SETAC Italian Language Branch

SETAC Italian Language Branch 2nd Workshop

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Book of Abstracts

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SETAC Italian Language Branch

2nd Workshop

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Chairs: *Paola Grenni, Elena Romano, Ginevra Moltedo*

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18.00 ***Concluding remarks***

Introduction

The ***Society of Environmental Toxicology and Chemistry*** (SETAC) was estabilished in 1979 in North America upon a set of founding principles currently defined as Multidisciplinary Approaches to Solving Environmental Problems, Science-based Objectivity and Multi-Stakeholders Involvement. SETAC's statutes provide for equal representation of research and education, industry and governance entities. SETAC's mission is to advance science and environmental management through collaboration, communication, education, leadership and organisations.

The ***SETAC Italian Language Branch*** (SETAC ILB) is a regional section of SETAC Europe, founded about 20 years ago to support the development of principles and practices for sustainable environmental quality, conservation and integrity of ecosystems through a multidisciplinary approach as foreseen by SETAC. The Italian section brings together the Italian-speaking members of SETAC Europe in Italy and abroad.

Since 2009, SETAC ILB has had a new organisational impulse, starting with a meeting with the most active members in Rome in the Marconi Room of the National Research Council. Since then, a number of even more significant opportunities for discussion have been created and the transposition of science into environmental policies has been facilitated through the sponsorship of thematic events, as well as the organisation of seminars and annual meetings as part of national training and dissemination events (e.g. Ecomondo, RemTech). To date, the SETAC ILB comprises about 150 members, distributed among Research Bodies, Universities and Industries in Italy and abroad. It also has a Scientific Council, whose current president is Ilaria Corsi (Siena Univ.). Through its social media channels and through the SETAC Europe, **the SETAC ILB sponsors and promotes, free of charge, various events at the national and the international level**, which are related to the SETAC mission and in which SETAC ILB members are involved[1].

In 2022, the 1st National Workshop was organized at the Siena University, under the presidency of Silvia Casini and vice-presidency of Ilaria Corsi, simultaneously to the XXXI Annual Meeting of the Italian Society of Ecology (S.It.E). Given the considerable success of the event, which was attended by numerous universities, research organisations, public institutions and companies, the SETAC ILB Scientific Council decided to organise the workshops annually. On 11 October 2023, the 2nd Workshop was hosted by the National Research Council at the Marconi Hall, the historical headquarters of the Research Institution in Rome, thanks to the precious contribution of Paola Grenni (National Research Council, Water Research Institute, CNR-IRSA) and under the presidency of Ilaria Corsi (Siena Univ.) and the vice-presidency of Stefania Marcheggiani (National Institute of Health, ISS). Very supportive was the scientific and organizing committee: Federica Cacciatore and Ginevra Moltedo (The Italian Institute for Environmental Protection and Research, ISPRA), Claudia Vaj (Corteva™ Agriscience), Camilla Puccinelli (ISS), Silvia Casini (Siena Univ.) and Massimiliano Scalici (Roma Tre Univ.). The 2nd Workshop was organised under the auspices of SETAC Europe: the current President, Bruno Campos, who spoke at the event with the opening speech, provided various insights into the mission of SETAC Europe and the importance of the regional branches in stimulating the scientific community towards the development and promotion of valuable solutions to counteract and mitigate the significant environmental changes, which we are witnessing in recent years, linked to the anthropic impact on natural ecosystems. His opening speech stimulated and encouraged the

[1] For SETAC ILB support to promote an event, please send an e-mail to the SETAC ILB President or a SETAC ILB Council Member (https://italianbranch.setac.org/council), clearly indicating topics, place and date of the event.

discussion and exchange of opinions that characterized the various session.
More than 140 members, mostly in person but also remotely, attended the 18 oral presentations and 12 virtual posters distributed in the following three main sessions:

a) **Ecological and human health risk assessment;**
b) **Ecotoxcology and stress ecology: from molecules to organisms up to population and ecosystem levels;**
c) **Environmental monitoring and exposure assessment including policy.**

During the Workshop, a competition took place for the "***Best Young Italian Scientist Award***", a prize established in 2018 which provides a free registration to the SETAC Europe Meeting of the following year. Fourteen young researchers took part in the scientific competition. 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 3-minute pitch during the Workshop. This year award went to the **PhD student Ifra Ferheen** (School of Advanced Studies, Cameriono University) for her work entitled "*Detection of ESBL-producing Klebsiella spp., adhering plastic debris in Bracciano Lake*".

Congratulations to her and her working group!

The Workshop concluded with the invitation to **meet again in 2024** for the next SETAC Europe Meeting in Seville (3-5 May 2024) and for the 3rd Workshop of the SETAC ILB, which will be held in **Milan on 7 and 8 October 2024**, hosted by the Milan University.

