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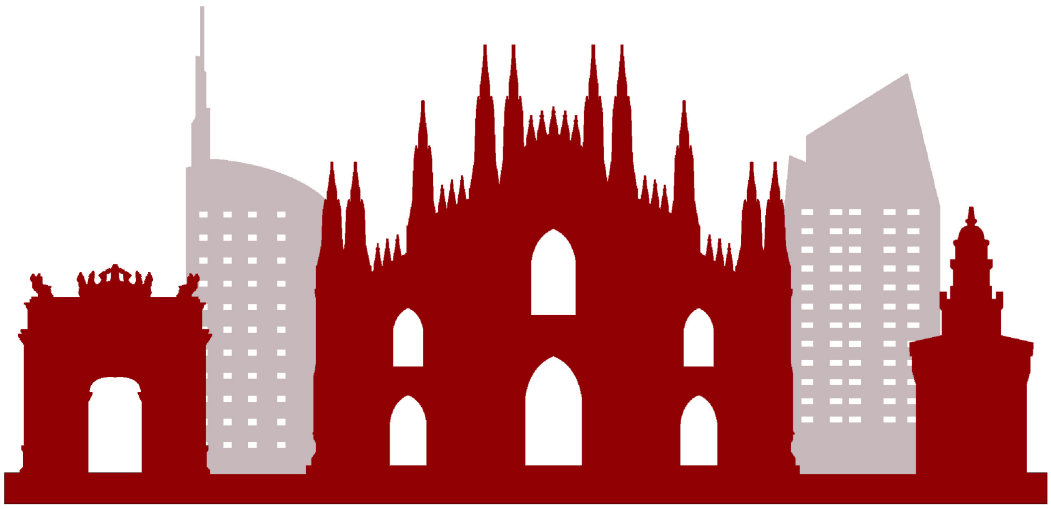

7 8 October 2024

Milan

SETAC Italian Language Branch 3rd Workshop

SETAC Italian Language Branch

Book of Abstracts

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Scientific and Organizing Committee

Andrea Binelli
(Univ. of Milan)

Camilla Della Torre
(Council Member of SETAC ILB, Univ. of Milan)

Stefano Magni
(Council Member of SETAC ILB, Univ. of Milan)

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Program

7th October 2024
12.30 - 14.00 Registration

14.00 – Welcome greetings from Dir. Sabine Apitz, President of SETAC Europe.
Opening of the workshop by Prof. Ilaria Corsi (Univ. of Siena, President of SETAC ILB) and Prof. Andrea Binelli (Univ. of Milan, Scientific and Local Organizing Committee).

14.15 – 16.00
Best Young Italian Scientist Award - pitches

Coordinator: Claudia Vaj (Corteva)
Chairs: Laura Marziali (IRSA-CNR), Stefano Magni (UNIMI)

1) **Andrea Masseroni (UNIMIB)**. Ecological fitness impairments induced by chronic exposure to polyvinyl chloride nanospheres in *Daphnia magna*.

2) **Emma Ferrari (UNISI)**. Antarctic threads: textile microfibers and chemical additives in the wild scallop *Adamussium colbecki* from the Ross Sea.

3) **Patrizia Romano (UNISI)**. Ecological risk assessment of manufactured nanomaterials: investigating the role of protein-corona formation in the coelomic fluid of the sea urchin *Paracentrotus lividus*.

4) **Lorenzo Federico (UNIMIB)**. A new ecological screening tool based on the aggregation of the terrestrial isopod *Porcellionides pruinosus* for assessing soil quality.

5) **Davide Rotondo (UNIUPO)**. impact of short-chain perfluoropropylene oxide acids on biochemical and behavioural parameters in *Eisenia foetida* (Savigny, 1826).

6) **Jacopo Fabrello (UNIPD)**. Effects of BPA analogues-contaminated diets in the crab *Carcinus aestuarii*.

7) **Andrea Ferrari (UNIMI)**. From molecules to bees: multi-level approach reveals negative effects of sewage sludge trace elements on honeybees.

8) **Sara Accardo (ENEA)**. A deep insight into eco(toxico)logical risk posed by metal contamination in the Alento River.

9) **Chiara De Carolis (UNIROMA)**. Evaluation of the effects of antibiotic and copper mixture on a plant-microbiome metaorganism.

10) **Antonio Morgillo (UNISI)**. Unveiling the effects of emerging contaminants on marine organism embryogenesis.

11) **Davide Gualandris (UNIUPO)**. Investigating sublethal responses of earthworms in PFAS-contaminated soils: a comprehensive study at Trelleborg fire fighting site in Sweden.

12) **Marco Evangelista (UNINSUBRIA)**. *In silico* prediction of the human transthyretin binding affinity.

13) **Isabella Calattini (UNISI)**. A holistic strategy for assessing the health and services of freshwater ecosystems.

14) **Silvia Giorgia Signorini (UNIMI)**. A multi-level approach to investigate potential adaptive mechanisms to ocean acidification in the limpet *Patella caerulea*.

16.00-17.30
Session A - Environmental chemistry and exposure assessment: analysis, monitoring, fate and modelling

Chairs: Sara Castiglioni (Mario Negri Institute), Antonio Di Guardo (UNINSUBRIA)

16.00-16.15 **Cristina Cremonesi (UNIMI)**. Temporal variability of floating plastic contamination in Lake Maggiore (Northern Italy): Implications for sampling strategies.

16.15-16.30 **Giulia Cesarini (IRSA-CNR)**. First evidence of microplastics in three invasive crayfish species in Lake Maggiore.

16.30-16.45 **Isabella Gambino (UNINSUBRIA)**. Textile chemicals of environmental relevance: from production to surface water.

16.45-17.00 **Stefano Tasselli (IRSA-CNR)**. Polluting while cleaning: the case study of polycyclic musk fragrances.

17.00-17.15 **Elisa Terzaghi (UNINSUBRIA)**. PAH hydroxy-metabolites on plant leaves: where do they come from? From field data to laboratory experiments.

17.15-17.30 **Matilda Porro (IRSA-CNR)**. DDT in Lake Maggiore: temporal variation from 1998 to 2023.

17.30-18.30
Poster session

1) **Antonio Calisi (UNIUPO).** Cytotoxicity and genotoxicity of ether perfluoro carboxylic acid PFAS congeners in *Eisenia foetida* coelomocytes.

2) **Aurora Mancini (ISS).** The application of Ecotoxicology in the Health Impact Assessment; a new legislative development relevant for the protection of human health.

3) **Barbara Billé (UNIME).** Evaluating the embryotoxic potential of the pesticide thiram in the sea urchin *Arbacia lixula*.

4) **Cristina Cavone (IRSA-CNR).** Bioaugmentation and Plant-assisted bioremediation for contaminated environment recovery.

5) **Giulia Tesoriere (UNIROMA).** *Drosophila melanogaster* a new ecotoxicological method for air pollutants.

6) **Ilaria Bernardini (UNIPD).** Integration of RNA and 16S rRNA amplicon sequencing for risk evaluation of pharmaceuticals on non-target species.

7) **Ilaria Savino (IRSA-CNR).** Tire rubber leachate: a threat to the gamete quality of the mediterranean mussel *Mytilus galloprovincialis*.

8) **Jana Krišković (University of Zagreb).** Investigation of the effects of 1,3-diphenylguanidine on *Daphnia magna*.

9) **Laura Giovanetti (UNISI).** A blood-based multi-biomarker approach reveals different physiological responses of common kestrels to contrasting environments.

10) **Luisa Albarano (UNINA).** It is not a party for the sea!

11) **Mario Carere (ISS)**. Effect-based methods in the EU Water Framework Directive: a new approach for the detection of mixtures.

12) **Melissa Barra (ISS)**. Pollinator pioneers: bee ecotoxicology unveiling effects of air pollutants.

13) **Roberta De Angelis (ISPRA)**. An integrated approach for the management of beach and recreational use of water.

14) **Silvia Casini (UNISI)**. Assessing the effectiveness of mitigation measures on pollinator decline: an integrated multi-biomarker approach (æm-polly project).

15) **Tommaso Campani (UNISI)**. Evaluation of toxicological effects of fungicides used in vineyards agroecosystem using *Apis mellifera* as a model species.

16) **Tommaso Zoli (UNIMI)**. *Aedes koreicus*: could an invasive mosquito be used as sentinel of heavy metal pollution?

8th October 2024

9.00 – 11.30
Session B - Environmental and human toxicology: from molecules to organisms, from omics to in vivo

Chairs: *Sonia Manzo (ENEA), Giovanni Libralato (UNINA)*

9.00-9.15 **Lara Nigro (UNIMI)**. Investigating the Effects of Some Water-Soluble Polymers on *Daphnia magna*: From Proteomic Changes to Transgenerational Epigenetic Inheritance.

9.15-9.30 **Giada Caorsi (UNIMI)**. Exploring the Impact of Commercial PVA-based Dishwater Pods: A Multi-Tier Analysis on *Danio rerio* Embryos.

9.30-9.45 **Riccardo Sbarberi (UNIMI)**. Evaluation of adverse effects induced by virgin and sampled plastics on *Chironomus riparius*.

9.45-10.00 **Stefano Magni (UNIMI)**. Effects of tire rubber-derived contaminants on aquatic and terrestrial organisms.

10.00-10.15 **Teresa Balbi (UNIGE)**. SSRI antidepressants in non-target marine invertebrates: multiple mechanisms of action of fluoxetine in the hemocytes of *Mytilus galloprovincialis*.

10.15-10.30 **Stefania Gorbi (UNIVPM)**. Mechanisms of molecular and cellular responses of human pharmaceuticals mixtures in non-target model species.

10.30-10.45 **Mariachiara Galati (UNIME)**. Impact of environmental doses of dexamethasone on the reproductive health of the Mediterranean mussel *Mytilus galloprovincialis*.

10.45-11.00 **Giuseppe De Marco (UNIME)**. Tissue and molecular alterations caused by the emerging contaminant dexamethasone in marine mussels and the use of ulvans to counteract its harmful effects.

11.00-11.15 **Maria Luisa Vannuccini (UNITUS)**. On the safe usage of copper sulphate in organic farming: evidence for genotoxic and enzymatic effects on a non-

target species, the Mediterranean stick insect *Bacillus rossius.*

11.15-11.30 **Safa Melki (UTM-Tunis)**. Effects of mercury exposure on oxidative stress biomarkers and the ovarian morphology of the ascidian *Ciona intestinalis* adults.

11.30-11.45
Best Young Italian Scientist Award ceremony

11.45-13.00
SESSION C - Ecological and human health risk assessment of chemicals, mixtures and stressors and risk mitigation strategies

Chairs: *Paola Grenni (IRSA CNR), Mario Carere (ISS)*

11.45-12.00 **Giovanna Meregalli (CORTEVA)**. Spot applications to control thistles in sugar beet fields – how to link the actual practice to the GAP table and the environmental risk assessment? An example case study.

12.00-12.15 **Maura Benedetti (UNIVPM)**. Stress priming tool: memory mechanisms to mitigate organisms vulnerability to multiple stressors

12.15-12.30 **Silvia Franzellitti (UNIBO)**. A meta-analysis of levels and physiological effects of pharmaceuticals in marine invertebrates.

12.30-12.45 **Simona Vezzoli (UNIBS)**. Employment of rapid bioassays for detection of toxicity following exposure to different organic substrates.

12.45-13.00 **Francesca Mauro (ISPRA)**. Public engagement in the industrial

13.00-14.20
Lunch

14.30-15.45
SESSION D - Moving beyond - Cross cutting, emerging and transdisciplinary themes

Chairs: *Camilla Della Torre (UNIMI), Stefania Gorbi (UNIVPM)*

14.30-14.45 **Arianna Sgariboldi (UNINSUBRIA)**. *In silico* screening of hazardous properties of Pharmaceuticals and Personal Care Products, and their associated potential risk after water treatment.

14.45-15.00 **Walter Cristiano (ISS)**. Listening to nature: how soundscapes can inform us about the health of fragmented habitats.

15.00-15.15 **Silvia Simonetti (UNISI)**. Adaptation/resistance to ocean acidification: the potential role of ATP-binding cassette transporters investigated in the polychaetes *Platynereis dumerilii* and *Platynereis cfr massiliensis.*

15.15-15.30 **Francesco Dondero (UNIUPO)**. Development of a behavioral test in the terrestrial oligochaetes *Eisenia fetida* exposed to Per and poly fluoro alkyl substances (PFAS).

15.30-15.45 **Camilla Puccinelli (ISS)**. Do diatom teratological forms work as a sentinel of antibiotic pollution in the aquatic ecosystem?

15.45-16.45
SESSION E - Ecotoxicology becomes stress ecology: from populations to ecosystems and landscapes

Chairs: *Andrea Binelli (UNIMI), Antonio Finizio (UNIMIB)*

15.45-16.00 **Agata Di Noi (UNISI)**. Biomarkers and cognitive analysis to assess the effects of a commercial fungicide and an herbicide, alone and in combination, on *Apis mellifera*.

16.00-16.15 **Laura Marziali (IRSA-CNR)**. Legacy mercury contamination in the Toce River (North Italy): from sediments to the terrestrial ecosystem.

16.15-16.30 **Renata Pacifico (ISPRA)**. EMAS tool for environmental risk management.

16.30-16.45 **Lucia Rigamonti (POLIMI)**. Life Cycle Assessment studies to support projects of waste treatment plants.

16.45-17.00
Wrap up and closing remarks from Prof. Andrea Binelli (University of Milan, Scientific and Local Organizing Committee) Prof. Ilaria Corsi (University of Siena, President of SETAC ILB) and Dr. Stefania Marcheggiani (ISS, Vice-President of SETAC ILB)

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Introduction

The Italian Language Branch (ILB) is one of several regional branches of SETAC Europe, founded about twenty years ago to inspire and support the development of principles and practices for sustainable environment quality, ecosystem preservation, and ecosystem integrity through a multidisciplinary approach according to SETAC provisions. The Italian Branch brings together the Italian-speaking members of SETAC Europe in Italy and abroad, including biologists, chemists, engineers, policymakers, students, stakeholders, and environmental scientists. It serves as a platform for discussing emerging and re-emerging issues in the ecological field and health, in line with SETAC's European objectives and mission. At the international level, the ILB actively contributes to SETAC Europe's annual meetings with scientific and technological contributions. At the regional level, the ILB advocates and encourages scientific discussions among academia, industry, research centres, and authorities, promoting the application of scientific knowledge to environmental policy. The ILB organises and supports training courses, conferences, and workshops, and actively encourages students to share their ideas and projects, inspiring and motivating them to contribute to sustainable practices. The SETAC ILB is a collaborative platform comprising 125 members distributed among research bodies, universities, and industries in Italy and abroad. As part of SETAC Europe, the ILB sponsors and promotes various events at the national and international levels, always free of charge. These events, which are related to the SETAC mission, are a testament to the collaborative spirit of our organisation, with SETAC ILB members actively involved in their planning and execution.

Since 2009, SETAC ILB has grown and expanded significantly thanks to new organisational impulse. It began with a meeting in the Marconi Hall of the National Research Council in Rome, setting the stage for our continued evolution and impact. That triggered the following initiatives: 1st National Workshop was hosted by the University of Siena in 2022 and followed in 2023 by the 2nd National Research Council in Rome, and the present 3rd workshop in 2024 hosted by the University of Milan. The 3rd Workshop opening ceremony started with a welcome speech of the new President of SETAC Europe Sabine E. Apitz and of the Deputy Rector of the University of Milan Giovanni Onida. The 3rd workshop hosted a total of 97 attendees, all in person, of which 64 from academia, 17 from National research centers (CNR, ENEA and many others), 3 from companies and 13 from National Agencies and Institutes. 30 oral presentations and 16 posters were distributed into several thematic sessions as follows: (i) Environmental chemistry and exposure assessment: analysis, monitoring, fate and modeling; (ii) Environmental and human toxicology: from molecules to organisms, from omics to in vivo; (iii) Ecological and human health risk assessment of chemicals, mixtures and stressors and risk mitigation strategies; (iv) Moving Beyond - Cross Cutting Themes, Emerging and Transdisciplinary; (iv) Ecotoxicology becomes stress ecology: from populations to ecosystems and landscapes. Several topics were covered during sessions including environmental occurrence and ecotoxicological effects of contaminants of emerging concerns addressing pharmaceuticals, micro-nano and bioplastics, PFAS but also legacy pollutants as mercury and metal-based pesticides; impact of climate changes on marine biodiversity (i.e., ocean acidification); regulatory topics as Environmental performance management and certification, environmental risk assessment and life cycle assessment.

The competition for the "Best Young Italian Scientist Award", a prize established since 2018 which provides a free registration to the SETAC Europe congress of the following year seen the involvement of 14 young researchers. The evaluation was based on the reviews of extended abstracts presented by candidates (with objective scientific criteria established by SETAC Europe) and on the oral presentation skills of the research, assessed through a 5-minute pitch during the workshop. The workshop ended with the invitation to attend the next SETAC Europe conference in Vienna in May 2025 and anticipation of the upcoming 4th workshop of the SETAC ILB, which will be held in Naples in 2025, hosted by the University of Naples Federico II.

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Oral Presentations

Session A - Environmental chemistry and exposure assessment: analysis, monitoring, fate and modelling

Temporal variability of floating plastic contamination in Lake Maggiore (Northern Italy): implications for sampling strategies

C. Cremonesi[1], S. Magni[1], R. Sbarberi[1], C. Della Torre[1], S. Galafassi[2], A. Binelli[1]

[1]Department of Biosciences, University of Milan, Milan, Italy. [2]National Research Council, Water Research Institute (CNR-IRSA), Verbania Pallanza, Italy.

The dangerous consequences for organisms associated with the presence of plastic waste in the environment have become increasingly evident in recent years, prompting the United Nations to declare this type of pollution one of the most critical emerging environmental issues of our time. Plastic is continuously released, particularly into aquatic ecosystems, with freshwaters represent one of the main environmental compartments affected by plastic pollution. In addition, approximately the 80% of plastic waste comes from terrestrial source and it is transported to the seas through lakes and rivers. Consequently, it is imperative to develop monitoring plans that offer a comprehensive understanding of plastic contamination in freshwater environments. For this reason, the aim of this project was the evaluation of the quantitative and qualitative monthly variations of floating plastics in Lake Maggiore, one of the largest aquatic ecosystems in Europe. Monthly samplings were carried out from January to December 2022 using a manta-net (100 µm mesh), along a transversal transect spanning from coast to coast. Every plastic particle was then quantified and characterized (shape, size, colour and polymer) using a µFT-IR. Results revealed low levels of plastic contamination in Lake Maggiore compared with other lakes worldwide, with a clear prevalence of large microplastics (size from 1 to < 5 mm) and microplastics (from 1 to < 1000 µm). Furthermore, a marked qualitative heterogeneity was observed, suggesting a prevalent secondary contamination source. Specifically, despite significant seasonal variations, we observed a predominance of polyester fibers and polyethylene and polypropylene fragments, suggesting secondary contamination primarily due to the release of plastics by wastewater treatment plants, atmospheric transport, and the fragmentation of plastic objects dispersed in the environment. Our data illustrates also a considerable quantitative variability, with 13-fold difference between the minimum value of 4375 plastics/km^2 (0.02 plastics/m^3) in September and the maximum value of 57.692 plastics/km^2 (0.29 plastics/m^3) in December. This study represents the first investigation of plastics in a lake through monthly samplings, highlighting the need to consider temporal variation to accurately assess the extent of plastic pollution.

First evidence of microplastics in three invasive crayfish species in Lake Maggiore

G. Cesarini[1], R. Sbarbieri[2], D. Schiavetta[1,3], M. Orlandi[1], L. Kamburska[1,4], S. Magni[2], A. Binelli[2], A. Boggero[1]

[1]National Research Council—Water Research Institute (CNR-IRSA), Verbania Pallanza, Italy. [2]Department of Biosciences, University of Milan, Milan, Italy. [3]University of Piemonte Orientale (UPO), Vercelli, Italy. [4]National Biodiversity Future Center, NBFC, Palermo, Italy

Invasive species and microplastics (MPs) pose significant threats to aquatic conservation, with their combined effects potentially causing severe ecosystem damage. Invasive are a major driver of global biodiversity decline, while MPs have numerous physical and ecotoxicological impacts on biota, also serving as vectors for environmental pollutants and pathogens. In Europe, crayfish are among the most common freshwater invaders, leading to local extinctions of native species, damage to freshwater resources, and disruption of productive activities. Lake Maggiore, characterized by intense urbanization and industrial activity, is an important study area for evaluating the combined

effects of these environmental pressures due to its biological, chemical, and organic pollution. This study aimed to assess the presence of invasive crayfish in Lake Maggiore and their bioaccumulation of MPs. Three invasive crayfish species were identified along the littoral zone: *Faxonius limosus*, *Pacifastacus leniusculus*, and *Procambarus clarkii*. Analysis of the digestive tracts of individuals of different sizes and sexes revealed MP accumulations of 0.07 ± 0.06 items/specimen in P. clarkii, 0.20 ± 0.10 items/specimen in *P. leniusculus*, and 0.37 ± 0.31 items/specimen in *F. limosus.* Microfiber shape made of polyester and polyacrylate were the most common in the three species. The digestive tract content of invasive crayfish may reflect the bioavailability of MPs in Lake Maggiore, providing an alternative to sampling abiotic matrices and simultaneously aiding in the removal of invasive species. Further research is needed to identify the most suitable crayfish species as indicators of MP pollution and to determine if other organs, particularly those involved in the food cycle such as muscle tissue, can accumulate more MPs.

