition after the dark incubation. In contrast, EE2 influenced cell growth as well as cell survival and germination after the dark phase (100-1000 fold reduction in germination rate compared to the control). Results indicated that contaminants mode of toxic action is not limited to vegetative growth phase and that contaminants may have different effects on different phytoplankton life phases. Investigation of contaminants effects on resting phases is required to better assess pollution impact on phytoplankton population dynamics and biodiversity loss.

The use of the zebrafish model for the evaluation of pesticide mixtures

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The presence of mixtures of chemical pollutants in the environment poses a risk for both human and environmental health, with effects often difficult to predict and evaluate. In particular pesticides, widely used to control pests for the prevention of crop loss and the spread of diseases, are in most cases recognized as harmful for non-target organisms, and their release in the environment is currently a cause of concern at the international

level. Moreover, their widespread and frequent application and their ability to persist in some cases for long periods of time after their use can lead to the formation of complex mixtures in water and soil. In this review, we aimed at summarise the current literature regarding the effects of simple and complex mixtures of pesticides. We focused on papers involving the zebrafish (*Danio rerio*) model, a freshwater teleost that finds many applications in biomedical research and is widely used for toxicity evaluation. Our search in literature included the last five years and takes into account four different databases, namely Pubmed, Google Scholar, Embase and Web of Science. Our query resulted in more than 1000 papers, that were later evaluated for inclusion by independent reviewers. Results show a prevalence of works focused on binary and ternary mixtures of pesticides, while only few papers involved more complex mixtures. Also, in the majority of the surveyed works the exposure time was limited to at most few days, highlighting a general interest for acute toxicity. The use of zebrafish was almost equally distributed between adult and larval stages, and the most frequently tested endpoints include mortality, developmental toxicity, cardiotoxicity, behavioural alterations and gene expression. Overall, this work highlights the interest that both pesticides and mixtures of pollutants in the environment gained in the recent scientific literature. Also, the zebrafish is confirmed as a strong and reliable model for toxicity evaluation.

From human use to aquatic impact: developmental toxicity of three psychoactive contaminants in *X. laevis* embryos

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The global increase in the use of psychoactive substances, combined with their incomplete removal during wastewater treatment and their documented toxicity at environmentally relevant concentrations, has led to their classification, along with their metabolites, as emerging environmental pollutants. Clonazepam, a widely prescribed benzodiazepine for anxiety and epilepsy, exemplifies the broader trend of rising consumption of psychoactive pharmaceuticals.

Anxiolytics like clonazepam are often co-used with other psychoactive compounds, including widely consumed legal substances such as caffeine and nicotine. This concurrent intake may result from lifestyle patterns or the intentional modulation of one drug's effects by another. Caffeine, a natural alkaloid found in tea and cacao plants, is the main stimulant in coffee and is also commonly present in tea, soft drinks, energy beverages, and over-the-counter medications. It is among the most frequently detected pharmacologically active contaminants (PhACs) in aquatic environments. Nicotine, the principal alkaloid in tobacco, is primarily absorbed through cigarette smoking and is one of the most widely consumed legal addictive substances worldwide. After ingestion, clonazepam, caffeine, and nicotine are metabolized and excreted predominantly via urine, entering municipal wastewater systems. Despite conventional treatment processes, these compounds are discharged into aquatic environments at concentrations ranging from nanograms to micrograms per liter. Their co-occurrence in surface waters, combined with their pharmacological activity even at trace levels, raises growing concern about potential ecotoxicological impacts on aquatic organisms and broader environmental risks. To investigate developmental toxicity, *Xenopus laevis* embryos were used as a model. Exposure began at the 4–8 cell stage following a modified FETAX (Frog Embryo Teratogenesis Assay–*Xenopus*) protocol, designed to better mimic environmentally relevant exposure conditions. A range of concentrations consistent with those detected in surface and wastewater was tested to assess dose-dependent effects. Preliminary results revealed significant developmental alterations, including cephalic and abdominal edema, intestinal malformations, bent tail phenotypes, and changes in dorsal pigmentation, together with altered early developmental gene expression. LC₅₀, EC₅₀, and Teratogenic Index (TI) values indicated notable teratogenic potential. These findings emphasize the urgent need for further ecotoxicological research and the development of specific regulatory frameworks to protect aquatic ecosystems and public health.