We hope to see even more SETAC ILB members next year in Milan and at the SETAC Europe Meeting in May 2024!

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

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Abstracts

Oral Presentations

Session A - Ecological and human health risk assessment

Environmental risk assessment (ERA) of veterinary medicines

L. Mancini

Dept. of Environment and Primary Prevention, National Institute of Health (ISS), Rome, Italy

Veterinary medicines have various pathways of interaction with ecosystems and human health.
From ecosistemic point of view the farmacological nature of the veterinary medicinise and their frequent use at large scale for animal production pose a risk for environmental and human health. Veterinary medicines are a large class of products (that in some cases share the active substances with products intended for human use) widely utilized for both pets and farm animals. There are several pathways that allow to these products to enter the environment, in particular through excretion in the urine and the faeces of the treated animals and through the use of contaminated manure.
Two types of complementary responses can be done. The first answer, which we can consider primary prevention, concerns the actions implemented before placing a veterinary medicinal product on the market and discusses compliance in the European Union with requirements and guidelines for an environmental risk assessment of veterinary pharmaceutical products. Applicants for new veterinary medicine must provide an ecotoxicity report according to guidelines based on a logical and stepwise approach with an intermediate threshold between an essential characterization of the veterinary medicine and a thorough assessment of its fate and ecotoxicological effects on battery tests. Based on the Environmental Risk Assessment (ERA), risk mitigation measures can be applied, such as limiting the application rate, the amount of contaminated manure spread on agricultural land, or the access of treated grazing animals to surface water. In this context, Italy, with the Ministry of Health, has established a national product evaluation commission, including environmental risk assessment, and the Istituto Superiore di Sanità is an integral part of it. Therefore, from a prevention point of view, the current legislation within the European Union (EU Regulation 2019/6) allows the effects of the product on the ecosystem to be assessed in advance and to develop mitigation measures.
The second answer concerns release/emission pharmaceutical products for human and animal use into ecosystems through various diffusion sources. Thousands of compounds and byproducts are released and discharged daily with well-documented consequences on human and ecosystem health. Some of these substances are present in the monitoring plans and, therefore, known in their diffusion, while others are yet to be included in the environmental monitoring plans based on the risk effective.
Emerging topic that requires institutional connections and dedicated studies as well as an integrated approach based on complexity and multidisciplinarity as well as a logical approach based on planetary health.

Environmental criticality: an expected or ongoing event can lead to potentially dangerous conditions for the environment. Intervention examples to prevent and/or mitigate environmental impacts

R. Pacifico, A. Sarni

ISPRA - Institute for Environmental Protection and Research, Area per le Emergenze ambientali sulla terraferma, Rome, Italy

An environmental crisis arises when an expected event could lead to potentially dangerous situations for the environment and it could require interventions to mitigate the potential risk. The emergency takes shape when a calamitous event,

of natural origin or deriving from human activity, determines a dangerous situation for people and for goods and the environment integrity and requires exceptional and urgent interventions to be managed and brought back to normal conditions. Both situations can lead to an environmental risk which can determine as a source of danger, damage to humans, assets and the environment itself, even in the deferred time. ISPRA, with the contribution of the operational network for environmental emergencies of the SNPA (National System for Environmental Protection), ensures an environmental risk assessment and monitoring activity, aimed at indicating the first prevention and safety measures. Critical situations are firstly reported by the Ministry, then ISPRA with SNPA takes action with inspections, technical investigations, following field activities, and issuing a preliminary assessment.The technical reports issued provide the competent Authorities with useful data and assessments to identify the site conditions and potential environmental threat situations and propose the consequent prevention, containment and safety measures.

The following examples represent three critical environmental situations.

1) Fire in an industrial storage of solvents, dyes for fuels and hazardous and non-hazardous special waste.

Environmental criticality: solvents, paints, and other burnt and unburned materials spreading mixed with extinguishing water on soils and roads nearby the burned plant.

2) Contamination of sea water caused by a depurator plant lacking measuring, sampling and / or analyzing wastewater (incoming and outgoing) instruments and maintenance activity, with the consequent halving of the original purification capacity.

Environmental criticality: discharge into the sea of untreated wastewater in case of overload of the 2 purification lines.

3) Leachate leaked from a dismissed landfill plant.

Environmental criticality: leachate leakage and contamination of reclamation channels surface water caused by water with leachates mixing.