Textile chemicals of environmental relevance: from production to surface water

Gambino[1], E. Terzaghi[1], G. Palmisano[1], G. Bergna[3], E. Baldini[2], A. Di Guardo[1]

[1]Department of Science and High Technology, University of Insubria, Como, Italy. [2]CTSS -Centro Tessile Serico Sostenibile, Como, Italy. [3] Lariana Depur, Fino Mornasco (CO), Italy

It is estimated that textile production is responsible for approximately 20% of global clean water pollution, due to dyeing, finishing products, and other chemicals. Indeed, textile wastewater are characterized by a variety of micropollutants (MPOs) of environmental concern. MPOs are organic chemicals either anthropogenic or of natural origin, found in the ecosystems generally at low concentrations (µg/L or ng/L). These chemicals are released into surface water bodies through wastewater treatment plants (WWTPs). This presentation has as its main objective to highlight the presence of textile MPOs discharge from textile industry (from raw materials to the final product) to WWTPs, and to the receiving surface waters. Their environmental fate and ecotoxicity was also addressed. A preliminary risk assessment was carried out with the aim of highlighting the potential environmental risks associated with the discharge of chemicals of textile interest. The research provided a list of 466 chemicals of textile origin (e.g. dyes, aromatic amines (AA), phthalates etc.). Some chemical groups are worth mentioning due to their relevant concentration and environmental concern. PFAS, used as water repellents in textile fabrics, represent one of the groups commonly found in WWTPs and sludge. Concentrations were higher in WWTP effluents rather than in influents, suggesting a scarce efficiency of conventional removal treatments. Long chain PFASs (PFOS, PFOA, PFDA, PFDoDA) were the most encountered compounds. PAHs used as dye and printing auxiliaries (dispersant), were among the most abundant contaminants of textile wastewater, especially in dyeing and printing processes. 3 and 4 ring PAHs were the main encountered chemicals. Akylphenols (APs) and alkylphenol ethoxylated (APEOs) were the third large group of chemicals found. Among them, nonylphenol (NP) and nonylphenol ethoxylate (NPEOs) were the most abundant compounds reported in WWTPs. Several other chemical class were also found in surface water affected by the proximity to the textile industries and the discharges of treated effluents of textile origin. Among all, dyes, APEOs, and OPEs showed relevant concentrations in surface water. Out of the 466 compounds emerged from the reviewed literature, more than one third were listed for their environmental relevance and for which acute toxicity EC(L)50 data were available. All reported measured environmental concentrations (MECs) were below the calculated PNEC, except for NP, NP2EO, TEHP and TPHP confirming, at least for single chemicals, that for most classes no appreciable risk seems to be evident, with MECs one or two orders of magnitude (or more) lower than corresponding PNEC.

Polluting while cleaning: the case study of Polycyclic Musk Fragrances

S. Tasselli, L. Guzzella

Consiglio Nazionale delle Ricerche – Istituto di Ricerca Sulle Acque (CNR-IRSA), Brugherio (MB), Italy

Polycyclic Musk Fragrances (PMFs) are compounds incorporated in detergents, perfumes, and house-cleaning products. Within PMFs there are Galaxolide, its oxidated metabolite Galaxolidone,

Tonalide, Celestolide and Phantolide. They are discharged every day into sewage systems and can enter aquatic ecosystems through wastewater treatment plant (WWTP) effluents. Since these chemicals proved to be lipophilic, persistent, and highly bioaccumulative compounds, the exposure of aquatic organisms to the continuous discharge of PMFs is raising many concerns about their possible adverse effects. The presence of PMFs in Italy is still not widely studied even if this country is the EU-Member State with the highest consumption of these chemicals. During last years, our group evaluated the presence and the fate of synthetic fragrances in various environmental metrices. First of all, PMFs were measured in some WWTPs in Northern Italy applying conventional systems for wastewater purification evidencing the incomplete removals of these compounds together with their accumulation in sludges and the increase in metabolite concentrations in the water matrix. Then, PMF presence was assessed in surface waters and sediments of the main tributaries of an Italian anthropized subalpine lake. PMFs were detected up to hundreds of ng/L especially in small rivers where dilution could not affect concentrations discharged through WWTP effluents. A common temporal trend was evidenced in all rivers, probably due to the effect of rainfall. The highest concentrations were recorded in the smaller rivers that cross anthropized areas. Sediments analysis showed accumulation of these compounds in the finest fraction together with a high variability of the measured concentrations. The analysis of two species of bivalves from the same lake, *Dreissena polymorpha* and *Corbicula fluminea*, confirmed the bioaccumulation capacity of these compounds, highlighted by the high concentrations measured in the soft tissues of individuals which reached values of over 1500 ng/g l.w. in sites close to the main inhabited centers on the lake shores. This scenario highlights not only the need to improve water purification systems in order to interrupt the continuous introduction of these compounds into receiving water bodies, but also the high potential for persistence and bioaccumulation of these substances both in the abiotic compartment and in the biota of water aquatic ecosystems and which could lead, in the future, to reaching levels of these chemicals extremely toxic for aquatic organisms and also the human health.

PAH hydroxy-metabolites on plant leaves: where do they come from? From field data to laboratory experiments

E. Terzaghi, D. Siniscalchi, E. Zanardini, A. Di Guardo

Department of Science and High Technology, University of Insubria, Como, Italy.

Polycyclic aromatic hydrocarbons (PAHs) constitute a class of organic contaminants, that can derive from the incomplete combustion of wood and fossil fuels and vehicular traffic (e.g., diesel-powered vehicles). Once emitted they can be found in the atmosphere in gaseous form or associated to particulate matter depending on their physico-chemical properties. In addition, PAHs can also be transformed through various chemical and biological processes, including photodegradation, chemical oxidation, nitration, and biodegradation by several microorganisms (i.e., bacteria, fungi, etc.). As a result of these transformation and degradation processes, several type of PAH metabolites are generated, through the substitution of one hydrogen atom by nitro-, oxy- or hydroxyl-functional groups (N-PAH, O-PAH, OH-PAH, etc.) or one carbon atom by sulphur (S-PAH). Moreover, PAH metabolites may be also directly emitted as primary pollutants together with parent PAHs, during the incomplete combustion and pyrolysis of fossil fuels, biomass, wood and coal. For instance, several studies indicate the coexistence of primary hydroxy-PAH metabolites and parent PAHs in air particulate matter. Plants can remove PAHs from the air through the so-called Forest Filter Effect accumulating these compounds in leaves and transferring them to soil. Moreover, the phyllosphere host significant amounts of bacteria that can contribute to the biodegradation of PAHs producing metabolites such as OH-PAHs. However, no studies are available so far about the presence and source of PAH metabolites in plant leaves. The main goals of this work were to investigate: 1) the presence of OH-PAH and di-OH-PAH metabolites on plant leaves of several plant species collected in field in different sites and, 2) the formation of OH-PAH metabolites mediated by light (photodegradation) and microorganisms (biodegradation) through a laboratory experiment. This was performed through target analysis and suspect screening of PAH metabolites using an

UHPLC-HRMS (LC-Orbitrap). Several OH- and di-OH-PAH metabolites were found in leaves of *Quercus ilex*, *Photinia glabra*, *Laurus nobilis* and *Ostrya carpinifolia* at ng/g dw level. Laboratory experiments showed that OH-phenanthrene can be produced in 14 days and its formation could be attributed to both photo- and biodegradation. The comparison of field and laboratory experiments highlighted a different distribution of three OH-phenanthrene metabolites indicating that leaves in field were continuously accumulating the metabolites from air, probably reflecting the composition of air PM, while in the experiment just the most favoured metabolites could be detected. Further studies are necessary to better investigate the source and the formation of PAH metabolites on leaves.

DDT in Lake Maggiore: temporal variation from 1998 to 2023

M. Porro, S. Tasselli, C. Roscioli, L. Guzzella

Consiglio Nazionale delle Ricerche – Istituto di Ricerca Sulle Acque (CNR-IRSA), Brugherio (MB), Italy

DDT (dichloro-diphenyl-trichloroethane) is one of the modern synthetic insecticides, highly hydrophobic but it has a great solubility in different organic compounds. It was used in the 1940s to contrast different insect pests along the crop and livestock production and it was also an efficient agent against different diseases such as malaria and typhus. Italy was an example of that: it was crucial to eradicate malaria in Sardinia, where the disease was endemic. In 1950s, DDT's toxicity started to be analyzed worldwide, like Rachel Carson did in her book where she talked about different toxic effects in some pesticides, DDT included. Thanks to that and other different studies DDT was banned in 1972 in USA and in 1978 in Italy. Due to its persistence and toxicity, it was included under the Stockholm's Convention as a POP (persistent organic pollutants). Even so, for its ability to bioaccumulate it's still found through the food chain along rivers and lakes. Talking about Lake Maggiore it was first detected in 1996, measuring different fish species where the level of DDT was above the legal limit for edible fish. It was seen that the source of pollution was a chemical plant that discharged for years the DDT in river Marmazza which is an effluent of the River Toce, one of the major affluents of Lake Maggiore. The first monitoring program started in 1998 revealed that DDT spread all over the entire lake environment. Pallanza bay, where the river Toce is situated, was found to be highly contaminated by this pollutant, thanks to the analysis made in a species of mollusk, *Dreissena polymorpha*. The mollusk is a great sentinel organism since it attaches to the substrate though a byssus and it can accumulate different pollutants by filter feeding. A part from mollusks, different matrices were analyzed, particularly sediment cores, tributaries sediments and fishes. This study wants to summarize the different effects of DDT in these matrices, focusing particularly on the biomagnification along the trophic chain in order to assess the actual level of contamination. In particular, the analysis is made on Lake Maggiore and in its tributary, River Toce and wants to highlight the difference between the last year of analysis, 2023 and the first contamination detected in 1998, focusing on the temporal variation of the contaminant.

Session B - Environmental and human toxicology: from molecules to organisms, from omics to *in vivo*

Evaluation of adverse effects induced by virgin and sampled plastics on *Chironomus riparius*

R. Sbarberi[1], C. Della Torre[1], L. Nigro[1], E. Tediosi[2], B. Ponti[2], M.C. Neri[2], A. Binelli[1], S. Magni[1]

[1]*Department of Bioscience, University of Milan, Milan, Italy.*
[2]*Chemservce s.r.l. - LabAnalysys group, Origgio, Italy*

Since the majority of ecotoxicological studies on the effects of plastics are focused on the impact of virgin polymers, there is a notable lack of knowledge on the toxicity of plastics directly collected in the environment, especially on benthic species. Therefore, this study addressed this gap by comparing the toxicity of three concentrations (22,400 plastics/kg sediment dry weight - d.w., 112,000 plastics/kg d.w., 224,000 plastics/kg d.w.) of virgin polystyrene (PS) microbeads with a size of 1 µm with the eventual effects induced by plastics sampled in water and sediments of four Po Rivers Tributaries (Ticino, Adda, Oglio, and Mincio Rivers). We investigated the toxic effects across two generations of *Chironomus riparius* benthic larvae from 0 to 56 days of exposure. Concerning PS, the OECD 233-*Chironomus* Life Cycle Test was adapted to determine the Effect Concentration 50 (EC_{50}), No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC). Additionally, we assessed biomarkers of oxidative stress (Superoxide Dismutase - SOD - activity and Reactive Oxygen Species - ROS - quantification), detoxification (Glutathione-S-Transferase - GST - activity), neurotoxicity (acetylcholinesterase - AChE - activity), and energetic metabolism (Glycogen – GLY - quantification) in the second generation exposed to PS. Subsequently, the OECD 233 was applied also to organisms exposed to river-sampled plastics, only measuring emergence ratio (ER), development rate (DR), fecundity (FEC), and fertility (FER), since no plastic concentration ranges were available for this test. Our findings revealed a high LOEC value of 22,400 PS kg/d.w. in the second generation, with no significant differences in biomarkers. However, plastics from Adda River (41 plastics/600 g wet weight - w.w. - of reconstituted sediment) induced a significant reduction in ER in the second generation. These results confirmed that the plastic toxicity is mainly modulated by physical factors, such as polymer type, size, and shape, rather than concentration

Effects of tire rubber-derived contaminants on aquatic and terrestrial organisms

S. Magni[1], F. Gagné[2], C. Gagnon[2], E. Eysseric[2], E. Roubeau Dumont[2], R. Sbarberi[1], L. Nigro[1], J. Požeg Krišković[3], C. Della Torre[1], A. Binelli[1]

[1]*Department of Biosciences, University of Milan, Milan, Italy.*
[2]*Environment and Climate Change Canada, Montréal, Canada.*
[3]*Department of Biology, University of Zagreb, Zagreb, Croatia*

Tires are a source of (micro)plastics and the additives used in tire production could induce adverse effects on biota, implementing the potential issue associated to plastic pollution. For this reason, the aim of this study was the evaluation of adverse effects induced by three different tire rubber-derived contaminants, represented by 1,3-dimethylbutyl-N′-phenyl-1,4-benzenediamine (6PPD), applied as antioxidant, its main metabolite 6PPD-quinone (6PPDq), and 1,3-diphenylguanidine (DPG), used as vulcanizer. We tested the chronic toxicity in different aquatic and terrestrial organisms, represented by larvae of *Danio rerio* (zebrafish, exposed for 0 to 120 hours post fertilization - hpf - at 500 ng/L of each chemical), *Daphnia magna* (exposed for 21 days to 500 ng/L and 50 µg/L of DPG) and earthworm *Lombricus terrestris* (adults and juveniles exposed for 28 days to 100 µg/Kg of each chemical). The eventual adverse effects were investigated through a wide battery of biomarkers and gel free proteomics. No acute effects were observed in exposed specimens of all considered species for the tested concentrations. Concerning zebrafish, the obtained results highlighted a low toxicity of tested chemicals, with a maximum of 9 proteins modulated by DPG treatment, as well as a significant increase of ethoxyresorufin-O-deethylase activity in specimens exposed to this additive. The absence of significant effect was

observed also in *D. magna* upon exposure to DPG. As for earthworms, all chemicals affected body growth of adult specimens and induced cytotoxicity. Besides, all chemicals inhibited the activity of alkaline phosphatase and DPG induced catalase and glutathione-S-transferase. Conversely, no effects were measured in juveniles. Results suggest that soil organisms might be more susceptible to the toxicity of these chemicals, nevertheless, other investigations are needed regarding their potential impact on aquatic environment, e.g., considering other ecotoxicological endpoints and investigating the tire additive fate in water.

SSRI antidepressants in non target marine invertebrates: multiple mechanisms of action of fluoxetine in the hemocytes of *Mytilus galloprovincialis*

T. Balbi[1], L. Canesi[1], C. Ciacci[2]

[1]Department of Earth, Environmental and Life Sciences-DISTAV, University of Genova, Italy. [2]Department of Biomolecular Sciences, University "Carlo Bo", Urbino. Italy

Due to their wide prescription and use, different types of antidepressants are detected in the aquatic environment and their ecotoxicological impact is of growing concern. Among the most common antidepressants, selective serotonin reuptake inhibitors-SSRI, Fluoxetine (FLX) is one of the most studied and detected in the aquatic environment, where it is found at ng-µg/L levels and has been shown to exert a number of effects in aquatic species at environmentally relevant levels. In mammalian systems, the use of FLX in treatment of depression is associated to its binding to presynaptic receptors preventing serotonin (5-HT) reuptake (high affinity for serotonin transporters SERT), resulting in an increase of 5-HT levels in the synaptic cleft, which potentiates serotonergic signaling. This MOA is presumed to occur also in non target aquatic vertebrates, as shown by the behavioural responses of fish to FLX exposure. With regards to invertebrates, components of the serotonergic system (receptors and related transduction systems) have been identified in bivalve molluscs. However, only one report is available on modulation of serotonergic components by contaminant exposure in bivalves, namely heavy metals. In different bivalve species, *in vivo* exposure to environmental concentrations of FLX has been shown to affect a number of biomarker responses in different tissues: immune responses, genotoxicity, oxidative stress, biotransformation and, more recently, lysosomal responses. However, no relationship has been described between the MOA of FLX in mammalian systems and the responses observed in bivalves. However, FLX has been recently described to have multiple mechanisms of action in mammalian cells: among these, FLX acts as a lysosomotropic agent, modulates autophagic and mitophagic processes and lipid metabolism. In this work, the mechanisms of action of FLX other than modulation of 5-HT signaling were investigated in *Mytilus galloprovincialis* cells utilizing the *in vitro* model of freshly isolated hemocytes in short term exposure conditions (1-4 h, 0.1-1-10 µM FLX). Data are presented on lysosomal parameters, immune responses, and autophagy processes. The results indicate that FLX acts on mussel hemocytes through multiple mechanisms of action similar to those observed in mammalian cells, although at much shorter exposure times. In particular, in mussel cells FLX acts as a lysosomotropic agent, induces frustrated phagocytosis and inflammatory responses, lipid accumulation, mitochondrial dysfunction and affects the autophagic flux, leading to lysosomal degradation of mitochondria. These data contribute to shed some light on the MOA of SSRI antidepressants in marine invertebrates.

Mechanisms of molecular and cellular responses of human pharmaceuticals mixtures in non-target model species

M. Mezzelani[1], M. Panni[1], G. d'Errico[1], F. Regoli[1], D.M. Pampanin[2], S. Gorbi[1]

[1]Department of Life and Environmental Sciences, Università Politecnica delle Marche, Ancona, Italy. [2]Department of Chemistry, Bioscience and Environmental Engineering, Faculty of Science and Technology, University of Stavanger, Norway

Pharmaceuticals are contaminants of emerging concern due to their biological activity and ubiquitous detection in environmental matrices. Wild organisms are continuously exposed to cocktails of these substances, that can interact producing unexpected effects in terms of toxicity or metabolic pathway changes. This study aimed at a multidisciplinary characterization of adverse effects caused on different model species by 15

different pharmaceuticals including Non-Steroidal Anti-inflammatory Drugs (NSAIDs), antidepressants, antiepileptics, antihypertensives, antidiabetics and lipid lowering agents dosed both alone and as mixtures. Analyses on bioavailability in *Mytilus galloprovincialis* were integrated with a panel of biochemical and cellular biomarker. Ecotoxicological effects were evaluated through a battery of bioassay with different endpoints and species (bacterial bioluminescence, algal growth, oyster embryotoxicity), while in fish hepatic (PLHC-1) and gill (RTgill-W1) cell lines, were investigated responses on cell viability, production of reactive oxygen species and changes in mitochondrial membrane potential. Interactive and competing mechanisms among tested drugs modulated bioaccumulation and biological responses in mussels, with both additive and antagonistic interactions depending on investigated mixture and metabolic pathways. Ecotoxicological bioassays highlighted different sensitivity of tested species toward selected drugs and mixtures, with major effects on the oyster embryotoxicity and algal growth. In cell lines, a major sensitivity of PLHC-1 compared to RTgill-W1 was observed, revealing the key role of liver tissue in the drugs-metabolization processes. Non-Steroidal Anti-Inflammatory drugs (NSAIDs) were shown as the less reactive therapeutic class. Paroxetine, Naproxen and Atenolol were the most toxic molecules for psychiatric drugs, NSAIDs and cardiovascular drugs, respectively. Single pharmaceuticals showed greater effects than their mixtures, where toxicity was not related to the exposure dose or the number of combined molecules, indicating the presence of some antagonistic mechanisms in the tested conditions. The application of the quantitative Weight of Evidence model, summarized the overall mechanisms of molecular and cellular effects caused by pharmaceuticals and mixtures, highlighting the added value of such multidisciplinary approach for an effect-based risk assessment of such molecules in marine ecosystem.