Embryonic neurodevelopment under pharmaceutical pollution: insights from a frog model exposed to Delorazepam

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Delorazepam is a psychotropic drug of the benzodiazepine class, widely prescribed for the treatment of anxiety, insomnia, and epilepsy. While effective for therapeutic use, its potential for misuse and abuse—particularly intensified in the aftermath of the COVID-19 pandemic—has raised significant public health concerns. Like other benzodiazepines, delorazepam exerts its effects by binding to the GABA-A receptor, enhancing GABAergic neurotransmission. Importantly, during early embryonic development, GABA also functions as a signalling molecule, guiding key neurodevelopmental processes such as cell migration, axon elongation, synaptogenesis, and intercellular communication. In parallel with its clinical implications, delorazepam is increasingly recognized as an emerging environmental pollutant. Due to its chemical stability and resistance to degradation, it is not efficiently removed by conventional wastewater treatment plants. As a result, delorazepam and related compounds are released into aquatic environments, where they can persist and bioaccumulate in non-target organisms. To assess the ecotoxicological impact of delorazepam, we employed *Xenopus laevis* embryos—a well-established model in developmental and environmental biology. As amphibians, they are particularly sensitive to environmental contaminants, especially during the early stages of development. Embryos were exposed from the 4–8 cell stage to three environmentally relevant concentrations of delorazepam (1, 5, and 10 µg/l), following a modified FETAX (Frog Embryo Teratogenesis Assay–*Xenopus*) protocol. A multidisciplinary approach was applied to assess developmental effects. Optical microscopy and morphometric analyses revealed structural alterations in the developing nervous system, while gene expression profiling identified changes in markers associated with brain development and function. Raman spectroscopy was used to explore

potential epigenetic modifications, and inductively coupled plasma mass spectrometry (ICP-MS) confirmed the uptake and bioaccumulation of delorazepam in the embryos. Notably, significant neurodevelopmental alterations were observed even at the lowest concentration tested, suggesting that early exposure to delorazepam can interfere with critical molecular and structural processes. Given the evolutionary conservation of key signalling pathways in vertebrate brain development, these findings raise both environmental and human health concerns. The persistence of psychotropic pharmaceuticals like delorazepam in aquatic systems highlights the urgent need for more effective wastewater treatment technologies and comprehensive environmental monitoring. Moreover, the potential for long-term impacts on wildlife and indirect risks to human health underscores the importance of re-evaluating the environmental footprint of commonly used drugs.

Double trouble: combined impact of microplastics and BPA on mussel health

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Microplastics (MPs) and bisphenol A (BPA) are prevalent marine pollutants that could simultaneously threaten marine organisms. MPs can adsorb BPA, potentially enhancing its uptake and toxicity. This study evaluated the individual and combined effects of polystyrene MPs (PS-MPs, 45 µm, 10 µg/L) and BPA (22.8 µg/L) on the Mediterranean mussel *Mytilus galloprovincialis*. Mussels were exposed to PS-MPs, BPA, or a combination of both (MPs+BPA) for three days, with sampling conducted after 1, 2, and 3 days. Key tissues, including gills, digestive gland, mantle, and gonads, were analysed to assess contaminant accumulation and oxidative stress response. Tissue accumulation analysis revealed significant MP accumulation in the digestive gland and mantle across all treatments. MP concentrations peaked after 1 day of exposure and declined over time, with accumulation levels higher in the MP-only treatment compared to the MP+BPA group. The antioxidant responses, including activities of

superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity (TAC) in mussel tissues, indicate that both MPs and BPA, individually and in combination, induce oxidative stress in a tissue- and time-dependent manner. In the gills, the transient increase in SOD activity followed by its decline, alongside delayed CAT and TAC activation, indicates an early but unsustainable antioxidant defense in response to combined MP+BPA exposure. The digestive gland exhibited a reduction in SOD activity under combined exposure, while CAT and TAC responses were primarily driven by MP exposure, indicating that BPA may suppress or alter the oxidative response when present with MPs. In the gonads, the consistent increase in CAT activity across all treatments and the sustained elevation of SOD in the MP+BPA group point to heightened sensitivity and a prolonged response to oxidative stress in reproductive tissues. Overall, these findings highlight complex, tissue-specific oxidative stress dynamics resulting from MP and BPA exposure, with combined exposure often eliciting distinct or enhanced responses compared to single contaminants. This underscores the need to consider mixture effects when evaluating pollutant toxicity in marine organisms.