In silico prediction of thyroid disruption-related endpoints: state-of-the-art analysis of the existing QSARs

M. Evangelista, N. Chirico, E. Papa

Dept. of Theoretical and Applied Sciences (DiSTA), Insubria Univ, Varese, Italy

Endocrine Disrupting Chemicals (EDCs) are structurally heterogeneous substances that may pose a threat to human and wildlife by interfering with the hormonal activity of the endocrine system. EDCs can be found in several consumer products, agricultural chemicals, and industrial substances, and have been associated with several adverse effects. Among EDCs, Thyroid Hormone (TH) Disrupting Chemicals (THDCs) are those interfering with the hypothalamic-pituitary-thyroid axis through multiple molecular targets involved in the TH signalling, biosynthesis, metabolism, excretion and binding with TH receptors. The correct functioning of this system is crucial for the brain development and metabolism regulation. For these reasons THDCs must be identified and correctly managed to reduce risks due to exposure to these contaminants for living organisms.

The use of *in silico* models based on Quantitative Structure Activity Relationships (QSAR) is suggested in the regulatory context as a valid approach to reduce the impact of animal tests performed to evaluate the effects of chemicals as well as to prioritize, rank and identify new potential hazards. In the last 15 years multiple endpoints related to the disruption of the Thyroid system (TS) were modelled using the QSAR approach. However, this knowledge has never been mapped before.

The present study provides a state-of-the-art analysis in the field of QSAR related to TS-disruption, by identifying available experimental data and existing approaches.

43 articles including 126 QSAR models were selected for eight endpoints (i.e., TRα and β, TTR, TPO, DIOs, TSHR, TRHR, NIS) directly involved with the TS. The number of articles ranges between 1 and 4 from 2007 to 2021, while the highest number was reached in 2022 (6). The modelled datasets included heterogeneous organic

chemicals or specific classes of halogenated compounds, such as PCBs, PBDEs, PFCs, etc. However, data are sparse and mostly suitable for classification. The number of published models per year is similar over the years (between 2 and 8), with four peaks in 2015, 2017, 2021 and 2022 (12, 16, 23 and 25 models, respectively). These observations show an increasing interest toward the modelling of TS-disruption using multiple approaches by the QSAR and the scientific community; however, additional effort should be made to provide experimental data for a consistent dataset of heterogeneous chemicals, allowing for comparison across results.

The Environmental Impacts in Aquaculture Production, Current Perspectives and Future Challenges

I. Bravo[1], S. Toniolo[2], P. Papetti[1]

[1]Dept. of Economics and Law, Cassino and Southern Lazio Univ., Italy.
[2]Dept. of Management, Verona Univ., Verona, Italy

Aquaculture represents the breeding of aquatic organisms (fish, crustaceans, mollusks and algae), under controlled and continuous conditions, without drawing on natural stokes. Although it is an ancient practice, developed above all in the Mediterranean basin, today it constitutes a very important economic sector for food production worldwide. According to the latest FAO Report “The State of World Fisheries and Aquaculture”, global aquaculture production registered a record in 2020 reaching 87.5 mill tons of aquatic animals for human consumption, with production value of 296.5 bill dollars, and it is expected to increase about 40% by 2030. Aquaculture is practiced mainly in Asia, with China being the world's largest fish producer, consumer and exporter; while in the EU accounts for around 20% of fish and shellfish supply.

However, if on the one hand the demand of seafood has grown, on the other it is suffering due to climate change, a shrinking wild fishery stock, inland water scarcity and consequent decline in economic and food production. Furthermore, aquaculture products present a significantly high consumption levels and make extensive use of the water and energy resources. At global level, the major challenges regard the water, a key resource whose use and pollution are related to the sustainability of the whole ecosystem, the availability of feed and energy. There are about 700 species currently cultivated around the world, but about half of the global production is made up of just 12 different types (salmon, tuna, shrimp, tilapia, carp, etc.), each one with specific nutritional needs and environmental burdens.

The aim of this research is to evaluate the environmental aspects of aquaculture, considering different issues related to water quality requirements, feeding management and the type of rearing. A literature review is presented and a descriptive analysis is carried out; finally, the articles are analyzed based on the environmental aspects highlighted. The results show that the different impacts largely depend upon the breeding method and technology adopted. This study explores the environmental impacts associated with the implementation of aquaculture, revealing current developments and the main challenges to face to improve its sustainability.

Session B - Ecotoxicology and stress ecology: from molecules to organisms up to population and ecosystem level

Impacts of contaminants of emerging concern on Mediterranean biodiversity: the role of the Univ. of Siena in the "Zero Pollution" Spoke 2 of NBFC

M.C. Fossi

Dept. of Physical, Earth and Environmental Sciences, Siena Univ., Siena, Italy

The main objective of this presentation will be to describe the role of the Univ. of Siena (UNISI) in the "Zero Pollution" Spoke 2 of NBFC in the study of the impacts of contaminants of emerging concern on Mediterranean biodiversity. The main focus of UNISI team will be to apply multiple approaches in diversified field conditions to validate the health tools and the effect-based monitoring for assessing the susceptibility to pollution-mediated loss of biodiversity in representative species, habitats, or anthropogenic activities. In detail UNISI team will use the health-tools (developed in WP2 laboratory activities) to characterize the susceptibility to pollution-mediated loss of biodiversity in key Mediterranean field scenarios (focusing mainly on endangered species) also providing guidelines for effect-based monitoring and normative implementation.