Impact of environmental doses of dexamethasone on the reproductive health of the Mediterranean mussel *Mytilus galloprovincialis*

M. Galati[1], G. De Marco[1], B. Bille[1], A. Cristaldi[2], M.C. Eliso[1], M. Terranova[1], G. Oliveri Conti[2], M. Ferrante[2], M. Maisano[1], T. Cappello[1]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy. [2]Department of Medical, Surgical and Advanced Technologies "G.F. Ingrassia", University of Catania, Catania, Italy.

The synthetic glucocorticoid dexamethasone (DEX) has been widely used for its anti-inflammatory properties in the treatment of arthritis, psoriasis and acute respiratory distress syndrome during the SARS-CoV2 pandemic. Its continuous use combined with the inadequacy of wastewater treatment plants in retaining it, aggravated its distribution in aquatic ecosystems. This has led to questions about the potential influence of DEX on the reproductive health of non-target aquatic organisms since its known role as an endocrine disruptor. Therefore, the aim of this study was to evaluate through a multi-biomarker approach the impact of four environmental doses of DEX, selected in the range of ng/L - µg/L, at different temporal checkpoints (T0, T3, T6, T12) for 12 days on the gonochorous species *Mytilus galloprovincialis*, a commercially edible bivalve mollusc, in order to understand the potential effects on the gonads of both sexes. The chemical data highlighted a higher tissue bioaccumulation of DEX at all time-points for the highest concentration tested (C4). Histomorphological analyses reported an early inflammatory response in both sexes given the presence of hemocytes distributed along the connective tissue with, in males only at T3 and T6 for C4 and at T12 for C2, infiltration within the follicle. Immunofluorescence showed an increase in positivity to the anti-FasL antibody at T3, while a raised response to the anti-PCNA antibody was recorded at T6, indicating a recovery in cellular turn-over. The Periodic Acid Schiff reaction showed an alteration of glycogen reserve attributable mainly to the vesicular connective tissue cells. The molecular analysis of key genes of the antioxidant pathway (Cu/Zn-SOD, Mn-SOD, CAT, OvoA) supported further the action exerted by DEX on both sexes. 1H NMR-based metabolomics demonstrated, differentially between sexes, the

impact on gonadal energy demand with increased metabolites such as acetoacetate and lactate. Overall, the results suggested that DEX exposes both male and female reproductive systems of organisms not directly targeted by the drug to a realistic danger, and since mussels are known bioaccumulator organisms and widely used in the Mediterranean diet, this could translate into a risk for human health.

Tissue and molecular alterations caused by the emerging contaminant dexamethasone in marine mussels and the use of ulvans to counteract its harmful effects

G. De Marco[1], A. Cristaldi[2], M.C. Eliso[1], D. Spagnuolo[1], G. Oliveri Conti[2], M. Galati[1], B. Billè[1], M. Terranova[1], V. Parrino[1], G. Genovese[1], T. Cappello[1], M. Ferrante[2], M. Maisano[1]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy. [2]Department of Medical, Surgical and Advanced Technologies "G.F. Ingrassia", University of Catania, Catania, Italy.

The discharge of various chemicals in the environment has significantly grown in the recent decades. Pharmaceuticals active compounds (PhACs), among the emerging contaminants, cause particular concern due to their ability to easily interfere with biological systems, besides the inadequacy of wastewater treatment plants to retain these compounds. Therefore, measures are urgently required to evaluate and mitigate the negative effects induced by these compounds on biota. Among the non-target organisms affected by PhACs, marine mussel *Mytilus galloprovincialis* (Lamarck, 1819) is a species that needs to be carefully monitored due to both its ecological and commercial importance. To this aim, marine mussel specimens were initially exposed for 12 days to four realistic concentrations of dexamethasone (DEX, 4 ng/l; 40 ng/l; 400 ng/l; 2 µg/l), the most widely utilised anti-inflammatory glucocorticoid worldwide, to assess its effects at the tissue and cellular level on gills, a key organ in gas exchange and nutrient filtering. Subsequently, a combined exposure of DEX (400 ng/l) and ulvans (100 mg/l) for 12 days was carried out on mussels to explore the antioxidant effect of these polysaccharides extracted from the algae belonging to the genera *Ulva spp*. Using a multi-biomarker approach, it was possible to observe the ability of the gills to uptake DEX as early as 3 days of exposure, with a time-dependent bioaccumulation in the specimens exposed to the higher tested DEX concentrations (400 ng/l; 2 µg/l). DEX exposure led to modifications in acid and neutral mucopolysaccharides in the gills, with a rise in the levels of the former and a drop in the latter. By using a molecular approach, the expression of the genes related to the cellular anti-oxidant response (Cu/Zn SOD; Mn SOD; ovoA) displayed a general down-regulation in the early stages of exposure, imputable to the DEX anti-inflammatory effect. However, at prolonged exposure time-points, this trend was followed by an upregulation, confirming the DEX's pro-oxidant effect, as previously observed in other studies. Preliminary results concerning the effects of ulvans to counteract the negative effects of PhACs confirmed the antioxidant properties of these algal extracts also on aquatic invertebrates, as well as a partial ability to mitigate the neurotoxic effects induced by DEX. Overall, the use of ulvans to counteract the environmental impact of emerging contaminants, including PhACs, has proven to be a promising approach that requires further investigations.

On the safe usage of copper sulphate in organic farming: evidence for genotoxic and enzymatic effects on a non-target species, the Mediterranean stick insect *Bacillus rossius*

M.L. Vannuccini[1,2], C. Pardo[1], R. Meschini[1], E. Rapiti[1], A. Bellati[1], D. Canestrelli[1]

[1]Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy. [2]Ichthyogenic Experimental Marine Center (CISMAR), Borgo Le Saline (Tarquinia), Italy

Copper sulphate is a widely used chemical in agriculture and industry, commonly employed in organic farming. Despite its extensive application as a fungicide, herbicide, and pesticide, environmental and biological impacts are not fully understood, particularly concerning non-target organisms. Insects play critical roles in ecosystems, therefore understanding their response to pollutants is crucial for assessing environmental health and sustainability. We investigated genotoxic and neurotoxic effects in the

Mediterranean stick insect (*Bacillus rossius*) exposed to copper sulphate to elucidate the extent to which this chemical affects genetic material and enzymatic functions. Animals were exposed to two different concentrations of copper sulphate over specified time frames to simulate both acute (short-term, high-dose) and chronic (long-term, low-dose) exposure scenarios. Genotoxicity was assessed by the comet assay technique to determine the extent of DNA damage. Enzymatic alteration was measured by quantifying changes in acetylcholinesterase (AChE) activity, a key enzyme involved in neurotransmission. The results showed a significant increase in DNA damage in *B. rossius* exposed to copper sulphate. The comet assay indeed revealed a significant increase in DNA strand breaks, confirming that copper sulphate is highly genotoxic under different exposure conditions. Furthermore, AChE activity resulted modulated under the selected exposure conditions. In conclusion, this study provides compelling evidence that copper sulphate poses significant risks to *Bacillus rossius*, highlighting the need for careful management of copper-based compounds in the environment where these and other insects are naturally present. The observed mild alteration of AChE activity, while not as severe as the genotoxic effects, still warrants attention, due to the potential long-term physiological effects in insects inhabiting agricultural ecosystems. These findings underscore the importance of further research into the environmental impact of copper sulphate and advocate for the development of effective strategies to mitigate its adverse effects on non-target organisms.

Effects of mercury exposure on oxidative stress biomarkers and the ovarian morphology of the ascidian *Ciona intestinalis* adults

S. Melki, S. Bejaoui, A. Azzouna, R. Ben Ahmed

Laboratory of Ecology, Biology and Physiology of aquatic organisms LR18ES41, Department of Biology, Faculty of Sciences of Tunis, University of Tunis El Manar, Tunis, Tunisia

Mercury is among the most damaging pollutants in the aquatic ecosystem. The purpose of the present study was to assess its effects when administered at gradual concentrations (1, 10, 50 and 100 µg/l) to evaluate antioxidant status in the gonadodigestive complex of the solitary ascidian *Ciona intestinalis* adults. The results showed an increase in malondialdehyde (MDA), protein carbonyl (PCO), glutathione (GSH) and Glutathione S-transferase (GST) levels. In addition, an increase in metallothionein (MT) level. Besides mercury induced a decrease in acetylcholinesterase (AChE) activity. Moreover, histological analyses shows that mercury induced various modifications on *C. intestinalis* ovarian morphology that involves irregular outline of nuclear membrane, less compacted cytoplasm, detachment of test and follicular cells from the oocyte membrane, yet same oocytes were destroyed after treatment with the higher concentration. Overall, this study demonstrated mercury toxicity on the redox status of *C. intestinalis* and supports the use of ascidinas as bioindicators of water quality.

Session C - Ecological and human health risk assessment of chemicals, mixtures and stressors and risk mitigation strategies

Spot applications to control thistles in sugar beet fields – how to link the actual practice to the GAP table and the environmental risk assessment? An example case study

G. Meregalli[1], E. Pilling[2], O. De Cirugeda[3], P. Havens[4], V. Guth[5], E. Rogissart[5], V. Bosco[1], M. Colombo[1], E. Garcia[6], P. Otero Sancho[6], A. Alix[2]

[1]Corteva Agriscience Italia s.r.l., Cremona, Italy. [2]Corteva Agriscience UK Ltd, Abingdon, United Kingdom. [3]Corteva Agriscience International Sàrl, Versoix, Switzerland. [4]Corteva Agriscience, Indianapolis, U.S.A.. [5]Corteva Agriscience France S.A.S., Guyancourt, France. [6]Corteva Agriscience Spain S.L.U., Pozuelo de Alarcon, Spain

The interest around the use of precision technologies for the application of plant protection products (PPP) in agriculture is increasing in Europe as, with precision applications (PA), only the required amount of a suitable product is applied where it is required to protect crop plants. EUPAF is the EUropean Precision Application task Force, a scientific platform created in June 2023 to gather knowledge and expertise on precision uses and PA techniques as well as to identify the implications of PA implementation on risk assessment and risk reduction. The current Environmental Risk Assessment (ERA) framework is designed for standard broadcast applications and may need to be adapted for a realistic assessment of the risk to environmental compartments following PA. In our case study, actual percent treated area data from spot applications of a herbicide in sugar beet fields to control thistles was gathered. A specialized spot sprayer was deployed on 39 fields, treating a total area of 458 hectares. These spot applications achieved between 24 and 76% savings in herbicide usage, with a mean 50% reduction compared to standard broadcast applications. The implications for description of the spot application in the GAP table, as well as for some environmental risk assessment aspects, will be illustrated.

Stress priming tool: memory mechanisms to mitigate organism vulnerability to multiple stressors

A. Nardi[1], M. Milan[2], D. Cesaroni[1], V. Tavolazzi[1], F. Regoli[1], I. Bernardini[2], G. Della Rovere[2], G. Monticelli[2], L. Peruzza[2], M. Benedetti[1]

[1]Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy. [2]Department of Comparative Biomedicine and Food Science, University of Padova, Padua, Italy

In the last decades heatwaves and chemical pollution severely affected bivalve inhabiting the Adriatic Sea, drastically reducing farmed and natural populations. Among the most impacted species, the Manila clam (*Ruditapes philippinarum)*, has been subjected to several mortality events impacting farmed and natural stocks, resulting in biodiversity loss and socio economic impacts. This study aims to investigate novel chemical and thermal priming strategies to ameliorate Manila clam performance under multiple stressful conditions. Priming consists in stimulating stress-memory mechanisms, providing a transient increased tolerance toward stress in organisms pre-subjected to a bout of a similar stress. In this context, chemical and heat priming were carried out on Manila clam alone or in combination for one week and, after a resting period of 15 days, animals were then exposed to multi-stressor scenario characterized by a simulated heatwave and chemical pollution with 2µg/L of perfluorooctanoic acid (PFOA). This multi-stressor scenario was chosen because it mimics the environmental conditions of the Venice lagoon, hence the results of our study carry a high biological and ecological relevance not only for Manila clams, but most likely also for other bivalve species of the lagoon. This study investigates molecular and cellular processes at the base of phenotypic plasticity derived from priming in this species. Preliminary results highlighted promising

and interesting adaptative mechanisms and a new point of view on actions/interactions of biological processes involved in the responsiveness and adaptation of marine organisms to environmental changes, opening new scenarios for research in applied biology, and for sustainable bivalve aquaculture management.

A meta-analysis of levels and physiological effects of pharmaceuticals in marine invertebrates

S. Franzellitti

Department of Biological, Geological and Environmental Science (BiGeA), University of Bologna, Ravenna, Italy

Pharmaceutical products are essential elements of the medical practice and their beneficial effects on human and veterinary health are widely acknowledged. However, we lack a global understanding on the biological impacts of these chemicals when they are discharged into the environment, either through consumption or as unused or expired products. A large body of literature data confirm that pharmaceuticals occur widely in marine and coastal environments; therefore, assessment of comprehensive risk to marine species needs to be elucidated. To this regard, marine invertebrates are key as intermediate consumers in the pelagic and benthonic food chains, hence they have been profitably used to assess the occurrence of pharmaceuticals in marine ecosystems worldwide and to infer their biological effects and potential ecological and human health risks. Drawing on the large body of available literature, this study will provide a comprehensive re-analysis of data on pharmaceutical accumulation in field biomonitoring surveys using marine invertebrates as sentinel organisms. Besides giving the overall distribution of pharmaceuticals in marine systems worldwide, results will infer possible hotspots of contamination by pharmaceuticals and find out the most detected therapeutical class. Furthermore, we conduct a meta-analysis of available biological data gathered through laboratory and field studies to examine how marine invertebrates deal with pharmaceutical pollution in terms of most relevant stress biological responses and shared effects related to the therapeutic modes of action of the compounds, while addressing the most vulnerable species according to geographical location and/or peculiar physiological and ecological studies. Overall, this study aims to collate perspectives developed during the past few years regarding the potential biological impacts of pharmaceuticals and to draw a snapshot of vulnerability of world marine systems towards pharmaceutical pollution.

Employment of rapid bioassays for detection of toxicity following exposure to different organic substrates

S. Vezzoli[1], M. Menghini[1], R. Pedrazzani[1,2]

[1]DIMI-Department of Mechanical and Industrial Engineering, University of Brescia, Brescia, Italy. [2]MISTRAL-Inter-University Research Center "Integrated Models for Prevention and Protection in Environmental and Occupational Health", DSCS, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy.

Contamination due to a cocktail comprising an increasing number of pollutants, with possible synergistic, additive, and antagonistic effects, requires monitoring studies in which traditional chemical analyses are supported by bioassays able to quantify the effects of these environmental mixtures. In view of the widespread adoption of effect-based monitoring strategies by the EU, rapid, reliable, robust, and reproducible toxicological diagnostic tools are needed. These tools can be useful not only for the scientific community but also for integrated water cycle companies, local environmental protection agencies, sludge treatment platforms, and many others. In this study, different kits were selected and applied to assess the potential toxicity of organic substrates on plants and bacteria. Specifically, potential baseline toxicity was assessed through a phytotoxicity test on the germination and root elongation of *Lepidium sativum*, *Sorghum saccharatum*, and *Sinapis alba*, as well as a colorimetric assay on a mutated strain of *Escherichia coli*. Additionally, genotoxicity was assessed through a short-term mutagenesis test, which provides an alternative to the classical Ames test with *Salmonella typhimurium* strains, and a colorimetric assay on an engineered *E. coli* strain. While there is evidence of inhibition of root elongation by some matrices, no toxicity was found in bacterial activity. On the other hand, no genotoxic effects were found in *E. coli*, whereas mutagenicity was found in an *S. typhimurium* strain.

Public engagement in the industrial odour management under the framework of Directive 2010/75/EU

F. Mauro

Department for Environmental Evaluation, Control and Sustainability, Italian Institute for the Environmental Protection and Research (ISPRA), Rome, Italy

Even though industries have brought economic well-being, there is scientific evidence that they can threat seriously both environment and public health. For this reason, in the European Union industrial operators are required to apply for an Integrated Environmental Authorization (IEA), according to the Directive 2010/75/UE. In Italy, the Legislative Decree n. 152/2006 regulates IEA procedure and states that ISPRA is responsible for the elaboration of the Monitoring and Control Plan (MCP) for large industrial plants. Among the industrial impacts, odour emissions are generally accounted to be responsible of several health disturbs. For this reason, in some MCPs ISPRA can include a specific odour monitoring, according to the Best Available Techniques. Currently, odour monitoring is mainly carried out through dynamic olfactometry, requiring the odour sampling. Even though such an approach has been accounted to yield more precise results, it can inevitably expose workers to occupational risks (especially in chemical and refining plants). Hence, with the aim to explore new solutions to avoid/limit risks for workers involved in odour impact assessment, an investigation of the most recent scientific literature has been conducted. More in detail, a systematic review was carried out to evaluate the public engagement (i.e. citizen science) in the industrial odour management. At an international level, citizen science has definitely spread out for several environmental monitoring purposes (e.g. biodiversity, land use, environmental pollution, marine littering etc.). Moreover, participatory approaches have been encouraged by artificial intelligence techniques, allowing quicker big data elaboration than traditional algorithms. However, with reference to odour emissions, only a few experiences of such a kind have been traced in scientific databases, thus showing the great novelty of such a theme. To sum up, this presentation aims at putting in light the advantages and limits of citizen science in the odour impact assessment and discussing the potential areas of research in such a field.

Session D - Moving beyond - Cross Cutting, emerging and transdisciplinary themes

In silico screening of hazardous properties of Pharmaceuticals and Personal Care Products, and their associated potential risk after water treatment

A. Sgariboldi[1], E. Postè[1], N. Chirico[1], A. Sangion[2], M. Evangelista[1], C. Morosini[3], A. Re[3], E. Papa[1]

[1]QSAR Research Unit in Environmental Chemistry and Ecotoxicology, Department of Theoretical and Applied Sciences, University of Insubria, Varese, Italy. [2]ARC Arnot Research & Consulting, Toronto, Canada. [3]Department of Science and High Technology, University of Insubria, Como, Italy.

Pharmaceuticals and personal care products (PPCPs) are emerging contaminants in the environment. Depending on their persistence and mobility after discharge through wastewater, PPCPs may reach different aquatic environments including surface, ground- and drinking waters, causing adverse effects to human and wildlife. The removal of these substances in wastewater treatment plants (WWTPs), and their behaviour in the environment, are of great interest for the scientific and regulatory community. Recently, the identification of chemicals that are persistent, mobile and potentially of concern for the quality of natural and drinking waters, has become a priority within the European Chemicals Strategy for Sustainability. In this study, multiple properties concerning Persistence, Bioaccumulation, Mobility and Toxicity (PBT and PMT properties) of several PPCPs found in WWTPs and in their effluents, have been investigated by combining their environmental persistence experimental information, quantified in terms of Removal Efficiency (RE) in WWTPs, and predictions generated by Quantitative Structure-Activity Relationships (QSAR) *in silico* models. QSAR models are efficient tools to predict unknown or incomplete information related to activities and properties of chemicals directly from their molecular structure. In this work, a dataset was developed from 32 literature studies representatives of different WWTPs in Europe, America, Asia, Africa and Oceania, which included experimental data like molecule identification, influent and effluent concentrations, RE and the WWTP treatment technology, for more than 200 PPCPs. The molecular structure of each substance was encoded by Simplified Molecular Input Line Entry System (SMILES) strings. This information was used as input for the application of *in silico* tools like QSAR-ME Profiler, EPISUITE, OPERA which were used for the prediction of multiple properties of the PPCPs like environmental persistence, organic carbon-water partition ratio (K_{OC}), water solubility, toxicity, in addition to other properties concerning metabolism and bioconcentration. After checking that predictions and chemical structure were within the applicability domain of the QSARs, the most hazardous compounds were prioritized on the basis of the information found in literature and of the *in silico* predictions. Seventeen substances were identified as potentially PMTs and /or PBTs. As a final analysis, the potential risk was assessed considering concentrations in WWTPs effluents reported in literature and the predicted toxicity values of all the studied chemicals. Forty-nine substances were associated to potential risk in relation to concentrations measured in WWTPs effluents. This work shows how the integration of non animal testing strategies, such as *in silico* QSAR models, in addition to existent data, is useful to screen undesired properties and the potential risks of environmental contaminants. This information is helpful to further address the regulation and the risk management of problematic compounds.