How microplastics modulate the effects of bisphenol A during zebrafish development

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In recent decades, plastic waste has become a serious environmental problem with implications for ecosystems and human health. When plastic is released, especially into water environments, it undergoes degradation, particularly by atmospheric factors such as ultraviolet (UV) radiation. This process breaks the plastic into smaller particles, known as microplastics (MPs). These small particles are found almost everywhere in the environment. Because of their small size and surface characteristics, microplastics can attract and carry other substances, such as chemical pollutants. Bisphenol A (BPA) is a synthetic compound commonly used in the production of polycarbonate plastics and epoxy resins, frequently detected in marine and fresh water. It is

classified as an endocrine disrupting chemical, and its toxicological effects have been widely documented. In a previous study, we demonstrated that exposure to polystyrene microplastics (PS-MPs) of 1 μm and 3 μm in size negatively affects zebrafish embryonic development. Specifically, PS-MPs were shown to induce morphological malformations, promote apoptosis, increase heart rate, and disturb redox homeostasis. Based on the results previously obtained and on studies in the literature, this work aimed to investigate the interaction between PS-MPs and BPA during the early developmental stages of zebrafish. Embryos were exposed for 72 hours to PS-MPs (1 mgL^{-1}) and BPA (25 μM) alone and in combination. We assessed some toxicity parameters, including hatching rate, spontaneous movement and heart rate, oxidative stress biomarkers, and the expression of development genes. The results showed that PS-MPs alone did not significantly alter the hatching rate, whereas BPA exposure delayed hatching. Both contaminants individually caused a reduction in spontaneous movements. Heart rate was increased following PS-MPs exposure but decreased with BPA. Interestingly, when embryos were co-exposed to PS-MPs and BPA, an antagonistic interaction was observed in several parameters: PS-MPs mitigated some of the toxic effects induced by BPA, particularly regarding hatching, spontaneous movement, and heart rate. Regarding oxidative stress, both single BPA and combined exposure resulted in alteration of redox homeostasis, and the combination exhibited a synergistic effect. Gene expression analysis revealed that both pollutants individually downregulated developmental genes, but their combined exposure showed an antagonistic effect where PS-MPs mitigated the effect of BPA alone. These results show that microplastics and bisphenol A can interact in different ways. It is important to understand how these substances affect each other to better evaluate the risks they pose to the environment and to support future research on pollution in water ecosystems.

The impact of abandoned fishing gear on mediterranean biodiversity: results from the STRONG SEA LIFE Project

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Abandoned, Lost or Discarded Fishing Gear (ALDFG) represents a growing threat to marine biodiversity, particularly in sensitive habitats such as *Posidonia oceanica* meadows and coralligenous reefs. All types of fishing gear that, for various reasons, are lost or abandoned at sea, cause significant environmental damage: they continue to fish passively, damage benthic habitats, and act as vectors for invasive species. The entire marine ecosystem is affected, with the most severe consequences observed in slow-growing, sensitive habitats with long recovery times. Over time, their degradation into microplastics releases contaminants that may enter the food chain, threatening both ecosystems and human health. The resulting ecological impact leads to biodiversity loss, affecting both protected and commercially valuable species. Though quantitative estimates remain limited, it is believed that nearly 2% of all fishing gear—including approximately 218 km² of trawl nets—is lost annually. The distribution of ALDFG depends on local fishing intensity and the hydrological and geomorphological features of the seabed. STRONG SEA LIFE project (Survey and TRreatment ON Ghost Nets SEA LIFE), funded by the EU LIFE Programme, aims to protect and restore *Posidonia oceanica* and coralligenous habitats in the Mediterranean Sea. The project implements an integrated approach combining mapping, removal, inactivation and monitoring. This contribution presents the outcomes of the first two years of project's field activities conducted in the Gulf of Asinara (NW Sardinia, Italy), focusing on the effects of ghost nets on marine environment. A total of 42 recovery and inactivation operations were carried out, targeting various types of fishing gear under a range of environmental conditions. These efforts led to the recovery of 11 tons of gear and the identification of 99 species across 16 taxa associated with the retrieved or inactivated material. Conservation outcomes included the improvement of 12 hectares of *P. oceanica* meadows and 5 hectares of coralligenous reef. Recovery efforts were guided by ecological assessments to minimize habitat disturbance. Special attention is given to inactivation as an

alternative to removal, when the gear is no longer fishing, is heavily colonized, not located in sensitive habitats, and hosts species of conservation value. In such cases, leaving the gear in place and monitoring it may better protect biodiversity. The project highlights the ecological relevance of context-specific strategies—such as selective inactivation—and the role of collaborative efforts in preserving Mediterranean marine biodiversity. Continued monitoring and stakeholder engagement are essential to ensure long-term conservation outcomes and policy effectiveness.