Early larval stages of the marine bivalve *Mytilus galloprovincialis* as a model for studying the possible impact of emerging contaminants

T. Balbi

Dept. of Earth, Environmental and Life Sciences (DISTAV), Genoa Univ., Genoa, Italy

Contaminants of emerging concerns (CECs) are becoming widespread contaminants in the aquatic environment, but are currently not included in regulatory/monitoring programs, and fate and biological impacts are poorly understood. Since many CECs are also detected in marine waters, assessment of seawater quality and of ecosystem health with respect to these pollutants is of particular concern, in particular in densely inhabited coastal areas, where the anthropic impact is higher.

The standardized 48 h embryotoxicity assay, based on microscopical determination of the percentage of normal D-larvae developed at 48 hpf, that involves exposure during the most sensitive stages of the organisms to environmental stress, have proven useful in the identification of those chemicals that represent a major threat to marine species. In the model invertebrate *Mytilus galloprovincialis*, application of the 48 hpf embryotoxicity assay showed significant effects of different CECs, in terms of dose-response and type of effect (mortality, retarded development, malformations).

Although the results can provide significant amounts of data potentially useful for regulatory purposes, the endpoint of this simple ecotoxicological assay does not allow for the identification of more subtle changes induced by contaminants that may affect subsequent development (i.e. alterations in shell biogenesis and neurodevelopment). However, embryotoxicity data can represent the basis for investigating the mechanisms of action of different CECs at environmental concentrations, from molecular to organism level, and can contribute drawing adverse outcome pathways (AOPs). Subsequent microscopical and molecular analyses are necessary in order to identify possible changes in physiological mechanisms involved in mussel early development that may play a role in key steps of development.

In early larvae, information of the physiological mechanisms of key processes of development, and how they can be affected not only by exposure to emerging contaminants, but also by changing in environmental factors, will contribute to the ability to predict how these organisms can respond and adapt to environmental change.

Molecular physiology of marine organisms under climate changes and emerging anthropogenic threats

S. Franzellitti

Dept. of Biological, Geological and Environmental Sciences (BiGeA), Bologna Univ., Ravenna, Italy

The identification of priority chemicals is a challenge for regulators, managers and researchers, so as the implications for health and economic policies. In European coastal regions, aquaculture of bivalve mollusks is facing the effects of climate changes and widespread sources of pollutants. Plastic litter and pharmaceuticals released through urban wastewater discharges are emerging as relevant threats, with potential health effects due to human consumption. As filter feeders, bivalves are particularly exposed to these pollutants within their food chain, with effects on growth rates, shell calcification, reproduction, increasing infections and diseases. Changes of environmental parameters due to global warming may act by altering the environmental fate and enhancing bioavailability of pollutants or exacerbating their physiological effects on organisms. These phenomena may impact several economic and social aspects related to marine food production, from productivity and quality, to safety and sustainability. Our research activity investigates hierarchical biological responses of mollusks (transcriptome, cellular biomarkers, whole-organism physiology) to address the environmental fate and biological risk posed by emerging pollutants in a climate change scenario. Laboratory experiments under controlled laboratory conditions and field investigations are employed. Bivalve farms and natural populations of the North Adriatic are used as field laboratories to explore the connection between animal health, pollutant exposure, and productivity. Data on bivalve biological responses and environmental parameters are integrated within a modelling analysis linking molecular initiator interactions to apical physiological outcomes on growth, shell biogenesis, reproduction. The ultimate goal is to establish advanced early-warning biomarkers of effect tailored to commercially relevant physiological traits (growth, reproduction, resistance to shell fracture, resistance to bacteria).

Impact of the anti-inflammatory dexamethasone on the cellular pathways of mussel *Mytilus galloprovincialis* under environmental scenarios

T. Cappello, G. De Marco, M.C. Eliso, M. Galati, B. Billè, M. Maisano

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It is known that the wastewater treatment plants (WWTPs) prevent the discharge of urban pollutants from their sludge into the environment. In recent years, nonetheless, it has been observed how certain classes of substances, such as the active pharmaceutical compounds (PhACs) are not adequately retained by the most common WWTPs, resulting in their release into the environment at concentrations ranging from ng/L to µg/L. This class of micropollutants can interact with non-target organisms, leading to cellular impairments. In this study, the biological impact of the anti-inflammatory dexamethasone (DEX) was therefore evaluated on marine mussels *Mytilus galloprovincialis* (Lamarck, 1819), following exposure at its environmental concentrations (C1: 4 ng/L; C2: 40 ng/L; C3: 400 ng/L; C4: 2 µg/L) during a time course of 12 days, with selected sampling time-points (3 days, T3; 6 days, T6; 12 days, T12). Among the recorded cellular impairments on mussel gills, a key organ in gas exchange, filter-feeding and osmoregulation of mussels, a time-dependent DEX-induced pro-oxidant effect combined with lipid peroxidation, altered cholinergic neurotransmission and haemocyte infiltration among the branchial epithelium were found, as revealed by a multi-biomarker approach including histology, enzymatic assays and protonic NMR metabolomics. Surprisingly, the impairments on mussel gills occurred from 3 days of exposure even when mussels were challenged with low DEX concentration as C2, with effects more pronounced at T12 particularly in specimens