Listening to nature: how soundscapes can inform us about the health of fragmented habitats

W. Cristiano[1], M. Gamba[2], V. Ferrario[2], F. Carugati[2], V. Torti[2], D. Valente[2], C. Giacoma[2], L. Mancini[1]

[1]Unit of Ecosystems and Health, Department of Environment and Health, Italian National Institute of Health, Rome, Italy. [2]Department of Life Sciences and Systems Biology, University of Turin, Turin, Italy

Habitat fragmentation is a threat to biodiversity loss and the depletion of ecosystem services, and it is driven by anthropogenic pressures, such as deforestation and pollution, in synergy with climate change. To investigate the effects of these pressures on threatened ecosystems and to restore degraded habitats, it is crucial to assess reliable environmental quality indicators. In recent years, the study of soundscapes has emerged as a valuable and innovative tool for gaining insights into the impacts of habitat fragmentation on biotic communities. Soundscape analysis employs the use of various techniques, including machine learning, and may offer a more sustainable approach for long-term studies compared to traditional methods. By depicting the relationships between different components of an ecosystem through sounds collected from a specific site, soundscapes can help assess fluctuations in ecological integrity within a changing environment. In this context, the use of soundscape analysis is particularly encouraged for ecosystems prone to collapse due to anthropogenic impacts and climatic variations, such as tropical rainforests. By applying multiple classification algorithms to acoustic features obtained from 24-hour environmental recordings collected across three neihbouring rainforest sites in Eastern Madagascar, we tested whether soundscapes could reflect differences in habitat fragmentation and anthropogenic pressures among these areas. We found that it is possible to predict the forest in which an environmental recording was collected with a high degree of accuracy, and this classification remains consistent both day and night. Additionally, we showed that the most critical variables explaining model performances are those corresponding to higher-frequency sounds. Our results reveal that acoustic differences are correlated with the varying pressures faced by the rainforests during both day and night. Moreover, soundscapes are likely to differ primarily in the composition of species emitting high-frequency sounds, which may benefit differently from one landscape to another. These findings support the idea that soundscape analysis can show how habitat degradation alters the acoustic features of a particular site. This approach may serve as a fundamental indicator of ecosystem health, especially in highly fragmented areas.

Development of a behavioral test in the terrestrial oligochaetes *Eisenia foetida* exposed to Per and poly-fluoro-alkyl substances (PFAS)

A. Calisi, D. Gualandris, C. Lorusso, D. Rotondo, F. Dondero

Department of Science and Technological Innovation, University of Eastern Piedmont, Alessandria, Italy.

Behavioral tests are considered a promising tool for ecological risk assessment and environmental toxicology. Their use can reveal direct effects on the nervous systems or sensory organs caused by different types of pollutants or mixtures. In recent years, successful behavioral ecotoxicological tests have been carried out on earthworms, indicating that the methodological approach may result particularly effective with this group of invertebrates. The avoidance behavior of earthworms is recognized as a valuable endpoint in the assessment of soil quality. The primary objective of this study was to standardize a behavior test using multiple locomotion variables to assess the behavior of earthworms (*Eisenia foetida*). The test aimed to evaluate the impact of three alternative PFAS congeners (HFPO-DA, PF4MOBA, PF3MOPrA) and PFOA, serving as a benchmark, across a concentration range of 0.6-229 microM through OECD Test No 207 contact exposure. The locomotion variables, including escape time, path length, speed, global activity, and immobility rate, were analyzed to investigate potential effects on earthworm behavior. Results indicate a significant impact of PFAS congeners on earthworm locomotion, with noteworthy dynamics observed for GenX and PFOA during the escape test, measuring the ability to withdraw from stressful situations. Compared to the well-known avoidance test, the behavior test employed in this study offers advantages by avoiding the limitations associated with the assessment of substance repellency. Instead, it provides a comprehensive evaluation of the earthworms' responses, avoiding potential biases related to intolerance or idiosyncrasy towards specific substances introduced into the soil. Additionally, analysis of a panel of almost 30 neurotransmitters highlighted alterations in the GABAergic and cholinergic systems in earthworms exposed to alternative short-chain perfluoropropylene oxide

acids (PFAS) congeners. This behavior test holds promise as a valuable tool for assessing the behavioral effects of PFAS exposure in earthworms. The outcomes of this study hold promising implications for the development and standardization of a behavior test designed to assess neurotoxicity in terrestrial oligochaetes and shed light on the suitability of utilizing earthworms as bio-indicators for neurotoxicity testing.

Do diatom teratological forms work as a sentinel of antibiotic pollution in the aquatic ecosystem?

C. Puccinelli, L. Mancini

Department of Environmental and Health, Istituto Superiore di Sanità (ISS), Rome, Italy

Biotic communities are the best sentinel of environmental global change, responding in different ways to the several pressures affecting aquatic ecosystems. Between the biological indicators, diatoms could be the most suitable to detect antibiotics pollution rapidly: they have the shortest life time cycle (30 days), being at first level of trophic chain and are more sensitive to chemical compounds. Furthermore, they are responsible of 25% of the global primary production. This work aims to evaluate the potentiality of diatoms as a sentinel of antibiotic pollution by observing the increasing of teratological alteration of cell wall (called frustule), after exposure to a panel of antibiotics, most frequently used in therapeutic practices and spread into aquatic ecosystems, through in vitro experiments. Four diatoms, widespread in freshwater ecosystems with different ecological features were selected: one planktonic *Cyclotella meneghiniana* and three benthic, one adnate living attached to substrates *Gomphonema parvolum*, and two motile species, *Navicula veneta* and *Nitzschia amphibia.* They were exposed to the following concentration of ciprofloxacin (0.1 mg/l; 0.5 mg/l; 1.0 mg/l C, plus negative control) for 30 days in an aerated incubator at 14 ± 1°C with a 12:12 h light-dark cycle photoperiod under cool-white fluorescent bulbs (3000 lx). Each sample was collected in a tube; 3-4 mL of sample was then treated to remove organic matter, according to Standard Procedure EN 13496 and permanent slides were prepared using mounting resin Naphrax. They have been exposed for their entire cycle life then, samples were analysed detecting the alterations of frustule like valve shape, apices, raphe features, pattern of striae trough morphological analysis. All species registered the presence of antibiotics with higher frequency of teratological forms, increasing along the ciprofloxacin concentration gradient. Most deformities were referred to frustule shape, the canal raphe and the striae patterns. A major increase of teratological forms were registered for benthic species, 30 % at highest concentration respect to control, with only 5 % for the planktonic one. Alterations can affect the survival of these organisms, deformed outline cell wall limiting them in colony forming; modified raphe system could limit the possibility to move from unstable environmental conditions. Further analysis for testing their response to amoxicillin are currently in progress.

Session E - Ecotoxicology becomes stress ecology: from populations to ecosystems and landscapes

Biomarkers and cognitive analysis to assess the effects of a commercial fungicide and an herbicide, alone and in combination, on Apis mellifera

A. Di Noi[1,2], I. Caliani[2], A. D'Agostino[3], G. Cai[4], M. Romi[4], T. Campani[2], F. Ferrante[5], D. Baracchi[6], S. Casini[2]

[1]Santa Chiara Lab, University of Siena, Siena, Italy. [2]Department of Physical, Earth and Environmental Sciences, University of Siena, Siena, Italy. [3]Department of Economics and Statistics, University of Siena, Siena, Italy. [4]Department of Life Sciences, University of Siena, Siena, Italy. [5]Department of Ecological and Biological Science, Tuscia University, Viterbo, Italy. [6]Department of Biology, University of Florence, Sesto Fiorentino, Italy.

Agrochemicals are crucial for protecting crops and enhancing agricultural yields by mitigating threats from pests, pathogens, and weeds. Various factors are able to affect the toxicological status of honey bees, including pesticides. While extensive research has primarily examined insecticides' lethal and sublethal impacts on individual bees and colonies, it is essential to acknowledge that fungicides and herbicides can also have effects on bee health. Unfortunately, honey bees are exposed to mixtures of chemicals rather than isolated substances in their natural environment. This study aimed to evaluate the effects of a commercial fungicide and a commercial herbicide, both individually and in combination, on honey bees. Mortality assays, biomarkers and learning and memory tests were conducted, and results were integrated to assess the toxicological status of honey bees. Neurotoxicity (acetylcholinesterase and carboxylesterase activities), detoxification and metabolic processes (glutathione S-transferase and alkaline phosphatase activities), immune system function (lysozyme activity and haemocytes count) and genotoxicity biomarkers (Nuclear Abnormalities assay) were evaluated. The fungicide Sakura® was found to activate detoxification enzymes and alter alkaline phosphatase activity. The herbicide Elegant 2FD, as well as the combination of both pesticides, showed neurotoxic effects and induced detoxification processes. Exposure to the mixture of herbicide and fungicide impaired learning and memory in honey bees. This study represents a significant advance in understanding the toxicological effects of commonly used commercial pesticides in agriculture and contributes to developing effective strategies to mitigate their adverse effects on non-target insects.

Legacy mercury contamination in the Toce River (North Italy): from sediments to the terrestrial ecosystem

L. Marziali[1], L. Valsecchi[1], E. Fasola[1], P. Volta[2], L. Ilahiane[3], M. Morganti[1]

[1]National Research Council, Water Research Institute (CNR-IRSA, Brugherio), Brugherio (MB), Italy. [2]National Research Council, Water Research Institute (CNR-IRSA, Verbania), Verbania Pallanza (VB), Italy. [3]Department of Environmental Science and Policy, University of Milan, Milan, Italy.

Mercury is still considered a contaminant of concern in freshwater ecosystems, due to the biomagnification potential in trophic chains. Mercury bioavailability was assessed in the Toce River (Northern Italy), were residual contamination deriving from the activity of a chlor-alkaly plant is present in sediments (values up to 0.4 mg/kg d.w.). The aim was to assess the role of benthic invertebrates as a vector of mercury from sediments to higher trophic levels, both in the aquatic (predator fish) and in the terrestrial ecosystem (insectivorous birds). Total mercury (THg) and methylmercury (MeHg) were analyzed in native benthic invertebrates belonging to different taxonomic/functional groups. Collectors, shredders and predators (up to 253 µg/kg d.w. for THg and to 88 µg/kg d.w. for MeHg) exhibited higher concentrations than grazers, confirming that exposure is mainly due to the ingestion of contaminated food resources (sediments or preys). Benthofagous fish (*Salmo trutta*, *Telestes muticellus*, *Squalius squalus*) showed values up to one order of magnitude higher than preys, and almost all mercury was in the organic form (MeHg).

Concentrations of THg analyzed in adult insects were close to those in aquatic stages, proving active mercury transfer even to terrestrial food chains. Here, samples of body tissues of the resident insectivorous passerine great tit (*Parus major*) were collected and showed the presence of mercury in feathers and blood of adult females (THg up to 2 mg/kg d.w. and 0.28 mg/kg w.w., respectively) and its transfer to their offspring (THg up to 0.04 mg/kg w.w. in eggs), as proved by significant correlations between Hg levels in eggs and maternal blood ($r=0.91$) or feathers ($r=0.76$, $p<0.05$). Many water bodies in Italy and Europe fail to achieve the good chemical status because of mercury concentrations in water and/or biota. In the Toce River, mercury in benthic invertebrates (up to 73 µg kg^{-1} w.w.), i.e., at the basal levels of the aquatic trophic chain, exceed the European Environmental Quality Standard for biota (20 µg/kg w.w. in fish), determining active cycling of the contaminant and posing potential risks for top predators.

EMAS tool for environmental risk management

M. D'Amico, B. Capra, A. Caputo, B. D'Alessandro, R. Pacifico, V. Tropea, S. Ubaldini

Department for Environmental Evaluation, Control and Sustainability, Italian Institute for the Environmental Protection and Research (ISPRA), Rome, Italy

EMAS (Eco-Management and Audit Scheme) is one of the voluntary instruments activated within the V EU Action Program for the Environment. The primary aim of EMAS is to contribute to the achievement of sustainable economic development, highlighting the roles and responsibilities of businesses. Organizations (companies, public bodies, ...) with sites (offices or industrial plants), in the territory of the European Community or outside it can join EMAS to evaluate and improve their environmental performance and provide the public and other interested parties with information on their environmental management. To obtain EMAS registration, an organization must adopt an environmental management system in which the following points are developed:

1. Environmental Analysis
2. Environmental policy
3. Environmental Program
4. Environmental Management System
5. Internal Environmental Audit
6. Environmental Statement
7. Verification and Validation of the Environmental Statement
8. Registration.

Within the management system, particular importance is given to the part of the system that allows emergencies to be addressed through the adoption of procedures that identify potential emergency situations including possible incidents that could have an impact on the environment, defining the kind of response. In fact, one of the main reasons why companies implement an EMAS environmental management system is to keep the environmental risks associated with their activities and services under control. The identification of environmental aspects and their evaluation helps to prepare effective responses and to reduce the environmental impacts on environmental matrix.

The management system relating to the technical activities of EMAS registration, accreditation and surveillance of Environmental Verifiers are carried out in compliance with ISO 14001:2015 standards.

The EMAS Environmental Statement and the implementation of Key Performance Indicators respond to the three most important today sustainability challenges: efficient use of resources, climate change, corporate social responsibility. Conscious of their environmental impacts, constantly EMAS Organizations constantly act to increase the environmental efficiency of their production system, introducing technologically innovative systems and adopting transparency in the communication of their environmental performance. The EU directive on industrial emissions (EU Dir. 75/2010 currently under revision) recognizes the EMAS scheme as the best available technique in terms of existing environmental management systems. Furthermore, EMAS membership can satisfy the requirements of the EU directive on corporate sustainability reporting (Dir. 2464/2022 CSRD) and the environmental statement can be used as a reference for the reporting itself.

Life Cycle Assessment studies to support projects of waste treatment plants

L. Rigamonti

Department of Civil and Environmental Engineering, Politecnico di Milano, Milan, Italy.

Nowadays during the authorization process for the construction of a new waste treatment plant a Life Cycle Assessment (LCA) study is increasingly required. In most cases the study is developed as a comparative study, between a reference scenario which does not envisage the construction of the plant and the scenario which instead includes the management of that certain type of waste through the plant under authorization. This is useful for the authority that shall decide to issue or not the authorization to understand whether the introduction of the plant will bring an improvement in the environmental performance of the waste management system or not, considering both the more easily understood direct impacts and the indirect ones quantifiable only by applying the principle of life cycle thinking. In some other cases the comparative study is used to show to the authorizing authority how the technology chosen in the project compares to other available technologies. In all cases, the LCA study is able to identify the hot spots of the waste management system. The presentation will show four recent case studies of LCA application in this context. All the case studies concern the request for authorization for new plants in the north of Italy. The first one is related to a combustion plant with energy recovery for the treatment of sludge generated in civil wastewater treatment plants. The sludge treatment scenario that involves the construction of the plant is compared with two scenarios that represent the current situation (i.e. sludge sent to agriculture or to landfill) and the possible future situation without the plant (i.e. sludge sent to agriculture or cement kilns or combustion plants outside Italy). The second case study deals with the construction of an anaerobic digestion plant dedicated to the treatment of livestock waste. The LCA was used to verify any benefits compared to the current management situation of this kind of waste, based on agricultural spreading. The third LCA study was conducted to compare different scenarios of municipal solid waste treatment where a new waste to energy plant is supposed to be built. The last one deals with a wastewater treatment plant and the construction of a new unit of biological treatment, that could be designed with three different configurations. The presentation will not enter in the details of the case studies but will focus on the challenges and opportunities of this kind of studies.

Best Young Italian Scientist Award

1st classified

A multi-level approach to investigate potential adaptive mechanisms to ocean acidification in the limpet *Patella caerulea*

S.G. Signorini[1,2,3], M. Munari[4], A.A.T. Weber[3], F. Crocetta[2,5], L. Nigro[1], I. D'Aniello[4], M. Giulio[3], R. Malagugini[4], C. Della Torre[1,2]

[1]Department of Biosciences, University of Milan, Milan, Italy.-[2]Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Naples, Italy. [3]Department of Aquatic Ecology, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switzerland. [4]Department of Biology, Stazione Idrobiologica Umberto d'Ancona, University of Padova, Isola San Domenico, Chioggia (Venice), Italy. [5]NBFC, National Biodiversity Future Center, Palermo, Italy.

Email: silvia.signorini@unimi.it

The continuous increase in anthropogenic carbon dioxide (CO_2) emissions in the atmosphere is one of the main factors that contribute to ocean acidification (OA). Indeed CO_2 is partially absorbed by oceans, where it alters the seawater carbonate chemistry and pH level, which is predicted to decrease from the current level of 8.1 to 7.7 by the end of the century. OA entails a primary and detrimental impact on calcifying organisms, due to the fact that it affects carbonate availability, making their calcareous structures more fragile and thinner. Nevertheless, the gastropod *Patella caerulea* is one of the few calcifiers that is able to inhabit naturally acidified sites, like the Castello Aragonese CO_2 vent system of Ischia Island.

Therefore, this study aims to deeper investigate what are the potential molecular, physiological, and metabolic mechanisms that allow *P. caerulea* to tolerate and survive under OA. To do so, morphology, physiology, antioxidant system, metabolomics, and genomics were investigated in specimens collected along the pH gradient (pH: N1 = 8.1; N2 = ~7.7; N3 = <7.4) of the Castello Aragonese vent system and from an ambient pH site (pH: 8.1) named San Pietro.

Limpets from the acidified sites displayed evident shell surface erosion. Conversely, in the most acidified sites organisms showed increased dimension, which might be related to different food availability and/or quality or to a reduction in predatory pressure. In support of this result, an increase in glycogen content was observed in the same organisms, but further analyses are still ongoing to better investigate this hypothesis. Physiological measurements taken across the year revealed an increase in respiration rate in the extreme acidified site N3 only in summer, suggesting a higher energetic demand to potentially cope with both OA and increasing temperatures. Untargeted metabolomics revealed an induction of carnitine metabolism in the acidified sites of the vent, pointing out a potential energy expenditure. A slight induction of oxidative stress was detected only in N3, through the activation of superoxide dismutase activity. Finally, whole-genome sequencing revealed a marked degree of genetic differentiation between the acidified population N3 and all the other sites, with a reduction in genomic diversity measured through the number of Single Nucleotide Polymorphisms and the genomic diversity index θ-pi relative, suggesting the presence of a strong selection.

Overall, our results suggest that multiple mechanisms may contribute to define tolerance to OA in the limpet *P. caerulea*, including ecological interactions, physiological functions, energetic performance, and potential genetic adaptation.

2nd classified

Effects of BPA analogues-contaminated diets in the crab *Carcinus aestuarii*

J. Fabrello[1], M. Roverso[2], S. Bogialli[2], V. Matozzo[1]

[1]*Departiment of Biology, University of Padua, Padua, Italy.* [2]*Department of Chemistry, University of Padua, Padua, Italy.*

E-mail contact: jacopofabrello@gmail.com

Introduction

The most important bisphenol is bisphenol A (BPA) and about 90% of the total manufactured BPA is used to produce plastic and plastic-derived products [1]. BPA is a chemical compound of very high concern due to its toxicity to the reproductive system. This has led to BPA restrictions, with its ban on the manufacture of baby bottles in several countries. Consequently, the replacement of BPA with other similar compounds - named BPA analogues - has begun. BPA analogues belong to a large chemical group composed of hundreds of compounds that share the same core structure (two phenolic groups). The main BPA analogues are bisphenol AF (BPAF), bisphenol F (BPF), and bisphenol S (BPS). These emerging contaminants can be found in food packaging, pipes, plastics, adhesives and thermal receipt papers [1]. The wide use of BPA analogues is causing their high release into ecosystems, even if their environmental concentrations are commonly close to few ng/l [2]. However, their concentration can reach hundreds and thousands of ng/l [2].

Materials and methods

A total of five experimental conditions (control, BPAF, BPF, BPS and MIX) were tested. Clams were exposed for 14 d to 300 ng/L of each bisphenol or 100 ng/L of each of them in the MIX treatment. After 14 d, the clams were dissected and used to feed crabs. After 7 and 14 d we collected the haemolymph, gills and hepatopancreas (HEP) to perform the analysis. We evaluated the total haemocyte count (THC), diameter and volume of haemocytes. Moreover, the haemocyte proliferation was evaluated using a kit and results were normalized to THC. After excision, both gills and HEP were homogenised to obtain supernatants (SN). Total superoxide dismutase activity (SOD) was expressed as U/mg protein [3]. Catalase (CAT) activity was measured at 240 nm and expressed as U/mg protein [3]. Glutathione reductase (GR) activity was measured at 412 nm and the enzyme activity was expressed as U/mg protein [3]. Glutathione S-transferase (GST) activity was measured only in HEP SN [3]. Selenium-dependent glutathione peroxidase (GPX) activity was measured at 340 nm [3]. The protein concentration was quantified according to the Bradford assay [3]. The biomarker results were compared with a two-way ANOVA analysis and the pairwise comparisons were performed using Fisher's LSD post-hoc test ($p<0.05$). Biomarker results are expressed as means ± standard deviation, n = 5. The bioaccumulation analysis was performed as reported by Fabrello et al. [3]. LODs were 40, 2 and 0,5 ng/g for BPF, BPS and BPAF, respectively. Results are reported as mean and standard deviation.