Microplastics and phytoplankton communities: interactions in monitoring programs

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In recent years, the marine environment has been increasingly impacted by anthropogenic activities, leading to a notable loss of biodiversity and a rise in emerging contaminants that compromise the delicate balance of marine ecosystems. In response to these issues, the European Union adopted the Marine Strategy Framework Directive (MSFD), formalized on June 17, 2008, and implemented in Italy through Legislative Decree No. 190 of October 13, 2010. The Directive aims to protect the marine environment across Europe while enabling the sustainable use of marine goods and services, by developing strategies to achieve Good Environmental Status (GES). To assess GES, the MSFD outlines eleven qualitative descriptors. The focus is specifically on Descriptor 1 (Biodiversity) and Descriptor 2 (Non-Indigenous Species), with particular attention to the phytoplankton component. However, this biodiversity is increasingly threatened by pollutants, in recent years, plastic pollution—specifically microplastics (MPs)—has been detected even in the most remote marine areas. MPs are particles ranging from 0.1 µm to 5 mm in size, which tend to bioaccumulate within organisms and enter the marine food web. Phytoplankton represent a fundamental ecological community, essential for biogeochemical cycling and serving as a primary food source for zooplankton and fish. The qualitative and quantitative assessment of the phytoplankton

community is conducted under Modules M1 and M3. This monitoring includes the determination of physico-chemical variables using a multiparameter CTD probe. Sampling in Module 1 is performed using a Niskin bottle. At each station, both surface water (0.5 m) and deep water (either at the depth of the Deep Chlorophyll Maximum or at 25 m) are collected. Module 3 involves deep water sampling using a plankton net. In the laboratory, known-volume sub-samples are preserved in Lugol's iodine solution, settled in counting chambers following the Utermöhl method, and subsequently analyzed qualitatively and quantitatively using an inverted light microscope at 40X/20X magnification. Results are expressed in cells per liter (cell/L). During these analyses, numerous fragments of microplastics were detected in the seawater samples intended for phytoplankton analysis. Highlighting this finding is of critical importance, as it underlines the need to investigate the potential interactions between microplastics and phytoplankton—particularly concerning their effects on growth rates and their implications for organisms that feed on phytoplankton. It is worth noting that zooplankton, as primary consumers of phytoplankton, may ingest microplastics due to the similar size range, potentially leading to significant biological impacts across the trophic web.

Paracetamol risks in global waters: an SSD approach

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Paracetamol (acetaminophen) is one of the most widely consumed pharmaceutical compounds worldwide, commonly used as an analgesic and antipyretic in both human and veterinary medicine. Despite its widespread therapeutic application and historical perception as an environmentally benign compound, recent studies have revealed its persistence and ecotoxicological relevance in aquatic environments. This work presents a comprehensive environmental and human health risk assessment of paracetamol based on global occurrence data and species