exposed to the highest concentrations (C3, C4). These results thus highlight the ability of PhACs to trigger cellular alterations on non-target species under realistic scenarios, therefore pointing out the urgency to enhance the existing WWTPs by innovative bio-recovery strategies and the use of effective eco-pharmacovigilance programmes.

Hydra vulgaris assay as early warning system to evaluate teratogenic risk in freshwaters

G. Cesarini[1,2], S. Secco[1], F. Spani[3], M. Scalici[1]

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Freshwater ecosystems are vulnerable to various anthropogenic impacts, including chemical pollution. The use of sentinel organisms, as required by the European Water Framework Directive (2000/60/EC), has enhanced the assessment and monitoring of chemical impacts on both environmental and human health. In this sense, the regenerative ability of Hydra vulgaris Pallas, 1766 a freshwater coelenterate (Cnidaria: Hydrozoa), is employed as early warning system to assess the teratogenic risk of freshwaters. Among the different teratogenic substances in freshwaters are present heavy metals, pesticides, emergent contaminants such as new formulations of drugs and plastics. The eco-toxicological index, Teratogenic Risk Index (TRI), is based on morphological biomarkers: the regeneration rate and the aberration frequency of the columna after the cut of head and tentacles. The TRI shows 5 qualitative classes of teratogenic risk level: from 1 (no risk) to 5 (very high risk). Besides morphological markers, eto-ecological responses can be provided by evaluating the feeding capacity and tentacular reactivity. The Hydra assay provides a rapid and cost-effective evaluation method able to detect the teratogenic risk of an unknown complexed mixture or one/more known substances. In our research, H. vulgaris was utilized to assess both the environmental teratogenic risk of riverine waters and potential teratogenic substances. Several rivers in the Lazio Region have been monitored for teratogenic risk over multiple years, revealing temporal variations in the levels of teratogens. Moreover, the Hydra assay has been employed to evaluate substances that have recently raised interest due to their potential detrimental effects on freshwater ecosystems. In particular, we tested the teratogenic effects of the rare earth element Gadolinium as different drug formulations, plastic particles of a copolymer of polystyrene and bioplastics composed of different ionic liquids. The Hydra assay showed significant results in all the various applications, emphasizing its effectiveness as an early warning system.

A multidisciplinary approach to assess the eco-safety of biogenic matrices for agricultural application

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The use of biogenic matrices as fertilizers and amendments is considered a good solution to improve soil properties and resilience. Nevertheless, there is a growing concern about their potential hazard to ecosystems and human health, due to the presence of toxic chemicals and pathogens. Criteria for agricultural application of biogenic matrices are based only on physicochemical properties and levels of some pollutant residues. Nevertheless, to ensure their safe environmental applications it is mandatory to develop *effect-based* tools, which can provide information on their toxic potential for natural ecosystems. In this scenario, this presentation will describe a study in which the eco-safety of two organic fertilizers as sewage sludge (SS) and defecation gypsum (DG) was evaluated through an integrated approach. Chemical analyses were carried out according to the Lombardy Regional Council Organizational Unit Executive Decree D.D.U.O. no. 6665 14/05/2019, about the reuse of biological sludge in agriculture.

In parallel, an ecotoxicological characterization was carried out, by means of a battery of standardized ecotoxicological tests coupled with the evaluation of the potential sub-lethal effects on the model organisms *Eisenia fetida* and *Danio rerio* (zebrafish) embryos. The composition of the bacterial community of soil treated with the matrices was also evaluated. Finally, the pot growth test on lettuce was carried out, according to the provisions of the Lombardy resolution D.G.R 16/04/03 no. 7/12764.

The concentrations of all physical and chemical parameters (inorganic and organic substances) comply with the limits established by regional legislation.

Regarding the ecotoxicity evaluation, both matrices caused acute and sublethal effects on earthworms and zebrafish embryos.

The microbiome analysis showed that the addition of both matrices changed the soil bacterial composition with an increase of bacteria belonging to Bdellovibrionota, Chloroflexi, Myxococcota, and Gemmatimonadota after 14 days of treatment. Besides, the treatment leads to a reduction of soil microbial biodiversity. Finally, both matrices were found to be suitable for agricultural application based on the pot growth test on lettuce (reaching the sludge a better score than defecation gypsum).