Results and discussion

Haemolymph and haemocyte biomarkers

Based on our results, the THC was significantly decreased by almost all the different diets after both 7 and 14 d. Similarly, a reduction in haemocyte number was observed in both molluscs and shrimps exposed to BPA [4,5]. Regarding diameter and volume, they were increased in crabs fed for 7 d with BPF-contaminated clams. Furthermore, the haemocyte cell proliferation was increased in crabs fed with BPS- and MIX-contaminated clams after 14 d. These results indicate that haemocytes were co-opted to phagocytize foreign materials increasing their size and reducing their number with a consequent increased cell proliferation to restore it.

Antioxidant biomarkers and bioaccumulation

The SOD activity in the gills of crabs fed with BPF-, BPS- and MIX-contaminated clams was reduced after 7 days. Moreover, a similar SOD activity inhibition was observed after 14 days in crab under the BPAF condition. In the HEP, we did not observe any alteration. Similarly, the CAT activity was

altered only in the gills in which there was a significant increase in crabs fed with BPAF- and BPF-contaminated clams for 7 d. Interestingly, the CAT activity was increased also in crabs fed with BPS- and MIX-contaminated clams after two weeks. Regarding the glutathione enzymes, the GR activity was not altered by any of the contaminated diets, while the GPX activity was significantly altered in the two tested tissues. Indeed, in gills there was a GPX decreased activity in crabs fed with BPF-contaminated after 14 d. In addition, in HEP, there was an increased activity caused by BPAF and BPF-contaminated clams after 7 d. On the contrary, there was a significant decrease caused by BPF-, BPS- and MIX-based diets after 14 d. Furthermore, the GST activity was significantly decreased in crabs fed with both BPAF and MIX-contaminated clams after 7 d, and BPF-contaminated clams after 14 d. An impairment of the antioxidant system in crustaceans was also recorded in animals exposed to bisphenol-contaminated water, even if higher concentrations were adopted. For instance, both SOD and CAT activity were significantly increased after 3 of exposure to 0.25, 0.5 and 1 mg/l of BPA in the crab *Charybdis japonica* [5]. Moreover, the GPX activity was increased by 0,5 and 1 mg/l after both 3 and 6 d of exposure [5]. In the crayfish *Procambarus clarkii*, an impairment of the antioxidant system was observed following exposure to BPS [6]. In detail, SOD activity significantly decreased following exposure to 1, 10 and 100 µg/l, while the CAT activity significantly decreased after 14 d of exposure to 10 µg/l and 100 µg/l [6]. Our bioaccumulation results indicate that only BPAF had a measurable concentration, while all the other tested compounds had a concentration <LOD. The BPAF concentration was very low (close to 1 ng/g).

Conclusions

The overall results on immune and antioxidant systems suggest that BPA analogues were able to exert detrimental effects also if provided via food. In addition, their toxicity appears to be like BPA as reported by previous researchers. Furthermore, the fact that the tested compounds were able to cause different effects in crabs fed with contaminated clams also suggests that they can have different modes of action. Lastly, crabs, at least under the experimental conditions tested, were able to bioaccumulate BPAF, even if at low levels.

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3rd classified

Ecological fitness impairments induced by chronic exposure to polyvinyl chloride nanospheres in *Daphnia magna*

A. Masseroni, S. Villa

Department of Earth and Environmental Sciences, DISAT, University of Milano-Bicocca, Milan, Italy

E-mail contact: a.masseroni2@campus.unimib.it

Introduction

Nanoplastics (NPs) are those plastic items ranging between 1 nm and 1000 nm [1]. The evaluation of the toxic effects of NPs has typically focused on engineered nanospheres of polystyrene (PS). However, as a wide variety of plastic polymers are found in the environment, it is essential to identify the specific hazard associated with each polymer to ensure appropriate risk assessment [2]. Polyvinyl chloride (PVC) is of particular interest as it is the third-most produced plastic polymer worldwide and it is considered a potential hazard for the environment [3]. The present study aimed to contribute to the production of evidence of the effects of PVC-NPs and PS-NPs on *Daphnia magna*, by evaluating the chronic effects resulting from a 21-day exposure to engineered PVC and PS nanospheres at environmentally relevant concentration. Survival, growth, physiological, reproductive and behavioural alterations were assessed. The evaluation and comparison of the chronic effects induced by PS-NPs and PVC-NPs can help elucidate the role of polymers in the onset of hazards, providing useful data for better characterisation of the ecological risk of NPs.

Materials and methods

Before the exposure experiments, PS-NPs and PVC-NPs were characterised by Scanning Electron Microscopy (SEM), Dynamic Light Scattering (DLS), Nanoparticle Tracking Analyses (NTA) and Raman spectroscopy to confirm the nominal size, shape, concentration and polymer type.

D. magna maintenance and 21-day reproduction tests were conducted in accordance with the OECD Test Guideline 211 [4]. Solutions of 10 µg/L of PVC-NPs and PS-NPs, along with controls (CTRL), were tested (n=12 for each treatment). Survival, morphological, physiological, reproductive and behavioral parameters were monitored daily. The population intrinsic growth rate was derived from the Euler–Lotka equation [5]. To analyse the effects of NP exposure on the investigated endpoints, one-way analysis of variance (ANOVA) or Kruskall-Wallis tests were performed using R software.

Results and discussion

The tests met the requirements specified in the OECD Test Guideline 211 [4], as the mortality observed in the CTRL treatment was below 20%. Resuls are presented in Table 1.

Morphological and physiological endpoints

PVC-NPs induced higher body dimensions than CTRL and PS-NPs, while both treatments increased the number of molts. These are new evidences for the PVC -NP induced effect, which could be linked to an impairment of ecdysone hormonal systems.

Reproductive endpoints

The impairment of *D. magna* fitness following exposure to PVC-NPs is caused by a delay in the timing of the first brood (Figure 1), which in turn resulted in a reduction in the number of broods and offspring produced (Figure 2). This led to a significant decline in the intrinsic population growth rate. In contrast, PS-NPS, although PS-NP exposure initially delayed offspring production, did not significantly impact population growth.

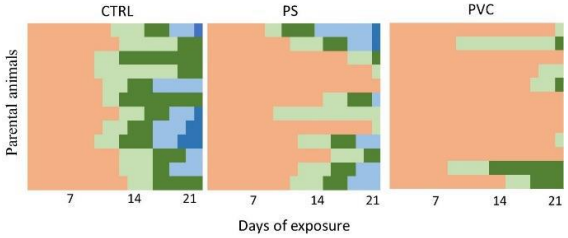

Figure 1. Number of broods produced by D. magna individuals (0: orange; 1st: light green; 2nd: green; 3rd: light blue; 4th: blue). Each row corresponds to a surviving parent animal.

*Table 1: Investigated endpoints measured in D. magna individuals over 21 days of exposure to CTRL, PS-NP, and PVC-NP treatments. Data are reported as mean ± standard deviation. (*p < 0.05; **p < 0.01; and ***p < 0.001 vs. CTRL).*

Endpoint	Unit of measure	CTRL	PS-NPs	PVC-NPs
Survival	Number of dead	0	0	0
Morphological	Length (mm)	2.9 ± 0.2	2.9 ± 0.2	3.2 ± 0.1**
Morphological	Width (mm	1.9 ± 0.1	1.9 ± 0.2	2.1 ± 0.1*
Physiological	Number of molts	6.0 ± 1.1	6.9 ± 1.6 *	7.3 ± 1.1**
Reproductive	Number of broods	3.0 ± 0.8	2.2 ± 1.3	0.83 ± 0.8***
Reproductive	Time of the 1st brood (day)	10.8 ± 1.4	14.7 ± 4.9	18.4 ± 5.1***
Reproductive	Number of offspring per parent	27.5 ± 18.4	25.9 ± 16.2	10.9 ± 11.4**
Behavioural	Velocity (mm/s)	12.8 ± 3.6	12.8 ± 4.2	12.3 ± 3.1
Behavioural	Acceleration (mm/s^2)	215 ± 45.9	206 ± 38.2	210 ± 37.4
Behavioural	Distance (mm)	313 ± 103	299 ± 125	297 ± 89
Behavioural	Activity (%)	79.6 ± 12.0	72.1 ± 11.7	79.0 ± 10.0
Population dynamic	Population growth rate (day-1)	0.41 ± 0.22	0.30 ± 0.18	0.10 ± 0.10***

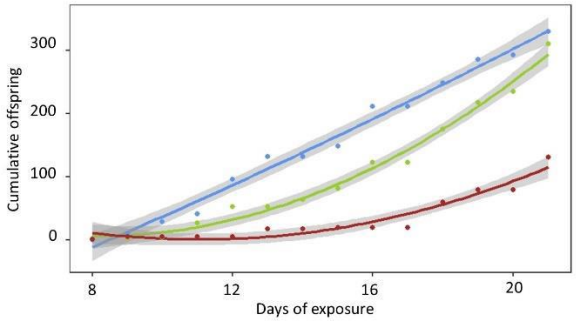

Figure 2. Cumulative offspring of D. magna parents over 21 days for the CTRL (blue), PS (green), and PVC (red) treatments. The shaded region represents the 95% confidence interval for the true value.

Behavioural endpoints

Regarding behavioural traits, the evaluation of swimming parameters did not reveal statistically significant differences among the different treatments. Regarding PS-NPs, the absence of swimming alterations is in line with evidence reported in the literature [6]. Conversely, we reported for the first time the evaluation of behavioral parameters after PVC-NP exposure in *D. magna*.

Conclusions

The role of nanoparticle polymer type in toxicity was highlited, as nanosized-PVC showed a greater environmental impact than nanosized-PS. PVC-NPs affected the population molting behaviour, morphometric parameters, and reproductive fitness. From an ecological perspective, the observed delay in reaching sexual maturity could have a significant impact in wild populations.

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Antarctic threads: textile microfibers and chemical additives in the wild scallop *Adamussium colbecki* from the Ross Sea

E. Ferrari[1], R. Russo[1], E. Bergami[2], Va. Ferrari[2], M. V. Barbieri[3], A. Balasch García[3], G. Birarda[4], L. Vaccari[4], E. Eljarrat[3], I. Corsi[1]

[1]Department of Physical, Earth and Environmental Sciences, University of Siena, Siena, Italy. [2]Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy. [3]Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Spain. [4]SISSI-Chemical and Life Science Branch, Elettra–Sincrotrone Trieste, Trieste, Italy

Plastic pollution has reached even remote regions like Antarctica, threatening biodiversity in the Southern Ocean. Microplastics, including synthetic textile microfibers released during washing and use of synthetic textiles, have been identified in Antarctic seawater, sediments, and organisms. This study investigates the occurrence of textile microfibers and associated chemical additives in the Antarctic scallop *Adamussium colbecki*, a key species in coastal ecosystems of Terra Nova Bay, Ross Sea. Specimens were collected near the Italian Mario Zucchelli Station during 2004 and 2019 expeditions. Textile microfibers were analyzed in gills and mantle tissues using µFTIR and µRaman spectroscopy, while chemical additives, including phthalates and organophosphates (OPEs), were assessed in whole individuals and reference textiles through LC-MS/MS.

Results showed that 91.6% of scallops contained microplastics, with an average of 6.6 ± 1.94 particles per individual. Microfibers, primarily black, blue, and red, were more concentrated in mantle tissues than gills. Comparisons between years revealed a decrease in microfiber presence over time, from 48 items in 2004 specimens to 31 in 2019. Red microfibers predominantly consisted of polyethylene terephthalate (PET), with some showing mixed semi-synthetic compositions. Analysis linked these fibers to textiles used by station personnel, indicating local human activity as the source of contamination.

Chemical analysis revealed notable concentrations of phthalates, alternative phthalates (PAEs), and OPEs in scallops, with compounds like DEHP and DEHA reaching levels significantly higher than those typically found in polar waters. OPEs such as tris(2-ethylhexyl) phosphate (TEHP) and triphenyl phosphate (TPHP) were also prevalent. These additives, commonly used in technical clothing, matched those identified in reference textile samples from the station, further confirming local sources of pollution.

This study provides the first evidence of synthetic textile microfiber contamination and associated chemical additives in *A. colbecki* from Terra Nova Bay. The findings highlight the vulnerability of even the most remote ecosystems to human impact and emphasize the need for stringent measures to minimize pollution from scientific activities in Antarctica. The results also raise broader concerns about the ecological and biological risks posed by synthetic textile-derived pollutants in marine environments worldwide.

Ecological risk assessment of manufactured nanomaterials: investigating the role of protein-corona formation in the coelomic fluid of the sea urchin *Paracentrotus lividus*

P. Romano[1,2], M. Morelli[3], R. Lucignano[3], A. Bellingeri[1], A. Carpentieri[3], D. Picone[3], I. Venditti[4], I. Corsi[1], S. Leone[2]

[1]Department of Physical, Earth and Environmental Sciences, University of Siena, Siena, Italy. [2]Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, National Institute of Marine Biology, Ecology and Biotechnology, Napoli, Italy. [3]Department of Chemical Sciences, University of Naples "Federico II", Naples, Italy. [4]Department of Sciences, Roma Tre University of Rome, Rome, Italy

The term engineered nanomaterials and nanoparticles (ENM/Ps) defines all those materials with dimensions between 1-100 nm. Due to their size and properties, they are widely used in everyday products, leading to environmental dispersion. Once in contact with natural/biological media, NPs have the property to adsorb biomolecules on their surface, particularly proteins. The complex of proteins adsorbed on the surface of NPs is called the "protein corona" (PC). This provides NPs with a new biological and environmental identity and affects NPs interaction with cell surface receptors, driving ecotoxicological outcomes. Therefore, the investigation of the structural and biological influence of the PC and their effects at the cellular level are relevant in the assessment of their environmental safety. This study examined the composition of the PC formed on 4 batches of NPs,

titanium dioxide ($nTiO_2$), functionalized polystyrene (PS-NH_2; PS-COOH) and functionalized silver (AgNPcitLcys), upon contact with the coelomic fluid (CF) of the Mediterranean Sea urchin *Paracentrotus lividus* and evaluates its ecotoxicological effects on the associated pool of immune cells (coelomocytes). The properties of the NPs upon corona formation were analyzed using dynamic light scattering (DLS) and transmission electron microscopy (TEM), which confirmed that NP dispersion improves over time in CF, especially for polystyrene NPs (PS-NPs), due to biomolecule adsorption forming PC. Proteomic analysis revealed a heterogeneously composed protein corona for each NP type, with many identified proteins being integral to the sea urchin immune system. The secondary structure of toposome, by circular dichroism spectroscopy (CD), remained stable initially but ongoing studies aim to explore changes following NP exposure. Protein-NPs effects on *P. lividus* immune cells were evaluated after *in vivo* exposure by analyzing cell viability and lysosomal membrane stability, and by measuring the amount of the red cells. Ecotoxicological assays indicated significant reductions in cell viability and lysosomal membrane stability in response to all NPs, except for PS-COOH at low concentrations. These effects were dose-dependent, with $nTiO_2$ displaying the most notable responses. Variability in red cell counts was observed, likely due to the short exposure time. The findings confirm that NP interaction with CF proteins leads to unique corona, affecting NP behavior and cellular responses. These interactions highlight the need for further studies to elucidate their ecological impacts. This research contributes to understanding the environmental risks of nanomaterials and offers insights for safer NP design.

A new ecological screening tool based on the aggregation of the terrestrial isopod *Porcellionides pruinosus* for assessing soil quality

L. Federico[1], G.S. Monti[2], S. Loureiro[3], S. Villa[4]

[1]DISAT, Department of Earth and Environmental Sciences, University of Milano-Bicocca, Milan, Italy. [2]DEMS, Department of Economics, Management and Statistics, University of Milano-Bicocca, Milan, Italy. [3]CESAM, Centre for Environmental and Marine Studies and Department of Biology, University of Aveiro, Aveiro, Portugal

Soil ecosystems are affected by stressors that can alter their stability or even induce regime shift conditions. Since soil formation rates are below 1 tons ha^{-1} $year^{-1}$, the loss of these ecosystems is irreversible. The development of new screening tools are therefore required to monitor and prioritize those soils under depletion, to protect them for sound environmental management. In that way, the behavioral alterations of edaphic organisms are considered as warning indicators of soil degradation induced by different drivers, such as xenobiotics or abiotic stressors. These behavioral alterations may lead to an important change at population or even higher ecological levels. In that contest, the aggregation behavior of the terrestrial isopods *Porcellionides pruinosus* is considered an interesting ecological trait at population level. The aim of our work was to assess the alteration in the aggregation behavior of *P. pruinosus* as a prognostic or diagnostic screening tools for monitoring the quality of soil ecosystems. As key studies, we present the effect of different physicals (Tire Particles, TPs) and chemicals (Benzotiazole (BT) and galaxolide (HHCB)) xenobiotics and an abiotic stressor (temperature) on the aggregation behavior of terrestrial isopods. As measure of disaggregation, the disaggregation index (DI) and the disaggregation in group (DG) were quantified for understanding the magnitude of population's fragmentation. Our results highlight how different soil stressors can modulate the gregarious behavior in terrestrial isopods, resulting in a reduction in the population density. Since they represent the main detrivores of soil litter, such alterations could have important consequences on the stability of production cycles. We suggest to take advantage of such endpoint as a rapid and non invasive screening tool at high ecological level.

Impact of short-chain perfluoropropylene oxide acids on biochemical and behavioural parameters in *Eisenia foetida* (Savigny, 1826)

D. Rotondo[1], D. Gualandris[1], C. Lorusso[1], A. Braeuning[2], A. Calisi[1], F. Dondero[1]

[1]Department of Science and Technological Innovation, University of Eastern Piedmont, Alessandria, Italy.[2] German Federal Institute for Risk Assessment, Berlin, Germany.

Per- and polyfluoroalkyl substances (PFAS) are a group of persistent organic pollutants that increasingly threaten environmental and human health. Soil serves as a long-term reservoir for PFAS, potentially affecting soil biodiversity and ecosystem functions. Earthworms, which play a crucial role in soil ecosystems, are particularly susceptible to PFAS exposure. In this study, we examined the sublethal effects of three short-chain (C4-C6) next-generation perfluoropropylene oxide acids on the earthworm Eisenia fetida, using perfluorooctanoic acid (PFOA), a legacy perfluoroalkyl carboxylic acid (PFCA), as a reference. We evaluated various biochemical markers, including those for oxidative stress (catalase and superoxide dismutase activity), immunity (phenol oxidase activity), neurotoxicity (acetylcholinesterase activity), and behavioral responses (escape test). The results indicate that all tested PFAS, even at sub-micromolar concentrations, caused significant effects across multiple physiological aspects. Notably, longer-chain PFAS generally provoked more severe responses. Our findings highlight the potential risks posed by both legacy and new PFAS to soil ecosystems, stressing the need for further research to understand the long-term impacts of PFAS contamination.

From molecules to bees: multi-level approach reveals negative effects of sewage sludge trace elements on honeybees

A. Ferrari[1], M. Sturini[2], B. De Felice[1], F. Bonasoro[1], C.F. Trisoglio[1], M. Parolini[1], R. Ambrosini[1], L. Canova[2], F. Maraschi[2], C. Polidori[1], A. Costanzo[1]

[1]Department of Environmental Science and Policy, University of Milan, Milan, Italy. [2]Chemistry Department, University of Pavia, Pavia, Italy

In agricultural landscapes, policies have been implemented to reduce the use of synthetic products and to promote circular economy practices such as the use of sewage sludge derived as soil amendment. However, sludge may contain toxic trace elements, such as heavy metals, due to suboptimal wastewater treatment. Apis mellifera Linnaeus, 1758 (Hymenoptera: Apidae), the common honeybee, is the most widely used managed insect pollinator in agroecosystems, providing the relevant ecosystem service of pollination. Laboratory studies have highlighted the negative effects of trace elements on honeybees; however, field studies investigating the potential negative effects of these agricultural practices on pollinators are lacking. Therefore, we collected honeybees from one area where sewage sludge is spread and one where it is prohibited; within the Parco Regionale Adda Sud (Lombardy, Northern Italy). First, the levels of twelve trace elements in bees and eleven in soils were quantified. Of these, we excluded the most highly correlated elements and therefore selected cadmium, chromium, lead, mercury and nickel to test their effects on oxidative stress and energy biomarkers, midgut epithelial health, body size and wing asymmetry. Overall, the trace element fingerprint of honeybees and soils differed between the two areas, suggesting that the element pattern may reflect local environmental concentrations of trace elements. We only found increased carbohydrate content in sites with higher cadmium levels, suggesting that bees may not be as biochemically stressed. However, increased histological damage to the midgut epithelium was recorded in the sewage sludge area and we found the presence of dark spherites in the epithelium of bees collected from the site with the highest lead levels. Thus, we can hypothesise that the presence of trace elements in these two areas has non-negligible effects on the midgut epithelium of honeybees. Finally, we found that honeybees with the highest lead levels were smaller and had smaller wings, and that wing fluctuation asymmetry, a proxy for larval developmental stress, was higher in sites with higher mercury levels. A reduction in body and wing size may reduce the dispersal ability of honeybees, with possible negative effects on pollination services. In conclusion, we have shown how trace element contamination can affect honeybees at biochemical, cellular, tissue and external morphological levels. Our study highlights the importance of studies based on organisms collected in the field and including analyses at different levels of biological organisation, which are still largely lacking.