sensitivity distribution (SSD) analysis. We collected occurrence data from over 200 peer-reviewed scientific publications published between 2000 and 2025, covering paracetamol concentrations across multiple aquatic compartments, including WWTP influents and effluents, hospital and industrial discharges, surface waters, groundwater, and drinking water. Acute toxicity values (EC₁₀, LC₁₀, NOEC) for 55 aquatic species were compiled from the ECOTOX and EnviroTox databases to develop an SSD. The fitted log-normal SSD yielded an HC₅ of 0.048 mg/L, from which a predicted no-effect concentration (PNEC) of 0.0096 mg/L was derived using an assessment factor of 5. Risk Quotients (RQs) were calculated by comparing the maximum environmental concentrations (PECs) in each matrix with the PNEC. The ecological risk characterization revealed extremely high RQs (>100) in WWTP influents and hospital discharges, and elevated RQs (often >1) even in treated effluents and surface waters—especially in low- and middle-income countries. Notably, approximately 90% of the environmental matrices analysed were classified as "high risk" for aquatic organisms, underscoring the need for targeted mitigation strategies and stricter effluent regulation. To complement the ecological assessment, we performed a preliminary human health risk evaluation using drinking water concentrations. Chronic Daily Intake (CDI) values were estimated for adults and children, considering standard water ingestion rates and body weights. Hazard Quotients (HQs) were then computed using an Acceptable Daily Intake (ADI) of 57 mg/kg/day. While all HQ values remained well below 1—indicating no immediate non-carcinogenic risk—the values were consistently higher in children due to their greater relative intake, highlighting age-based susceptibility. Our findings demonstrate that paracetamol, though not currently regulated as an environmental contaminant, poses significant ecological risks and a measurable, albeit low, potential for human exposure through drinking water. The study underscores the importance of including widely used pharmaceuticals in monitoring programs and supports the development of environmental quality standards (EQS) and improved wastewater treatment technologies. Further research is needed to evaluate mixture toxicity, long-term sublethal effects, and age-specific health impacts

associated with chronic low-dose pharmaceutical exposure.

Effects of short-term exposure to polystyrene nanoparticles and water-soluble diesel oil fraction alone and combined in the ascidian *Styela plicata*

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Plastic and oil pollution are considered as two major critical environmental issues to marine ecosystems. Polystyrene nanoparticles (PS-NPs; 1–100 nm) contribute to oxidative stress, DNA damage, and histopathological abnormalities in marine life. Similarly, oil pollution can disrupt vital physiological processes and increases mortality in aquatic species. Ascidians, both in their adult and developmental stages, are widely used as models in ecotoxicology due to several key characteristics. As filter-feeding organisms, they are highly vulnerable to contaminant bioaccumulation due to their constant water filtration and tunic absorption of soluble pollutants. However, the combined effects of PS-NPs and the water-soluble fraction of diesel oil (WFDO) on ascidians remain unexplored. The current study investigates the acute exposure of environmentally realistic concentrations of PS-NPs (50 µg/L) and a low concentration of WFDO (5%), both individually and in combination, on the ascidian *Styela plicata*. Oxidative stress biomarkers as well as histological alterations were evaluated. Our findings show an increase in acetylcholinesterase (AChE) activity. In contrast catalase (CAT) and glutathione-S-transferase (GST) activities were reduced. Histological analysis revealed normal tissue structure in groups exposed to polystyrene nanoparticles or water-soluble fraction of diesel oil alone, while the combined exposure group exhibited significant structural alterations.

EMAS and the energy efficiency challenge: a case study in the energy sector

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The EMAS (Eco-Management and Audit Scheme) environmental management system is a strategic voluntary tool to promote a continuous improvement of the environmental performance, with an increasing focus on energy efficiency. EMAS registered organizations, thanks to the structure of the system, are already aligned with many requirements of the ISO 50001 standard, encouraging the adoption of an energy management system. EMAS registered organizations are striving to continuously improve their environmental performance, to identify the best available practices and the most effective strategies that promote the rational use of resources, to mitigate environmental risk through the management of environmental aspects/impacts. This study analyses a homogeneous group of small, medium and large power plants, that have obtained EMAS registration. The analysis of their Environmental Declarations (DA) has identified the implemented energy efficiency measures and the improvements obtained over the time. The results show an improvement in the energy performance of companies, highlighting how the integration between EMAS and energy strategies concretely allows to optimize the use of resources, reduce consumption and contribute effectively to the transition towards a sustainable energy system and to the mitigation of the climate change. The analysis is part of the regulatory framework updated by the Legislative Decree 14 July 2020, n. 73, which implements the Directive 2018/2002/EU on energy efficiency (EED), strengthening the obligations for large companies, including those with environmental management systems, in terms of energy diagnosis. Environmental management systems, such as EMAS, are therefore configured as fundamental tools for achieving energy saving objectives, thanks to a structured approach to the evaluation, monitoring and improvement of performances.