Our study highlights how ecotoxicity could provide relevant information in terms of ecological hazards posed by substrates intended for agricultural applications on terrestrial and aquatic environments and the importance to combine acute assays with sub-lethal endpoints. The integration of biological data with results related to chemical characterization provides an overall and representative view of the real effect of these matrices. Though this study contributes to fill the gap of knowledge on the effect-based approach in this context, conclusive deductions could be drawn only once further investigations will be performed and reference standards will be defined.

Development of an Adverse Outcome Pathway to understand the mechanism of action of amino-modified polystyrene nanoplastics during the embryogenesis of *Ciona robusta*

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The growing concern around nanoplastics (NPs, <1 µm) has led to a wide investigation on their effects on marine life. Nowadays, the main point is to understand their mechanism of action for assessing and predicting the toxicological effects at organism and population levels. To do this, here we applied an integrative approach to develop an Adverse Outcome Pathway (AOP) upon acute exposure to 50 nm amino-modified polystyrene particles (PS-NH_2; 10 and 15 µg/mL), as proxy for NPs, during the embryogenesis of the chordate *Ciona robusta*. In detail, two -omic approaches, transcriptomic and metabolomic (^{1}H-NMR) analysis, and in vivo experiments were assembled into two putative AOPs, which identified the adhesion of PS-NH_2 as (molecular) initiating event, followed by oxidative stress. At cellular level, several genes involved in glutathione metabolism, immune defense, nervous system, transport by aquaporins and energy metabolism resulted deregulated, while metabolites of the cholinergic synapse (e.g. choline, acetylcholine, acetate) and of the energy metabolism (e.g. glucose, glucuronate, malonate, lactate) resulted affected. Instead, morphological defects, increase in reactive oxygen species level and impaired swimming behavior were identified as response at organ and tissue level. As final adverse outcomes, altered larval development, reduced metamorphosis and inhibition of hatching were identified. Overall, this work provided new insights into the understanding of PS-NH2 mechanism of toxicity on early stages of marine life, resulting in a highly sensitive and effective tool for environmental risk assessment purposes.

From plastics to rubberized materials: a new line of research on the ecotoxicity of tire particles

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Plastics represent a well-known global issue, but the pollution by non-conventional plastics, as biobased and water-soluble polymers as well as rubberized materials, is still poor considered by scientific community. In this context, only in Europe, about 3.4 million tons of tires reach the end-of-life every year. Consequently, the End-of-Life Tires (ELTs), which represent a special waste, are reduced in granule (Gr; 0.8 < size < 2.5 mm) and powder (Pw < 0.8 mm) to produce other materials or used in rubberized asphalts to absorb the traffic noise. The aim of this study was the evaluation of sub-lethal effects induced by the leachates obtained by ELT Gr and Pw on Danio rerio (zebrafish) embryos. The exposures were performed in triplicate, to 0.1, 1 and 10 mg/L of Gr and Pw leachates, in static conditions and from 0 to 120 h post fertilization (hpf). The chronic toxicity was evaluated through an integrated ecotoxicological approach of biomarkers, transcriptomics and proteomics. We observed a significant increase ($p < 0.05$) in swimming activity and heart rate in specimens exposed to Pd leachates, as well as significant modulations of the detoxifying enzymes ethoxyresorufin-O-deethylase (EROD) and glutathione-S-transferase (GST), probably due to the released chemicals by ELTs. Despite no effects were observed with transcriptomics on specific genes encoding detoxifying enzymes and metallothionein, proteomics highlighted the modulation of many proteins by Pw leachates (108 proteins by both Pw 0.1 and 1 mg/L and 102 proteins by Pw 10 mg/L) and a lowest, but dose-dependent, modulation by Gr (37 by Gr 0.1 mg/L, 87 by Gr 1 mg/L and 106 by Gr 10 mg/L). Detected proteins are involved in the metabolism of aromatic and nitrogen compounds, probably derived from traffic activity or used during the tire production. Obtained results suggest that the toxicity of ELTs is associated to the release of chemicals in the leachates, with a highest toxicity of Pw, compared to Gr leachate. However, considering the tested concentrations (mg/L), selected to investigate the ELT mechanism of action, other investigations are needed to clarify the effects of these materials, whose recycling is a pivotal aspect in the context of circular economy. As future perspectives, considering the release of tire particles during the activity of transport means, the next step of this research will be focus on the toxicity contribution of tire rubber used as additives in asphalts.