A deep insight into eco(toxico)logical risk posed by metal contamination in the Alento River

S. Accardo[1,2], S. Schiavo[1], L. Parrella[1], S. Manzo[1]

[1]ENEA CR PORTICI, Portici (NA), Italy. [2]International PhD Programme / UNESCO Chair "Environment, Resources and Sustainable Development"

E-mail contact: sara.accardo001@studenti.uniparthenope.it

Introduction

Anthropogenic activities (industry, agriculture, urban development) generate contaminants that could have a direct impact on the environment. Aquatic ecosystems provide essential benefits to our global society and human wellbeing. Food, drinking water, cultural services, and recreational fishing are among the obvious ecosystem services provided by river catchment. Contaminants may flow into water systems that are located close to residential areas, causing risks to humans and ecosystem health, creating fragmented habitat zones [1]. Continuously entering aquatic systems, these contaminants pose a significant threat due to their hazardous characteristics, eventually ending up to sediments. The main goal is to understand the environmental impacts on river quality and to identify the ecotoxicological and ecological risks in the studied area. In the initial phase, the study focused on water matrix characterization, producing interesting results that suggested an existing ecological risk due to contamination by mixtures of metals. Subsequently, a deeper evaluation of sediment toxicity was conducted.

Materials and methods

An analysis of existing data of the area and GIS analysis were used to integrate available territorial and environmental data to select sampling areas to be monitored. A seasonal survey of the waters at five different sites along the river (upstream to downstream: Al1, Al2, Al3, Al4bis, and Al5), was conducted. Chemical (As, Cd, Cr, Hg, Ni, Pb, Zn by ICP-MS) and ecotoxicological analyses (Alivibrio fisheri bioluminescence inhibition test ISO 11348-1997) [2], Raphidocelis subcapitata growth inhibition test, OECD 201 2006 [3], Daphnia magna immobilization test, OECD 202 2004) [4] of the collected samples were performed. The same evaluation was performed on eluates (1:4 dry w/V, ISPRA, MLG 67/2011) [5] of annually collected sediments. In addition, chronic effects (survival and reproduction) were evaluated by a Daphnia magna tailored test. All results were integrated to obtain a Toxicity Battery Index (TBI) [6].

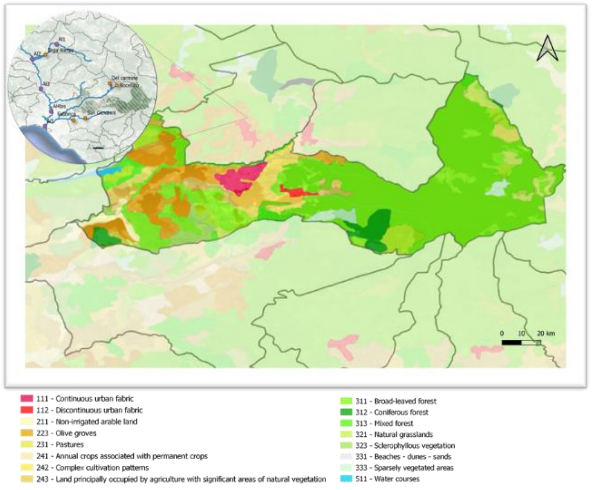

Figure 1 GIS analysis of the area of study and river catchment sampling points.

In depth analysis of metals occurrence in sediments and waters and associated risk was carried out by the Hazard Quotient (HQ) approach, defined as the ratio between exposure and effect concentration [7] and results were mapped by GIS. Moreover the risk posed by chemicals on Alento catchment with the measured ecotoxicological effects in the view of ES protection were estimated following procedure by Maltby et al. [8].

Results and discussion

The Alento River catchment, situated in Vallo della Lucania and Novi Velia municipalities area is located in the National Park Parco of Cilento and Vallo di Diano, hosts a combination of cottage industrial, domestic/touristic, and agricultural activities: in particular land cover map highlights olive oil cultivation as the primary activity, following by pasture areas and vineyard cultivation. Moreover an integrated system of storage dams is present (Fig 1). The water quality assessment showed the occurrence of Cu and Zn for all the season and a moderate ecotoxicological risk (TBI) in winter season that in part was confirmed by ERA values (Fig 2).

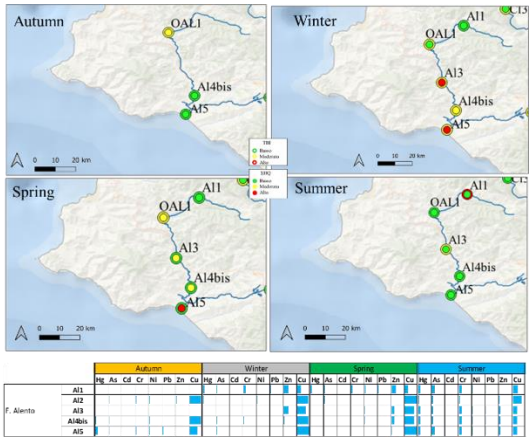

Figure 2 Map of TBI and HQ values for the water matrix across different sampling seasons. Green indicates points with no eco(toxico)logical risk, yellow indicates moderate eco(toxico)logical risk, and red indicates high eco(toxico)logical risk.

The evaluation of sediments showed a moderate ecotoxicological risk specifically for Al1 and Al5 (Fig 3). Regarding ecological risk, most sites fall into the first risk class (low), although further evaluation is deemed necessary due to the limited amount of available data. The survival rate of D. magna chronic exposure was 100%, except for Al2 and Al5 (20% of effect). Instead, *D. magna* reproduction was generally affected with Al2 sample showing the most significant effects (>50%). The most impacted ecosystem services related to metal contamination (Fig. 3) were related to fish surviving and to fishing, although data availability may limit the resolution of the assessment of some ES.

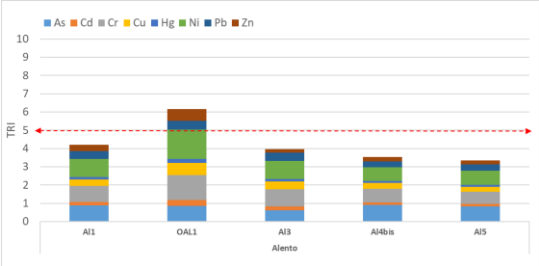

Figure 3. Metal amounts measured in Alento river sediments. OAL1 represents the most impacted site

Conclusion

This study investigated the impact of anthropogenic activities of the Alento River basin. Sediment analysis revealed moderate ecotoxicological risks for samples Al1 and Al5, while ecological risks were generally low but require further data for comprehensive assessment. Chronic effects on Daphnia magna

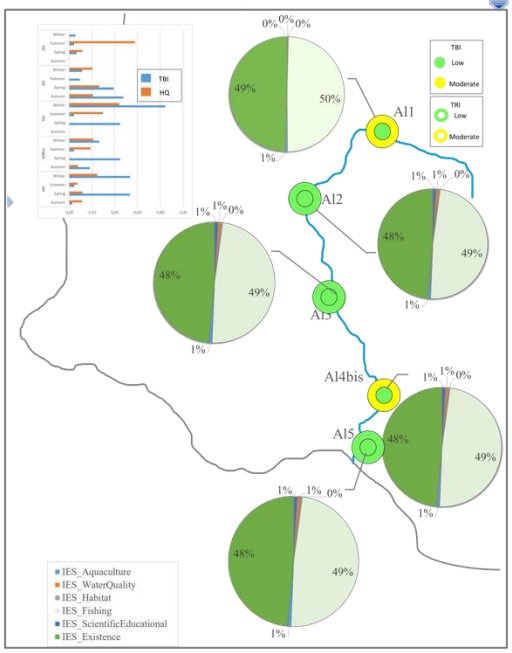

Figure 4. Results of impact upon ES, IES most impacted were fishing and existence due to limited data availability

highlighted significant reproductive impacts, emphasizing the need for continued monitoring and management of river quality in this biodiverse region.

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Evaluation of the effects of antibiotic and copper mixture on a plant-microbiome metaorganism

C. De Carolis[1,2], L. Cidamidaro[3], M. Chalot[3], A. Narciso[1,4], P. Grenni[1], T. Couraud[3], F. Spataro[5], J. Rauseo[5], L. Patrolecco[5], A. Barra Caracciolo[1]

[1]Water Research Institute, National Research Council (IRSA-CNR), Montelibretti (Rome), Italy. [2]Department of Environmental Biology, Sapienza Universita di Roma, Roma. [3]Chrono-environnement Universite de Franche-Comte CNRS, France. [4]Department of Ecological and Biological Sciences, Tuscia University, Viterbo, Italy. [5]Institute of Polar Sciences - National Research Council (ISP-CNR), Montelibretti (Rome), Italy.

Antibiotics are emerging contaminants often found in agroecosystems due to the use of soil amendments such as manure or reclaimed water. Indeed, only a minority of antibiotics administered to animals are metabolised, with the majority excreted as residues through urine and faeces.
Overall ABs may pose a risk to public health, as well as causing adverse effects on biota, as well as on crop growth and productivity. The bactericidal action of antibiotics can alter the natural microbial community, leading to a reduction in microbial diversity with a consequent loss of important ecosystem functions, as well as stimulating the spread of antibiotic resistance genes among natural soil populations, animal, plant and human microbiomes.
Copper (Cu) is used for fungicide treatments, including in organic farming, and it can contaminate soil from the same pollution sources of antibiotics. Consequently, agricultural soils can be environmental sinks for both contaminants, and their accumulation in plants and eventual transfer through food chains to animals and humans needs to be investigated.
This work was aimed at studying the effects of 3 antibiotics (sulfamethoxazole, chlortetracycline and ciprofloxacin, 7.0 mg/kg each) and Cu (10 mg/Kg) on the growth and development of lettuce plants (*Lactuca sativa*) and their associated rhizosphere microbial communities, in presence/absence of compost (1%) as an organic amendment. A 46 days experiment in microcosms with lettuce was conducted in a growth chamber. Soil microbial communities were evaluated in terms of total abundance (DAPI counts), and enzymatic activities (dehydrogenase, phosphatase, β glucosidase). The plant growth and physiology were assessed as biomass, number of leaves, leaf area, root elongation and chlorophyll content.
For all conditions an increase in soil microbial abundance and activities was found at the end of the experiment (36 days), compared to the initial time. At 36 days the highest values of microbial abundance were observed in the conditions amended with compost. No significant differences were observed for phosphatase. The β glucosidase values were higher in compost presence and at 36 day in antibiotic and copper co-presence. Microbiological parameters were in accordance with plant biomass, number of leaves, leaf area, root elongation and chlorophyll content. The compost condition presented the highest values, followed by Soil+plant, Soil+Cu+plant and Soil+ABs+Cu+compost+plant. The co-presence of ABs and Cu had a detrimental effect on the lettuce plants. However, the addition of compost buffered the negative effect of the antibiotic. The plants grew healthy where bacteria numers and activity were high.

Unveiling the effects of emerging contaminants on marine organism embryogenesis

A. Morgillo[1,2], F. Salatiello[2,4], E. D'Aniello[2], L. Riva[3], C. Punta[3], A. Spagnuolo[2], I. Corsi[1], F. Ristoratore[2]

[1]Department of Physical, Earth and Environmental Sciences, University of Siena, Siena, Italy. [2]Department of Biology and Evolution of Marine Organisms, Stazione Zoologica AntonDohrn, Napoli, Italy. [3]Department of Chemistry, Materials, and Chemical Engineering "G. Natta" Politecnico di Milano, Milano, Italy. [4]Department of Life Sciences, University of Trieste, Trieste, Italy

The increasing presence of contaminants of emerging concern (CECs) in everyday applications necessitates urgent examination of their impact on environmental and human health.
Pharmaceuticals active compounds (PhACs) and micro/nano-sized fibers (MNF) are CECs found in aquatic environments thus prompting to explore their interaction within marine ecosystems. This research proposes to investigate the effects of Venlafaxine and two types of cellulose nanofibers, non-oxidized (CNF) and TEMPO-oxidized (TOCNF), during the embryogenesis of two filter feeder animals, the urochordate Ciona robusta and the bivalve *Mytilus galloprovincialis*. Through

phenotypical, molecular and histochemical approaches we aim to shed light on the impacts of CNF, TOCNF, and Venlafaxine on these marine experimental models, both offering evolutionary significance for comparative analyses.

Investigating sublethal responses of earthworms in PFAS-contaminated soils: a comprehensive study at Trelleborg firefighting site in Sweden

D. Gualandris[1], D. Rotondo[1], C. Lorusso[1], A.Calisi[1], F. Dondero[1]

[1]*Departiment of Science and Technological Innovation , University of Eastern Piedmont, Alessandria, Italy.*

Fire training sites and airports are the principal sources of per- and polyfluoroalkyl substances (PFAS) contamination in various environmental matrices, including water and soil. This contamination arises from the widespread use of Aqueous Film-Forming Foam (AFFF), which is primarily composed of PFAS. Despite recent global policies restricting the use of PFAS congeners with more than eight carbon atoms (C8), the persistent legacy of Perfluorooctanesulfonic acid (PFOS) and Perfluorooctanoic acid (PFOA) continues to impact numerous ecosystems due to their environmental persistence and bioaccumulation potential.
Current AFFF formulations often incorporate short-chain PFAS and their oxidizable fluoralkylamide precursors, posing further risks to ecosystems. These short-chain PFAS, while less bioaccumulative, are still highly mobile in the environment and can lead to widespread contamination. Terrestrial ecosystems, in particular, face significant threats due to this unforeseen and unexpected contamination. The critical services provided by these ecosystems, such as soil fertility and biodiversity, are under major threat.
In terrestrial ecosystems, earthworms play a vital role in soil formation processes, organic matter decomposition, and nutrient cycling. They serve as essential bioindicators of soil health and quality. This study aims to comprehensively assess the impact of PFAS-contaminated AFFF soils on the earthworm species *Eisenia fetida*. By examining the physiological and ecological responses of *E. fetida* to PFAS exposure, we aim to highlight the broader ecological consequences and emphasize the urgent need for sustainable AFFF policies. This research underscores the importance of developing and implementing environmentally friendly alternatives to PFAS-containing AFFFs to protect and preserve terrestrial ecosystems and their essential services.

In silico prediction of the human transthyretin binding affinity

M. Evangelista, N. Chirico, E. Papa

Department of Theoretical and Applied Sciences (DiSTA), University of Insubria, Varese, Italy.

The identification, assessment and management of endocrine disruptors has become a priority in the last years, due to their ubiquitary presence in the environment and their ability to negatively affect the endocrine system in living organims. The use of only *in vivo* approaches to define the potential endocrine disrupting activity of existing chemicals and mixtures is not sustainable in terms of costs (time, money and animal lives). Therefore, the use of alternative *in silico* approaches such as Quantitative Structure-Activity Relationships (QSARs) is highly suggested to fill data gaps and to support the identification of potentially hazardous chemicals. This work aims to develop new QSAR models using Multiple Linear Regression by means Ordinary Least Squares (MLR-OLS) method for the prediction of the binding affinity of exogenous chemicals with human transthyretin (hTTR), which is an important transport protein to guarantee the correct delivering of the thyroid hormone thyroxine (T4) to target tissues. In particular, the predicted endpoint is the Relative competitive Potency (RP), which reflects the binding affinity of a compound towards the hTTR, compared to the binding affinity of the thyroxine. The here proposed models were developed using two new curated datasets previously created by merging the available experimental values of binding affinities and RPs published in the literature. These values were measured using two different *in vitro* assays based on the fluorescence displacement, from hTTR, of 8-anilino-1-naphtalenesulfonic acid (ANSA) and of a conjugate fluorescein isothyocyanate (FITC)-T4, respectively. Each dataset is composed of data measured by the same *in vitro* method. Simple molecular descriptors were generated from harmonized SMILES (Simplified

Molecular Input Line Entry System) and were used as variables in QSAR modeling. The development of the QSARs followed the OECD guidance for their assessment and acceptance in a regulatory context, in order to guarantee the internal statistical reliability and external predictivity of the models when applied to new chemicals. These QSARs can be used as tools to support and refine the hazard identification and risk assessment of chemicals with potential thyroid-disrupting activity, which could represent a severe threat for human health and wildlife.

A holistic strategy for assessing the health and services of freshwater ecosystems

I. Calattini[1,2], T. Campani[1], F. Pulselli[1], D. Giani[1], M.C. Fossi[1], L. Marsili[1], P. Centorrino[2], T. Fiaschi[3], C. Angiolini[3], L. Patrolecco[4], P. Grenni[5], S. Casini[1]

[1]Department of Physical, Earth and Environmental Sciences, University of Siena, Siena, Italy. [2]Associazione LaGorà, Colle di Val d'Elsa (Siena), Italy. [3]Department of Life Sciences, University of Siena, Siena, Italy. [4]Institute of Polar Sciences, National Research Council (ISP-CNR), Rome, Italy. [5]Water Research Institute - National Research Council (IRSA-CNR), Rome, Italy.

Freshwater ecosystems are essential for humans and the planet. Over time, anthropogenic pressure on rivers has significantly increased, leading to rising stress levels and a gradual decline in freshwater quality. This situation has escalated to the point where rivers are now among the most threatened ecosystems worldwide. The main causes of their degradation are climate change and mismanagement of land and water resources. For these reasons, the aim of this study was to propose an integrated methodology based on a holistic approach. The state of health of the river will be assessed considering from the chemical-physical analyses of the environmental matrices to the toxicological effects on organisms. The ecosystem services provided by the river in the different stretches will be defined, with the final aim of seeing how they influence or are influenced by, the state of health of the river. The Elsa River is a tributary of the Arno River in Tuscany (Italy). It was chosen as a case study because of the presence of a river park, agricultural and industrial activities and some towns. Some chemical parameters of the water (macronutrients, pH, etc.) were sampled with a citizen science project involving the local population and schools. These data are integrated with ecological indices based on benthic macroinvertebrates (STAR-ICMi), macrophytes (IBMR), diatoms (ICMi) and river functionality (FFI). Squalius squalus was used as a fish bioindicator for the ecotoxicological analyses: microplastic ingestion, contaminant concentration and a battery of biomarkers were evaluated. Microplastics have been analysed in water, some pharmaceutical residues are measured in water, sediments and organisms, and microbiological characterisations of water and sediments have been done. The ecosystem services will be evaluated in different sections. Preliminary results show a general negative trend from upstream to downstream from a chemical and ecological point of view. The microplastics isolated in the water were all fibres, mainly blue and ranging from 1-2.5 mm in size. The polymers found were kayocel, polyester and viscose. The ecotoxicological analyses on fish underline the presence of a neurotoxicity effect in the last downstream sampling station and a genotoxicity effect increasing from upstream to downstream. This holistic approach will allow us to have a complete vision of the state of health of the river as a whole. This, when compared to the ecosystem services provided by the river in its different sections, will allow us to develop different management scenarios and to identify the most sustainable one.

Posters

Cytotoxicity and genotoxicity of ether perfluoro carboxylic acid PFAS congeners in *Eisenia foetida* coelomocytes

A. Calisi, D. Gualandris, C. Lorusso, D. Rotondo, F. Dondero

Department of Science and Technological Innovation, University of Eastern Piedmont, Alessandria, Italy.