Advanced chemical analyses integrated to experimental and modelling ecotoxicological-approach to study natural toxic mixtures from river waters affected by WWTP

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The contamination of natural waters by complex toxic mixtures originating from wastewater treatment plants (WWTPs) represents a critical environmental and public health challenge. These effluents often contain thousands of pollutants, including pharmaceuticals, heavy metals, pesticides, and other anthropogenic compounds, which enter the aquatic environment and may exert combined toxic effects on ecosystems and human populations. The risk assessment of such mixtures has a high level of uncertainty due to potential additive, synergistic, or antagonistic interactions among components, which are not adequately accounted for when assessing each substance separately. To address this complexity and to assess the overall hazard posed by environmental mixtures, an integrated approach combining chemical analyses with ecotoxicology is essential. In this work we combined the exposure assessment, obtained from wide chemical target analysis of pharmaceuticals and trace metals, and the ecotoxicological assessment, including both experimental methods, carried out with a battery of toxicity tests, and modelling approach, to study the natural toxic mixture from two WWTP-impacted river-waters. The samples were collected from Lambro and Molgora rivers, both downstream from two WWTPs in the province of Monza-Brianza in northern Italy. The bioassays included the classical test organisms: alga (*P. subcapitata*), crustacean (*D. magna*) and fish (*D. rerio*). As a strategy to compare the concentrations of organic compounds with the toxicity attributable at this class of chemicals, the 72h-alga and 48h-daphnia tests were performed on organic extracts obtained through the enrichment sample procedure by the SPE-treatment. The calculated median effective concentrations (EC₅₀) were expressed as Relative Enrichment Factor (REF). The Toxicity Equivalency method (TEQ), a special case of the Concentration Addition (CA) model, was used for the integration of chemical concentrations and ecotoxicological data. Preliminary results showed the presence of some dozens of pharmaceuticals while slight - moderate toxicity was measured with the three bioassays in

the natural waters from both rivers. However, a strong toxic effect on algae was found in the adsorbable organic fraction with 72h-EC₅₀ values of 8.8 REF and 13.3 REF in Lambro and Molgora samples, respectively. By translating these effects in toxic equivalent concentrations, we can directly compare the effect levels and the exposure levels, trying to quantify the actual contribution to the measured toxicity of the individual organic compounds analytically detected in the SPE extracts. Our finding, even at a preliminary level, can represent a promising approach to challenge the complexity of toxic mixtures in natural waters affected by WWTP discharges.

Sediments as sources of contaminants, implications for aquatic organisms and human health: a systematic review

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Sediment in marine and freshwater ecosystems plays a crucial role in the dynamics of chemical pollution, acting both as long-term storage and as a potential source of contamination. Over time, it can accumulate a wide range of traditional and emerging contaminants, including heavy metals, pharmaceuticals, and PFASs, which can be released back into the environment due to human activities (e.g. dredging) or environmental events. The contaminants can enter in the food chain through benthic organisms and accumulate via bioaccumulation and biomagnification in edible species, ultimately reaching human consumers. This systematic review aims to provide an updated overview of the scientific literature (2014-2024) concerning the presence of several contaminants in sediments, its transfer to edible organisms, and its implications for human health. For this work, we performed extensive research with the aim of summarising and discussing the current state of knowledge on the topic, we have taken into account scientific articles retrieved from the Embase database, selected through a two-step process: an initial screening, followed by a full-text review. The research produced a large data set from studies covering different geographical regions and environmental contexts, with a major number of studies deriving from Asia. The data

analysis extrapolated information on pollutant concentrations in sediments, edible organisms, and human health risk. In particular, the methods that are considered for the evaluation considered trophic transfer indices such as Bioaccumulation Factor (BAF), Biota-Sediment Accumulation Factor (BSAF), Trophic magnification factor (TMF); also, health risk indicators such as the Target Hazard Quotient (THQ) were considered. The results highlighted the presence of several traditional and emerging pollutants, with a widespread detection of heavy metals and PAHs often exceeding environmental threshold values or food safety limits. Despite the heterogeneity of sampling approaches and regulatory frameworks, the results highlight the need for harmonized monitoring and assessment strategies that integrate sediment, biota, and health risk analyses. These strategies are key aspects to understand the environmental fate of the contaminants in aquatic environments, to apply measures for the sediment contamination and implement general guidelines to protect ecosystems and human health.