Session C - Environmental monitoring and exposure assessment including policy

Imposex phenomenon in coastal and transitional waters

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Tributyltin (TBT) and triphenyltin (TPhT) have been used worldwide as antifouling biocide in marine environments. They are responsible for imposex, a phenomenon consisting of the superimposition of male sexual characters onto gonochoristic gastropod females. This phenomenon, considered as the best example of endocrine disruption in wildlife, affects more than 260 gastropod species, showing a significant correlation between levels of morphological alterations and Organotin (OT) environmental concentrations. Thus, imposex has been used as a specific biomarker of OT pollution around the world.

Along Italian coast, first imposex studies were carried out in late 90s early 2000s and they found this phenomenon to be widespread. ISPRA in Chioggia (VE) has been monitoring Butyltin pollution, as well as imposex, in Italian transitional and coastal waters. Recent surveys confirmed imposex is still affecting lagoon, coastal and offshore gastropods populations in the Northern Adriatic Sea, even after more than ten years from the EU Regulation 782/2003. Indeed, despite restrictions, OTs are still worldwide present, probably due to slow degradation rates in sediments, new sources, or unregulated and illegal use in antifouling paints. Thus, OT global contamination and related effects are not yet a solved problem and deserve sustained attention.

OTs are indicated as priority hazardous substances according to the European Water Framework Directive 2000/60/CE (WFD) and its daughter Directives 2008/105/EC and 2013/39/EU. Environmental Quality Standards for TBT in transitional and coastal waters are lower than the OT levels that can be routinely measured. Thus, the use of gastropods for OT monitoring has been further promoted (e.g.: WFD Technical Advisory groups in the Northern/Atlantic Europe).

The ISPRA working group on Ecology in transitional waters proposed an indicator for assessing bioavailable OTs by imposex in gastropod populations of *Nassarius nitidus* and *Hexaplex trunculus* in Venice Lagoon and the Northern Adriatic Sea. The new approach has aimed at defining new boundaries for the ecological status classes to assess OTs by imposex. The method has been suddenly applied also in Brazilian coastal waters with several species of gastropods. As future perspectives, the next step of this research will be focus on new surveys in order to display a more robust dataset and to use it as a support to chemical analyses whenever high sensitivity analytical instruments are not available.

Application of UAV platforms and sensors to environmental monitoring: design of UAV based operational protocol for multi-relief monitoring in urban ecosystem

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Urban agglomerations are also often characterized by the close and sometimes contiguous presence of sensitive receptors and emissive sources responsible for emitting different types of pollutants into the ambient air. Emission sources can be, for example, vehicular traffic, civil heating systems but also artisanal and industrial production activities, port areas and phenomena of illicit burning of waste materials. It therefore becomes of paramount importance to be able to precisely identify which paths the plumes of pollutants take, circumstantiating them

with respect to the conditions present in the surrounding area (weather-climatic conditions, orography, topography etc.). In this way it will be possible to provide decisive support for the development of policies for the protection of sensitive receptors, for the control of the territory and for the implementation of further monitoring strategies, implemented by public administrations and control bodies.
At present, the monitoring networks, continuous or discontinuous, dedicated to the measurement of concentrations of pollutants in the airborne matrix are based on surveys from ground, via fixed or mobile stations, sometimes activated in an automated manner with respect to signals coming from citizen science protocols. However, ground systems, whether fixed or mobile, are affected by some spatial limitations. Currently, the use of UAV systems for this kind of tests is limited to a check in the optical or thermographic field aimed at quickly and punctually identifying any illegal emission phenomena.
The project team will be testing the use of UAVs for environmental protection and public health protection in urban ecosystems, via the use of sensors and software which until now have been used for monitoring contaminated sites or industrial areas.
This work describes the methodology underlying the design of a UAV-based monitoring protocol composed of multiple surveys over a large time scale. It should be noted that, in order to proceed with a technically correct design of the monitoring and considering the size of the site being measured, the complexity of the environments, the diversity of emissive sources and sensitive receptors, it is of fundamental importance to outline an accurate picture of the site specific details right from the start. The correct design of the monitoring campaign will be one of the fundamental prerequisites for a valid execution of the campaign. The added value of a correctly designed analysis will be substantiated by an excellent spatial coverage of the acquired data.