Emerging pollutants represent a serious hazard to environment and ecosystems. From different type of pollutants Per-and polyfluoroalkyl substances (PFAS) are found in the environment due to their various industrial uses (e.g. anti-fire foams, fluoropolymer resins such as Teflon; separation processes; textiles; cosmetics) and their high persistence. Soil is considered as a reservoir and a long-term source of PFAS, in fact, once they have arrived in this matrix they can spread to others (water, air), spread even to regions further away from the source of contamination. As they are then absorbed by plants from the soil, they also enter the trophic network, reaching human beings and bringing serious problems to human health and the entire ecosystem. On the other hand PFAS in soil represent a serious hazard to organisms living in belowground and aboveground systems. earthworms are among the species most frequently used in standard laboratory and field tests as bio-indicators of soil contamination with different classes of contaminants. Earthworms are common in a wide range of soils and may represent 60±80% of the total soil biomass, and traditionally they have been considered to be convenient indicators of land use and soil fertility. This makes them one of the most suitable bioindicator organisms for testing chemicals in soils. Aim of this work was to investigate the effect of these substances from an eco-toxicological point of view on non-target species, using PFAS as a reference standard. For this purpose, our study focused on assessing the potential cytotoxicity and genotoxicity of four different PFAS congeners on immune system cells of sexually mature earthworm Eisenia fetida in a range concentration of 0.6-229 µM. Toxicity tests were carried out according to OECD Test No207 (by filter paper test) to assess the cytotoxic and genotoxic effect in earthworms coemolocytes. Results showed significant alteration of the investigated patterns. An increased enlargement of granulocytes was usually observed in exposed earthworms with respect to control group. A hormetic pattern was observed. The enlargement was quantified by measuring the area of 2D digitalized granulocyte images. A decrease of oxidative burst and an increase of micronuclei frequency were also seen. The results of this study will lead to the construction of an ecotoxicological database on numerous alternative PFAS congeners allowing a weight of evidence risk assessment analysis of these substances. These data can contribute to regulation and restriction of PFAS at national and European level.

The application of ecotoxicology in the health impact assessment; a new legislative development relevant for the protection of human health

A. Mancini, I. Lacchetti , M. Carere, W. Cristiano, L. Mancini

Ecosystem and Health Unit, Environmental and Health Department, Italian National Institute of Health, Rome, Italy

Health Impact Assessment (HIA) is a systematic process used to evaluate and prevent the potential health effects on populations from the impact of large industrial enterprises, particularly large combustion plants, , i.e. large combustion plants (>300 MWth) or particularly those projects that may have significant environmental impacts. This process aims to protect public health by anticipating potential health outcomes and identifying ways to mitigate negative effects. In Italy, the HIA procedure in accordance with the European Union's Directive 2014/52/EU, on the assessment of the effects of certain public and private projects on the environment. This directive integrates health considerations into the Environmental Impact Assessment (EIA) process. In Italy the HIA procedure has been included in the legislative decree N. 104/2017 with a key role of ecotoxicology.

The integration of an ecotoxicological approach into the Health Impact Assessment (HIA) procedure in the Italian legislation represents a significant advancement in addressing the

potential health impacts of large industrial enterprises. This approach aims to detect toxic effects caused by unknown or not-monitored contaminants or mixtures in ecosystem components affected by emissions, discharges, and accidental releases. Serving as a critical bridge between environmental and human health, ecotoxicology is particularly valuable during the scoping and monitoring phases of the HIA procedure, functioning as an early warning system and screening tool. After a brief period of adapting to the new rules, currently 80% of enterprises that applied HIA provides robust, integrated, and detailed documentation regarding the ecotoxicological assessment.
The legislative inclusion of ecotoxicology in the HIA procedure is an important development for the protection of human health. In the future, a more accurate procedure for triggering values/evaluation criteria for interpretation of results will be necessary. This is also an important step that will be considered at European level in the context of the application of effect based methods in the EU Water Framework Directive. Moreover, the innovative ecotoxicological approach included in the HIA in Italy, plays a crucial role in early warning and screening, and helps to implement the necessary preventive measures to eliminate or reduce the potential effects and impacts of industrial emissions of chemicals in air, water and soil.

Evaluating the embryotoxic potential of the pesticide thiram in the sea urchin *Arbacia lixula*

B. Billè[1], M.C. Eliso[2], G. Genovese[1], D. Iannazzo[3], T. Cappello[1], M. Morabito[1], M. Maisano[1]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy. [2]Department of Investigative Marine Ecology, Stazione Zoologica Anton Dohrn, Naples, Italy. [3]Department of Engineering, University of Messina, Messina, Italy

Dithiocarbamates (DTCs) represent a class of chemical compounds widely utilized in agricultural practices. They are mainly used as insecticides, herbicides, and fungicides to protect fruits and vegetables from pests and diseases. Due to their extensive application, these compounds readily infiltrate aquatic environments, posing a potential threat as pollutants to a variety of biological communities. Among the DTCs, thiram is notably prevalent in both soil and aquatic ecosystems. Recent attention has been drawn to this pesticide because it is classified by the European Union as a category 1 potential endocrine disruptor, indicating its high potential to interfere with hormonal systems. Moreover, thiram acts as a pro-oxidant, inducing the formation of reactive oxygen species (ROS), which can lead to oxidative stress and cellular damage. As a result of these concerns, different studies have been undertaken to unravel the mechanisms underlying thiram's toxicity on non-target organisms, with the final aim to provide a comprehensive assessment of its environmental and ecological impact. In light of this, the effects of thiram were assessed on the embryonic development of the sea urchin Arbacia lixula (Linnaeus, 1758) within the project SAMOTHRACE. Embryos were exposed for 72 hours post-fertilization (hpf) to four different environmental concentrations of thiram (0.24 µg/L, 1.2 µg/L, 6 µg/L, and 30 µg/L). Preliminary findings indicated morphological impairments in embryos even at the lowest tested concentration of thiram, highlighting a delay in the development. Overall, these data indicate the necessity of in-depth investigations into the toxicological mechanisms exerted by this pesticide on biota, as well as the urgency to find potential green applications, such as products derived from algal biomass wastes, to mitigate the toxic effects of thiram on aquatic organisms.

Bioaugmentation and plant-assisted bioremediation for contaminated environment recovery

C. Cavone[1,2], A. Bucci[2], G. Naclerio[2], A. Barra Caracciolo[3], I. Savino[1], L. Rolando[3], P. Grenni[3], V.F. Uricchio[1], V. Ancona[1]

[1]Water Research Institute, National Research Council, Bari, Italy .[2]University of Molise, Department of Biosciences and Territory, Pesche, Italy. [3]Water Research Institute, National Research Council, Rome, Italy

Inorganic and persistent organic pollutants (POPs) pose a significant environmental and human health threat to terrestrial and aquatic ecosystems. Bioaugmentation and plant-assisted

bioremediation (PABR) can be effective and sustainable tools for recovering environmental pollution resulting from these chemicals.
The IRSA-CNR research group is focused to the development of advanced and environmentally friendly remediation strategies for various contaminated ecosystems. The proposed strategy relies on collaborative actions of microbial populations with plant species, promoting metabolic breakdown and removing contaminants from an ever-growing number of contaminated environments.
The effectiveness of the bioaugmentation with a microbial consortium composed of four hydrocarbon-oxidizing bacterial strains belonging to the *Acinetobacter*, *Rhodococcus,* and *Gordonia* genera was evaluated for recovering a soil contaminated by heavy metals and polychlorobiphenyls (PCBs). Furthermore, the contribution to the decontamination process of a medicinal plant (*Lavandula angustifolia* Mill.), alone or in combination with the hydrocarbonoclastic bacteria, was also assessed.
In detail, the experimental microcosm setup included: a (1) negative control represented by a historically contaminated soil; (2) contaminated soil pots where *L. angustifolia* alone was planted; (3) contaminated soil pots inoculated with the microbial consortium; (4) contaminated soil pots bioaugmented with bacteria and planted with the medicinal plants.
Chemical analysis of soil properties (pH and EC) and contaminants concentrations were performed on soil samples collected at two times of investigations (45 and 90 days) in each microcosm experimental condition. At the same time, microbial analyses were carried out to assess total microbial abundance (DAPI counts), and microbial dehydrogenase activity; moreover, 16S rRNA gene sequencing was carried out to assess soil microbial community composition. PCB and HM analyses are ongoing on biomass samples (roots, leaves, shoots) for evaluating the occurrence of contaminant translocation/bioaccumulation phenomena, as well as assessing secondary metabolites produced by plants.

Drosophila melanogaster **a new ecotoxicological method for air pollutants**

G. Tesoriere[1,2], F. Vernì[2], I. Lacchetti[1], L. Mancini[1]

[1]Ecosystem and Health Unit, Enviromental and Health Department, Italian National Institute of Health, Rome, Italy.
[2]Department of Biology and Biotecnology "Charles Darwin", Sapienza University of Rome, Rome, Italy

Air quality in urban sites is a matter of growing concern, with significant implications for public health and the environment. Many cities have recently reached alarming levels of air pollution that exceed the limits set by European and international regulations, causing concern among experts and citizens. The impact of air pollution on ecosystems and human health is also one of the complex aspects addressed by the WHO, which is working to ensure the improvement of air exposure quality through the achievement of three SDGs of Agenda 2030 (3, 7 and 11). It is essential to develop new methods and bioindicators that can identify early signs of air quality impoverishment, which is increasingly linked to climate change. Standardized protocols for test organisms in ecotoxicology on air quality are very limited, especially when applied directly to air samples. The aim of this work is to fine tune a simple but effective method that could allow us to make *Drosophila melanogaster* a valid standard model system to assess the toxic effects of air pollutants.
D. melanogaster has proven to be an effective model system in scientific research due to its many advantages; fruit flies, in fact, have low maintenance costs, they have a short lifespan (12 d), they are easily raised and manipulated; moreover 75% of the genes related with human diseases have homologs in *D. melanogaster.*
The proposed method involves the use of drosophila 3rd instar larvae, since it has proven to be the most sensitive phase to environmental changes compared to adults. 30 larvae at least are exposed in each test to different volatile substances, such as ethanol (EtOH), in order to identify the lethal and sublethal endpoints and to verify the feasibility of the method. Particular attention was given to neurotoxicity endpoints through the study of behavior. We analyzed the locomotor activity of the larvae through different environmental conditions (such as light/dark, temperature, exposure time, etc.) and with the help of Ethovision software we identified and quantified the effect observed compared to the control larvae.

Preliminary results have confirmed that *Drosophila melanogaster* is an excellent test organism for ecotoxicological research, we have also demonstrated the feasibility of the proposed method, identifying the most crucial and easily quantified parameters. In the future, more substances will be tested and the method will also be conducted in the field, exposing organisms directly to the mixture of air pollutants present in urban contexts.

Integration of RNA and 16S rRNA Amplicon Sequencing for risk evaluation of pharmaceuticals on non-target species

I. Bernardini[1], M. Mezzelani[2], A. Nardi[2], O. El-Idrissi[1,3], G. Dalla Rovere[1], L. Peruzza[1], M. Babbucci[1], T. Patarnello[1], S. Ferraresso[1], F. Regoli[2], M. Milan[1]

[1]Department of Comparative Biomedicine and Food Science, University of Padova, Legnaro (PD), Italy, [2]Department of Life and Environmental Sciences, Università Politecnica delle Marche, Ancona, Italy. [3]UMR CNRS 6134 Sciences pour l'Environnement, Università di Corsica Pasquale Paoli, Corte, France

Nowadays, the massive and continuous use of pharmaceuticals means they are consistently poured into waste waters and consequently introduced into aquatic environments, leading to growing concerns about their environmental impact. These biologically active compounds are designed to interact with specific physiological pathways, therefore their mode of action on non-target species needs to be investigated. In this regard, the integration of RNA and 16S sequencing analyses may be adopted as valuable tool for understanding potential effects of emerging contaminants. This approach was applied on Mytilus galloprovincialis chronically exposed to different classes of pharmaceuticals. Specifically, two laboratory experiments were conducted to investigate transcriptomic and microbial community changes in mussels exposed for 30 days to environmental concentrations of i) gemfibrozil, metformin, ramipril, venlafaxine and ii) ibuprofen and paroxetine both in single and in combination each other. These treatments were followed by a 14-day depuration period to assess the potential recovery.
Overall, the greatest transcriptomic and microbial changes were detected in mussels exposed to the combination of drugs. Except for ramipril, specific responses of each single compound mirrored known drugs' mode of actions in humans: gemfibrozil altered fatty acid metabolism, metformin impaired homeostatic maintenance, venlafaxine modulated ion regulation. In the second experiment, ibuprofen significantly affected proliferation, stress response, and immune functions, with lingering effects post-depuration, while paroxetine impacted pathways related to the serotonergic system. In addition, changes in microbiota composition were observed, including the over-representation of potential opportunistic pathogen species in mussels exposed to a few of tested drugs. However, considering all the transcriptional responses, the depuration period allowed to decrease the risk to the single and mixtures of investigated pharmaceuticals.
Overall, the integration of these data allowed to evaluate the hazard on gene expression and microbiota community in non-target marine organisms of these such concerning contaminants.

Tire rubber leachate: a threat to the gamete quality of the mediterranean mussel *Mytilus galloprovincialis*

I. Savino[1], A. Nobahar[2], J.P. Da Silva[2], P. Cotugno[3], R. Notariale[4], G. Corriero[5], V.F. Uricchio[1], A. Gallo[4]

[1]Water Research Institute, Italian National Council of Research, CNR-IRSA, Bari, Italy. [2]Centre of Marine Sciences (CCMAR/CIMAR LA), University of Algarve, Campus de Gambelas, Portugal. [3]Chemistry Department, University of Bari "Aldo Moro", Bari, Italy. [4]Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Naples, Italy. [5]Department of Biosciences, Biotechnologies and Environment, University of Bari Aldo Moro, Bari, Italy.

Micro-sized tire particles originating from tire wear, recycled tire fragments, and debris tire repair, significantly contribute to microplastic environmental pollution. It is estimated that thousands of tons of micro-sized tire rubber particles enter the marine environment, each year, threatening the health of organisms. Moreover, tire rubber contains many additives and their by-products, which can leach into seawater and induce toxicity. Referring to marine invertebrate species, that adopt external fertilization as a reproductive strategy, this exposes gametes to chemical contaminants in seawater, which could

compromise their quality. In this regard, the present study aims to analyse the chemical profile of seawater leachates produced by end-of-life vehicle tire particles and their negative effect on the quality of *Mytilus galloprovincialis* gametes. The comprehensive screening of organic and inorganic compounds in leachate was conducted using advanced techniques, such as Liquid Chromatography-Mass Spectrometry and Inductively Coupled Plasma Mass Spectrometry. Gamete quality assessment was performed by analysing several physiological parameters including viability, mitochondrial membrane potential, intracellular pH, oxidative state, and sperm motility, after both 1-hour and 2-hour of exposure to leachate. The chemical characterization undeniably demonstrated the leaching of some metals, particularly zinc, and organic compounds such as antioxidants, vulcanizing agents, and protective agents from car tire particles in seawater. The ecotoxicological analysis highlighted the impact of these compounds on *M. galloprovincialis* gametes. The exposure of mussel oocytes and spermatozoa to tire particle leachate caused a significant increase in mitochondrial activity in both gametes. Moreover, a reduction in sperm motility was detected. These results indicate the adverse effects of end-of-life tire particles on gamete quality, which is a key factor for species' suitability and survival. Indeed, the observed changes in physiological parameters may result in fertilization failure and, in turn, mussels' reproductive success. However, due to the "cocktail effect" of the chemicals in tire particle leachate, it is challenging to determine which is responsible for the harmful effects in assessing reproductive risk. Therefore, more research efforts are needed to ensure risk mitigation and environmental safety.

Investigation of the effects of 1,3-diphenylguanidine on *Daphnia magna*

J. Požeg Krišković[1,2], S. Magni[1], L. Nigro[1], G. Caorsi[1], C. Cremonesi[1], A. Binelli[1], C. Della Torre[1]

[1]*Department of Biosciences, University of Milan, Milan, Italy.* [2]*Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia.*

1,3-diphenylguanidine (DPG) is an organic compound, primarily used as a secondary accelerator in the vulcanization of rubber, process crucial for manufacturing vehicle tires. Consequently, DPG can enter the environment through wear and tear from vehicular traffic. It can be found in high concentrations along highways, on rubberized playgrounds, and in indoor parking lots. Moreover, rain can further mobilize DPG, causing road run-off into urban receiving waters and potentially drinking water supplies. DPG-containing particles were even found in dust particles in European, American, and Asian households. Therefore, assessing the effects of this contaminant on the environment is important, as understanding these impacts is essential for developing effective mitigation strategies in the future. This study investigates the effects of DPG on the freshwater crustacean *Daphnia magna*, a widely used model organism in ecotoxicology. The acute effects were assessed according to the OECD 202 protocol, showing the absence of toxicity up to 1 mg/L. Organisms were subsequently exposed for 21 days to negative control, DMSO control, and two concentrations of DPG (500 ng/L and 50 µg/L) according to the OECD 211 protocol. Several endpoints were measured at different levels of biological organization, such as reproduction, heart rate, behaviour, filtration rate, and biomarkers of neurotoxicity and oxidative stress. There were no significant effects in most of the endpoints investigated. Therefore, our findings suggest that DPG is not toxic at the evaluated concentrations, yet additional research is required to conclusively eliminate any concerns regarding its ecotoxicity.

A blood-based multi-biomarker approach reveals different physiological responses of common kestrels to contrasting environments

L. Giovanetti[1], I. Caliani[1], G. Damiani[2,3], G. Dell'Omo[2], D. Costantini[3,4], S. Casini[1]

[1]*Department of Physical, Earth and Environmental Sciences, University of Siena, Siena, Italy.* [2]*Ornis Italica, Rome, Italy.* [3]*Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy.* [4]*Unité Physiologie Moléculaire et Adaptation, CNRS-Muséum National d'Histoire Naturelle, Paris, France.*

The increase of urbanization and agricultural activities is causing a dramatic reduction of natural environments. As a consequence, animals need to physiologically adjust to these novel environments,

in order to exploit them for foraging and breeding. The aim of this work was to compare the physiological status among nestling common kestrels (Falco tinnunculus) that were raised in nest-boxes located in more natural, rural, or urban areas in a landscape with a mosaic of land uses around Rome in Central Italy. A blood-based multi-biomarker approach was applied to evaluate physiological responses at multiple levels, including antioxidant concentrations, immunological functions, genotoxicity, and neurotoxicity. We found lower concentrations of glutathione and GSH:GSSG ratio values and higher proportions of monocytes in urban birds compared to the other areas. We also found higher DNA damage in rural compared to urban and natural krestels and inhibition of butyrylcholinesterase activity in urban and natural birds compared to rural area. Finally, we found similar values among study areas for respiratory burst, complement system, bactericidal capacity, and plasma non-enzymatic antioxidant capacity. These results suggest that (i) city life does not necessarily cause physiological alterations in kestrels compared to life in other habitats, and (ii) environmental pressures are likely to differ in typology and intensity across habitats requiring specific responses that a multi-biomarker approach can help to detect. Further studies are needed to assess which factors are responsible for the physiological differences among city, rural, and natural birds, and whether these differences are consistent across time and space.

It is not a party for the sea!

L. Albarano[1], G. Lofrano[2], V. Romano Spica[2], M. Guida [1,3,4], G. Libralato[1]

[1]Department of Biology, University of Naples Federico II, Naples, Italy. [2]Department of Movement, Human and Health Sciences, University of Rome "Foro Italico", Rome, Italy. [3]Centro Servizi Metrologici e Tecnologici Avanzati (CeSMA), Naples, Italy. [4]NBFC, National Biodiversity Future Center, Palermo, Italy.

Microplastic and microfiber contamination from party items such as balloons and straws has become an increasingly significant issue in recent years, attracting growing attention. These often-overlooked materials contribute substantially to marine and coastal pollution. Plastic fragments resulting from the breakdown of these items and the microfibers released during production and disposal are significant sources of contamination for aquatic environments. It is estimated that a considerable amount of microplastics and microfibers from party items are released into the marine environment each year. Since they are made of non-biodegradable plastic materials, these fragments and fibers persist in the environment for extended periods, causing damage to marine ecosystems and posing a threat to wildlife. In this study, aging experiments in saltwater were conducted on two party items, balloons and straws, to evaluate the release of microfibers and microplastics and their potential environmental impact on the marine crustacean Artemia franciscana. The results showed a time-dependent release of organic materials, composed of fragments and fibers of various colors, distributed across four size ranges: 0.6213 nm, 142 nm, 1283.12 nm, and 5560 nm. Spectrophotometric analyses identified the chemical nature of the released plastics, with UV absorption peaks between 260 and 290 nm, attributable to the presence of Styrene-Butadiene Rubber (SBR), and peaks between 290 and 450 nm, indicative of the presence of polycarbonate and polystyrene. Toxicity experiments revealed that leachates generated after prolonged exposure to synthetic saline solution showed a decrease in survival and phototactic activity, particularly in larval stages. This highlights a greater vulnerability of larvae to the ingestion of microplastics. Genotoxicity tests on *A. franciscana* further showed that leachates produced after 5 and 6 months caused significant molecular stress in all larval stages and adults. This stress is correlated with the down-regulation and up-regulation of genes involved in immune response and protein protection from environmental stress. Overall, the results indicate that microplastics and microfibers released from party items can negatively affect marine organisms, altering their gut microbiome and significantly reducing survival rates, with potentially harmful impacts on the entire marine ecosystem. These findings underscore the need for greater attention to the management and disposal of plastic materials used in celebrations to reduce their environmental impact.