Exploring the link between Ecosystem Services and Environmental Risk Assessment in marine ecosystems

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Environmental Risk Assessment (ERA) is a commonly applied tool for evaluating the impacts of human pressures on marine ecosystems. However, traditional approaches often focus on individual-level endpoints without explicitly considering the consequences for ecosystem services (ES), i.e. the benefits that nature provides to humans. This gap could reduce the relevance of risk assessments for environmental policy and marine management, especially in contexts where human well-being is closely tied to the health of marine ecosystems. Marine environments are characterized by complex and dynamic interactions among a multitude of variables, including physical, chemical, and biological factors. Contaminants and their mixtures contribute to ecological stress, often causing ecotoxicological effects on organisms that impact ecosystem

functions. Combined different environmental pressures make it difficult for traditional ERA methods to fully assess ecosystem changes and their effects on ES. This work represents a starting point for integrating ES into the ERA approach, based on the assumption that higher environmental risk is generally associated with a greater loss of ES. Recognizing and potentially quantifying this relationship can help filling the gap between ecological risk assessment and human well-being, offering a more holistic perspective. The proposed approach focuses on identifying key ecological functions (e.g., primary production, nutrient cycling, habitat provisioning) that support the generation of different categories of ES (provisioning, regulating, and cultural) and that can be linked to specific stressors and ecological risk endpoints. Through this integration, we aim to support the assessment of the potential loss of ES under increased levels of environmental contaminants. Potential applications of such integrated framework could allow more effective communication of environmental risks and trade-offs, in support of sustainable marine management strategies, and a stronger link between ecological risk, ES loss, and informed decision-making in marine conservation and management.

Impact of Nanocellulose in *Homarus gammarus*: a sub-chronic *in vivo* study with early life stages of an economically and ecologically important Mediterranean species

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Biodegradable and renewable materials like nanocellulose (NC) within growing market use and associated low environmental impact, deserves a proper ecological risk assessment for an environmental safety disclosure. Recent findings of cellulose-based textile fibres bioaccumulation and ecotoxicity in marine invertebrates worldwide raise concerns about NC potential hazard to marine life. Herein we tested for the first time newly synthesized TEMPO-oxidized cellulose nanofibers (TOCNF) through an *in vivo* sub-chronic

exposure study on early life stages of the European lobster (*Homarus gammarus*), an economically and ecologically important decapod species that has been subjected to conservation programs for decades. By resembling realistic exposure scenarios for marine waters, lobster larvae were exposed for 7 days to 1µg/L and 10µg/L of TOCNF and survival as well as growth along with neurotoxicity and oxidative stress were assessed after 48 hours and 7 days. Rhodamine B-labelled TOCNF were also used for disclosing uptake and biodistribution along the exposure. Results showed a slight increase in mortality in TOCNF exposed larvae but no effects on growth of those survived. An increase over time in the accumulation of TOCNFs within the cephalothorax apparatus was observed, suggesting a time- and concentration-dependent trend. Overall findings reveal that nanocellulose can be taken up by early life stages of lobster with onset of potential toxicity which deserve further attention and ecotoxicological study.

Thanks are extended to the University of Naples Federico II for hosting the 4th SETAC ILB Workshop at the Complesso dei SS. Marcellino e Festo (Naples, IT).



This book of abstracts summarised the contributions received within the 4th Workshop of the SETAC Italian Language Branch (ILB), hosted in Naples on October 13–14, 2025, that served as a key multidisciplinary forum for environmental science and policy. Organized at the historic University of Naples Federico II, the workshop focused on topics aiming to bridge the gap between academic research and regulatory frameworks. The scientific program highlighted critical topics such as the preservation of Mediterranean biodiversity, the management of emerging contaminants (including microplastics and PFAS), and the impacts of climate change on ecosystem and human health. By integrating sessions on Life Cycle Assessment (LCA) and advanced ecotoxicological modelling, the workshop fostered collaboration among academia, industry, and public authorities. This event supported Italy's commitment to sustainable environmental quality and the development of innovative risk mitigation strategies to protect our natural resources.

Giovanni Libralato è Professore Ordinario di Igiene presso l'Università degli Studi di Napoli Federico II, Federica Cacciatore è Ricercatrice presso l'Istituto Superiore per la Protezione e la Ricerca Ambientale, Silvia Franzellitti è Professoressa Associata di Fisiologia presso l'Università degli Studi di Bologna, Claudia Vaj è Regulatory Manager presso Corteva Agriscienze Italia e Stefania Marcheggiani è Primo Ricercatore presso l'Istituto Superiore di Sanità.

ISBN: 978-88-6887-428-5

DOI: 10.6093/978-88-6887-428-5