Effect-based methods in the EU Water Framework Directive: a new approach for the detection of mixtures.

M. Carere, A. Mancini, I. Lacchetti, L. Mancini

Deparment of Environment and Health, Unit Ecohealth, Italian Institute of Health, Rome, Italy

Thousands of chemicals substances released in the ecosystems are considered emerging because are not regulated, the exposure data are limited and risk assessment data are scarce as highlighted in the context of the EU Chemicals Strategy for Sustainability. These chemicals, together with traditional water pollutants, can also form mixtures with a potential synergistic combined effect and risks for ecosystems and human health. Legal requirements need to be consistently in place to ensure that risks from simultaneous exposure to multiple chemicals are taken into account. The effects caused by the mixtures of substances in the environment may not be predictable on the basis of chemical analyses alone and Effect-Based Methods (EBM) are considered one the main methodologies for the detection of synergistic effects. In the current European Commission proposal Directive on environmental quality standards for the priority substances in the aquatic environments it is foreseen, for the first time, the inclusion of Effect-Based Methods (bioassays in vitro and in vivo; biomarkers) for the evaluation of the effects of the current and future priority substances. In this proposal there is a possibility for the Member States to monitor, through the use of bioassays, the effects of the widespread endocrine disrupting chemicals E2, EE2 and E1. Furthermore the definition of the environmental quality standards for the good chemical status will include also trigger values based on the use of effect based methods; these trigger values should protect the aquatic life and human health. This proposal will update the EU Water Framework Directive (WFD). The inclusion in the EU legislation of EBM has been possible also thanks to the activity co-chaired by Italy in the context of the WG Chemicals of the Common Implementation Strategy (CIS) of the WFD. It is useful that this approach must be in future strengthened considering also other EBM and the other main effects in the aquatic environments such as genotoxicity, neurotoxicity and oxidative stress. In conclusion it is necessary that the Proposal Directive will be endorsed and published as soon as possible because the use of EBM can represent an important screening and early warning system for the mixtures detection in European waterbodies with important benefits for aquatic ecosystems and human health.

Pollinator pioneers: bee ecotoxicology unveiling effects of air pollutants

M. Barra, M. Carere, L. Mancini, I. Lacchetti

ISS, Italian National Institute of Health, Department of Environment and Health, EcoHealth Unit, Rome, Italy.

The increasing population and the growing use of industrial techniques, fossil fuels, and greenhouse gas emissions are causing significant climate changes. These and other human activities contribute to the decline in air quality. The most common pollutants are particulate matter (PM10 and PM2.5), sulphur dioxide, nitrogen oxides, ozone and its precursors, carbon monoxide, and others which have been increasing in recent years. Furthermore, one of the most significant problems associated with atmospheric pollutants is the formation of heat islands in urban environments, which amplify the effects of high temperatures and worsen air quality.

In this context, we propose a new method for analysing atmospheric particulate matter (PM) and pollutants in urban and rural environments using bee larvae (*Apis mellifera*). Bees are effective bioindicators due to their natural role in collecting data on air and environmental quality, providing valuable insights into various types of contamination and their potential impacts. However, their application is often limited to pesticide assessment, and ecotoxicological tests are rarely used to analyse air quality.

This study aims to examine the effects of particulate matter and various relevant atmospheric contaminants on bee larvae for 96 hours. Specifically, we propose to adapt OECD guideline 239 to study atmospheric pollutants by utilising endpoints such as mortality and behavioural changes. Additionally, by varying parameters such as temperature, it is possible to assess the impact of global temperature increases on bees, offering a new and important perspective for understanding the effects of climate change on

urban ecosystems. A comparison between urban characteristics and a rural area was considered, and preliminary results indicate a correlation between increased mortality and locomotor activity with the presence of pollutants.
These findings suggest an integration of monitoring to assess lethal and sublethal responses in bees following exposure to pollutants and atmospheric particulate matter, thereby enhancing our understanding of long-term environmental impacts.

An integrated approach for the management of beach and recreational use of water

R. De Angelis[1], M.G. Andrisani[1], E. Beccaloni[2] M. Benzi,[3] L. Bergamin[1], P. Borrello[1], V. Fuscoletti[2], C. Maggi,[1] C. Mazziotti[3], M. Lezzi,[3] E. Nerone[4], S. Recchi[4], F. Scaini[2], F. Silvestri[1], E. Spada[1], A. Tornambè[1], A. Vecchio[1], E. Romano[1]

[1]ISPRA. Italian Institute for Environmental Protection and Research, Rome, Italy. [2]ISS Istituto Superiore di Sanità, Rome, Italy. [3]ARPAE. Agenzia regionale per la prevenzione, l'ambiente e l'energia dell´Emilia-Romagna, Cesenatico, Italy. [4]IZS Istituto Zooprofilattico Sperimentale Abruzzo e Molise, Termoli, Italy.

The recent challenges caused by increasingly numerous global climate and environmental changes are making us rethink human health as something inseparable from the state of natural resources and environmental variables on which health depends, converging on the "one health" principles. The global recreational use of water in coastal areas, often associated with many leisure activities, determines the ever-increasing need to guarantee these areas in an ever improving quality status. Over the years, the European Union has adopted directives and decisions that set water quality objectives, even if they have been incompletely effective. The Directive 2000/60/EC provides for monitoring and preventing pollution, even accidental, to achieve a “good” environmental status, protecting water resources, natural ecosystems, and biodiversity. Moreover, 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 Directive 2000/60/EC. In recent years, also risk analysys is applied for 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 contaminants, like emerging pathogens (including antibiotic-resistant ones) and of some chemical contaminants (heavy metals, PAHs and PCBs, among the others) in bathing areas exposed to anthropogenic pressures (industrial, urban, and agricultural discharges), thus integrating health skills with environmental ones. In particular, an integrated chemical, ecotoxicological and microbiological characterization is being conducted on two recreational coastal sites of the Tyrrhenian and Adriatic Sea. This characterization, carried out in three matrices (water, sediment, and biota), will allow the development of a risk assessment methodology to estimate the population's exposure to contaminants through different routes: ingestion, dermal contact and dust inhalation from the beaches; ingestion, dermal contact and inhalation of marine bioaerosols from bathing water; direct contact with marine sediments and the evaluation of their resuspension in bathing waters and transfer through the trophic web.

Assessing the effectiveness of mitigation measures on pollinator decline: an integrated multi-biomarker approach (ÆM-POLLY project)

S. Casini[1], T. Campani[1], I. Caliani[1], G. Manieri[1], A. Franchi[1] , A. Di Noi[3], L. Giovanetti[1], M. Mazzei[2], L. Cabiddu[2], L. Lenzi[2], F. Lami[2], R. Costantino[2], F. Sgolastra[2]

[1]Department of Physical, Earth and Environmental Sciences, University of Siena, Siena, Italy. [2]Department of agricultural and food sciences, Universiy of Bologna, Italy. [3]Department of Life Science, University of Siena, Siena, Italy

The aim of the ÆM-POLLY PRIN project is to develop and validate an integrated protocol as a tool to verify if the mitigation measures foreseen by the EU Green Deal for agricultural practices, are effective in halting and reversing the decline of wild pollinator biodiversity. This monitoring protocol will integrate endpoints in terms of presence, abundance and diversity of wild

pollinator species with endpoints able to assess the state of health at the sub-individual, individual and population level. These endpoints are selected and designed to diagnose biological alterations in wild pollinators due to different pressures: chemical stress from pesticides, stress linked to climate changes, food and water deficiencies, habitat loss and diseases. Such an integrated approach using a set of biomarkers has never applied before to wild pollinators. The monitoring protocol is applied in 4 orchards and 4 vineyards characterised by the presence or absence of mitigation measures, and in 4 adjacent natural areas. Surveys for assessing pollinator diversity are performed once in spring and once in late summer. During this activity individuals of the most abundant/representative species are sampled for chemical (pesticide levels), morphological (body size and fluctuating asymmetry variations) and biomarker analysis (immune, reproductive, and nervous systems, oxidative stress, metabolism, detoxification processes and genotoxicity, energy mobilisation and feeding performance). From our project we expect to: 1) obtain a dataset of baseline biomarker values related to the health status of representative pollinator species; 2) assess the effectiveness of the mitigation measures by comparing the pollinator diversity and the biomarkers related to their health in two types of crops; 3) define a final integrated monitoring protocol to assess the health status of wild pollinators and their biodiversity; 4) develop user-friendly guidelines to assess the effectiveness of the different mitigation measures in different agroecosystems, to guide decisions and inform policies.

Evaluation of toxicological effects of fungicides used in vineyards agroecosystem using *Apis mellifera* as a model species

T. Campani[1], I. Caliani[1], A. Di Noi[2], L. Giovanetti[1], G. Manieri[1], S. Casini[1]

[1]Department of Environmental Earth and Physical Science, University of Siena, Siena, Italy. [2]Department of Life Science, University of Siena, Siena, Italy.

Agroecosystems provide habitats, food and water, for many species, but are areas where pesticides and fertilizers are widely used. Pesticides are the most important threat to pollinator insects that live in agroecosystems, especially due to their intensive use in agriculture. Many European regions are famous for wine production, with land covered by vineyards where the biodiversity is absent and to preserve wine production many pesticides are used. The European Directive 128/2009/CE and No 540/2011 regulate the use of pesticides in agroecosystems. Although many active principles have been banned, some substances such as those used in viticulture are already permitted. Breed bees and wild bees come in contact with the pesticide used in vineyards collecting the morning dew from vine leaves and using sugar from the grapes, especially during increasing periods of drought. Our study aimed to investigate the toxicological effects on honeybees, used as model species, of the commercial formulation of two fungicides: 1)Folpet-based and cymoxanil+copper-based, alone and in combination and the toxicological effects of 2)copper. Laboratory experiments with fungicides and copper and a 2 years beehive survey in two vineyards with different types of management (conventional and organic) during the use of this type of fungicide were carried out. A multi-biomarker approach was applied to test the neurotoxic effect (AChE, CaE), metabolic alterations (ALP), detoxification system (GST), efficiency of the immune system (LYS, proPO, PO and hemocyte count and genotoxicity (NA assay). The results of the laboratory experiment with Folpet-based, and cymoxanil+copper-based showed induction of GST activity in all treatments except the mixture at the lowest concentrations and a decrease in the ALP activity. LYS activity increased in the groups exposed to the Folpet-based fungicide, to the lower concentration of cymoxanil-copper-based and the mixtures. The pesticide interaction model showed that the mixture of the two fungicides had subadditive effects. The beehive monitoring showed no esterase inhibition during and after the treatment of the vineyards compared to the organic vineyard and metabolic alterations and effects on the immune system were found in bees sampled during the vineyard treatments. This study confirms the need to deeply investigate the sublethal effects of commercial pesticides, in particular those used in vineyards, to protect wild and farmed pollinators.

Aedes koreicus: could an invasive mosquito be used as sentinel of heavy metal pollution?

T. Zoli, I. Arnoldi, S. G. Signorini, L. Soresinetti, G. Naro, A. Negri, P. Gabrieli, C. Bandi, C. Della Torre, S. Epis

Departiment of Biosciences, University of Milan, Milan, Italy.

Heavy metals could represent an environmental hazard when they accumulate in organisms following contact to contaminated environmental substrates, such as soil, water, and air. Contamination can occur due to massive and/or mismanaged anthropic activities, like mining, vehicular traffic, and manufacturing. Due to their persistency and the ease with which they are eventually transferred to food products, high heavy metal concentration poses a threat also for human health.

Metal contamination is usually stronger in urban environment where the sources of pollution are more common, nevertheless, heavy metals can also be found in more rural areas, where metal rich sewage and water treatment sludges are commonly used as fertilisers in agriculture. Mosquito larvae are frequently found associated both with artificial containers and natural pools. They have already been used as bioindicators to detect pollutants, due to their capillary spread in both urban and peri-urban territories. We have recently characterised the distribution of the invasive Korean bush mosquito species *Aedes koreicus* in the Lombardy region (Northern Italy), particularly in some highly industrialised peri-urban areas and farmlands. In this study we evaluated the possible use of *Ae. koreicus* larvae as sentinels for heavy metal pollution in these localities. To this aim, larvae were exposed *in vivo* to Cu, Cd and Pb at 0.1 and 0.5 mg/L for 7 days. Morphological, biochemical, and molecular endpoints were investigated to select potential markers of metal exposure and to determine if this species could be useful for future environmental monitoring.

Morphological analysis using larvae body length as a parameter are being conducted using Leica stereoscope camera. A suite of biochemical biomarkers related to oxidative stress (content of reactive oxygen species, activity of catalase) detoxification (Glutathione-S-transferase) and neurotoxicity (activity of acetylcholinesterase) was also applied. To detect possible alteration in larvae metabolism we selected three genes: Trehalase 1, Chitin synthase 2, Chymotrypsin 1 and analysed respective expressions via RT-qPCR. Preliminary results show a significant damage to metabolism and physiological functions in *Ae. koreicus* larvae exposed to heavy metals.

The Team

Andrea Binelli – Scientific and Local Commettee

Andrea Binelli was a Member of SETAC ILB Council from 2012 to 2021. He is Full Professor of Ecology at the Department of Biosciences, University of Milan, since 2017 and currently his research is focused on the evaluation of the effects of micro- and nanoplastics, as well as water-soluble polymers (WSPs) in different biological models.
Since 2020 he has been a Member of the Restricted PhD Course board in Environmental Sciences of the University of Milan and since 2024 he has been a Member of the PhD Course of National Interest (DIN) on Biodiversity. Since 2022 he has been an elected Councilor on the Executive Board of SITE (Società ITaliana di Ecologia), and from 2021 to 2023 he was the President of the ASN (Abilitazione Scientifica Nazionale) Committee.
He is the author of more than 120 articles in international scientific peer-reviewed journals.

Camilla della Torre – Member of SETAC ILB Council

Camilla della Torre is a Council member since 2018. Associate Professor in Ecology at University of Milan. Her research deals with understanding the molecular and physiological mechanisms involved in the response to environmental and anthropogenic disturbances in aquatic organisms. Visiting scholar at Scripps Institution of Oceanography California University San Diego in 2012, at Laboratory of Molecular Ecotoxicology Division for Marine and Environmental Research Rudjer Boskovic Institute, Zagreb, Croatia, in 2009 and at Norwegian University of Science and Technology (NTNU), Trondheim in 2007. Italian member of the COST action "Conservation of Freshwater Mussels a Pan European Approach". Member of the Editorial board of Frontiers in Marine Sciences Marine Pollution section and of Environmental Toxicology and Pharmacology.

Stefano Magni – Member of SETAC ILB Council

Stefano Magni is a Council member since 2018. He is a Researcher of Ecology at the Department of Biosciences of the University of Milan. His research focuses mainly on the impact of (micro)plastics and tire particles in aquatic environments through the application of biomarkers and omics techniques. In 2017, thanks to the Brusarosco Award assigned by the Italian Ecological Society (S.It.E.), he performed a period as visiting researcher in the laboratory of Dr. François Gagné at Environment and Climate Change Canada (Government of Canada) to investigate the neurotoxicity of (micro)plastics. He is in the editorial board of the Journal of Xenobiotics and has authored and co-authored more than 50 publications in international scientific peer-reviewed journals and books.

Ilaria Corsi – President of SETAC ILB

Ilaria Corsi is the President of SETAC ILB since June 2023, vice-president in 2021-2022 and member of SETAC Europe since 1999. Member of the scientific committee of the SETAC Europe Annual meetings in 2021 (virtual) and in 2024. Associate Professor of Ecology and Ecotoxicology at the Univ. of Siena (IT) and Head of the Ecotoxicology and Biological Monitoring Lab (EcoBiomLab). Expert in ecotoxicology of contaminants of emerging concern including nanomaterials and nanoplastics. Co-chair of the PLASTICS Action Group of the Scientific Committee for Antarctic Research (SCAR) since 2018 and of the Marine Ecotox Focus Group of the Nanosafety Cluster of the European Commission from 2011 to 2017. Currently member of the Expert Group on plastic pollution of the International Science Council. FULBRIGHT fellow at the Stanford University in 2002-2003 and two times fellow of the King's College of Cambridge in 1994 and 2017. Co-chief editor of Frontiers in Marine Sciences, Marine Pollution section and member of the Editorial board of several peer-review journals and guest editor of special issues. https://docenti.unisi.it/it/corsi-0.

Stefania Marcheggiani – Vice President of SETAC ILB

Stefania Marcheggiani, Council member since 2022 and Vice President of SETAC ILB since 2023.As a researcher at the Dept. of Environmental and Health of Istituto Superiore di Sanità, she has proven experience on: water-related pathogens, antibiotic resistance, biotechnology sampling strategies, and climatic changes. Principal BlueAdapt H2020 and actively involved in European research projects (PULVIRUS, IntCatch H2020, BlueHealth H2020, µAQUA -FPVII, µPAD-FPV). Expert for: WG Ecological Status (ECOSTAT) CIS WFD 2000/60/CE since 2009; WG WATER Reuse, WG Water Scarcity and Drought, DG Santè subgroup OH AMR and IAMPHI subgroups Planetary Health and One Health and Extreme Weather Events. She is a supervisor of Master and PhD students and chair and co-chair of national and international conferences and training courses.

Claudia Vaj – External Advisor of SETAC ILB

Claudia Vaj, member of SETAC Europe since 2015 and chair of several sessions throughout the years; Member of SETAC ILB Council since 2016; President of SETAC ILB (May 2019-May 2021) and currently External Advisor and coordinator of the SETAC ILB Young Scientist Award; Member of SETAC Europe Council since May 2022. She has a background in ecotoxicology and environmental sciences, and she works in the Regulatory and Stewardship Dept. of Corteva Agriscience Italia as Country regulatory Manager for Italy and Malta and focal point at Country level for environmental topics (e.g. mitigation measures of PPP, water, sustainable uses). She is also part of various working groups in Italian Crop Protection Association.

Paola Grenni – External Advisor of SETAC ILB

Paola Grenni, Member of SETAC Europe since 2004 (Chair of different sessions of EU meetings), co-Chair of the SETAC EU Annual Meeting 2018 (Rome); from 2020, part of Steering Committee of Global Soils Interest Group, GSIG. Member of the SETAC ILB Council, President of SETAC ILB (May 2015-May 2017) and currently External Advisor. Senior Researcher at Istituto di Ricerca Sulle Acque, Consiglio Nazionale delle Ricerche (IRSA-CNR) in Rome (IT). Expert in Microbial ecology of contaminated environments and in ecotoxicology. Official Representative of IRSA-CNR in the Antimicrobial Resistance (AMR) Multi-Stakeholder Partnership Platform. Member of NORMAN Network. Scientific and Organizing Committee of RemTech EU 2023 and 2024. Awarded with Elsevier Atlas Award and MARIE CURIE Fellowship. Editorial board, Specialty Chief Editor and Guest editor of various peer-review Journals.

Federica Cacciatore – Member of SETAC ILB Council

Federica Cacciatore is a Council member since 2016 and more recently Social Media Manager of the SETAC ILB. She is a marine biologist expert on ecology of transitional waters, working at ISPRA as a Researcher. Among major topics: monitoring and assessment of contamination in biota, water and sediments of transitional and marine waters; Imposex; environmental quality assessment; ecological restoration assessment; statistical data analysis; scientific and technical support to the Italian Ministry of Environment. Member of: WG Ecological Status (ECOSTAT) CIS WFD 2000/60/CE; WG SO VI/09-06 Eutrophication – classification criteria; WG ENCA – Climate change adaptation - Coastal Wetland restoration. Coordinator of Monitoring actions (D.1, D.6) of LIFE Lagoon Refresh. Editorial board, Topic and Guest Editor of peer-review Journals. Supervisor of Master students.

Silvia Franzellitti – Member of SETAC ILB Council

Silvia Franzellitti is a Council member since 2022 and more recently Social Media Manager of the SETAC ILB. She is an Associate Professor at the Univ. of Bologna with a background in animal physiology and environmental science. Her research activity deals with molecular mechanisms behind physiological plasticity and stress response in marine invertebrates related to climate changes and emerging pollutants, including pharmaceuticals and microplastic. She is Associate Editor of Environmental Toxicology and Pharmacology, Frontiers in Physiology, and Frontiers in Marine Sciences.