Pesticides in surface and groundwater - Italian monitoring data 2020

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The 2020 monitoring of pesticides in waters involved 4,388 monitoring points and 13,644 samples. Pesticides were found in surface waters in 55.1% of the 1,837 monitoring points, in groundwater in 23.3% of the 2,551 points. 406 substances were searched and 183 were found. These are some of the data that emerged from the National Report on pesticides in waters. Monitoring of pesticide provides information on water quality and environmental risks that were not foreseen in the authorization phase. Although the EU has one of the most comprehensive regulatory frameworks on pesticides, the overall result indicates a widespread presence of pesticides. The 2020 monitoring results are presented in terms of the detection frequency and concentrations distribution, where the mean measured concentrations are compared with the Environmental Quality Standards fixed by European and national legislations to obtain levels of contamination. The frequency of pesticides overall increased in the last ten years of the survey, in a manner related to the extension of the monitoring points and the number of searched substances. The interpretation of the monitoring data is in fact strongly influenced by the effectiveness with which the monitoring was performed, bringing out a contamination not detected in the past. The increase of the detection frequency was more pronounced for surface water than groundwater where hydrological dynamics are very slow. The measured concentrations were generally fractions of µg/L (parts per billion), but the harmful effects of substances can also occur at very low concentrations. Moreover, pesticide residues were detected in deep aquifers, which are less exposed to percolation.
The pesticides most often detected at concentrations that did not comply with environmental regulatory limits were the herbicides glyphosate, metolachlor, imazamox, bentazone and their metabolites. The data

showed the presence of mixtures in the waters. With an average number of 4.3 substances and a maximum of 31 substances in a single sample. It must therefore be taken into account that humans, like other organisms, are often exposed to mixtures of chemical substances, the composition of which is unknown and the risk cannot be assessed.

Diatoms as rapid indicators of environmental changes: twenty years of research and future perspectives

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Diatoms can represent a rapid respond to environmental change, they colonize all aquatic ecosystems worldwide, are main component of the benthic and planktonic assemblages and present characteristic of biological indicators. They rapidly register environmental changes being at first level of trophic change and having a short generation time.
Diatoms are useful indicators of eutrophication, organic matter and hydromorphological alterations thanks to strict ecological requirements regarding physical variables and chemical parameters. Moreover, they are able to register the contamination of heavy metals, pesticides, that uptaken within cell can induce malformation at silica cell wall (frustule).
In this work we present our twenty-year work on diatoms of research, technical and scientific support for Ministry of Environment and training. We deepen the knowledge about distribution of diatoms species on Italian rivers, lakes and transitional waters; sampling protocols and method for the assessment of ecological status have been developed to meet the requirements of Water Framework Directive WFD 2000/60/EC. We participated to Environment Ministry Working Group for the harmonization of ecological methods and to European Commission Geographical Intercalibration Working Groups.
Training courses and technical scientific support on diatom analysis were provided to operators of the Environmental Agencies.
Furthermore, a patented rapid method for their identification, the crucial step in diatom studies, was realized. To improve the reliability of their method, we accredited the hole analytical process including sampling phase according to ISO/IEC 17025. We also have improved the application of diatoms in forensic medicine, to ascertain the death by drowning, set up a protocol analysis. We used this community to assess the aquatic ecosystem health during environmental emergencies.
Using diatom as sentinel organisms with analysis of teratological forms, we studied the impact of nanoparticles of polystyrene. Actually we are investigating their response to antibiotic exposure, with two target species widespread in river and lake ecosystems, experimental phase is still in progress.
These species have been exposed to different concentrations of Ciprofloxacin, for 30 days (life cycle of diatoms) under controlled growth conditions. After time exposure, Alterations of frustule like valve shape, apices, raphe features, pattern of striae have been studied trough morphological analysis.

The integration of site reclamation and environmental damage disciplines as a key strategy for environmental management upgrade

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To verify the presence of negative environmental externalities from a site, the procedures contained in Part IV of the TUA (D.lgs. 152/06) are applied: they concern the reclamation of contaminated sites. The focus of these procedures is limited to the soil/subsoil and groundwater and to the extensive list of substances for which the CSC are determined in Annex 5 to Part IV of the TUA.
To obtain a more reliable overview of the effects a site may have on the environment, it would be advisable to adopt an integrated approach that also considers the principles of environmental damage (Part VI of the TUA) transposed into Italian law from the Directive 2004/35/CE. It represents a very effective tool for protection of the main environmental matrices from human caused pollution for three main factors:

- the scope of application is much broader compared to that of the reclamation discipline, including protected species and habitats, protected areas, surface waters, and coastal marine waters;
- for protected resources (excluding soil), it also includes innovative remediation measures. Complementary r. is carried out when it is not possible to achieve a complete restoration at the compromised site and may be implemented at an alternative location. Compensatory r. takes into account the period of time during which the resource was unusable due to the damage (from the moment of the damage occurrence until its complete restoration);

It includes a powerful instrument known as "threat of damage" This refers to a sufficiently probable risk of environmental damage occurring in the near future. It serves as an effective prevention tool that allows (or rather obliges) the operator to take action when the damage has not yet occurred, but there is a high risk that it might happen. It shifts the focus from merely responding to environmental damage that has already occurred to proactively preventing potential harm.

An integrated approach between the reclamation discipline and the environmental damage discipline can provide an important contribution not only for an accurate estimation of negative externalities of a site towards all environmental matrices but also for preventing the realization of negative effects on them. It is a way to promote sustainable practices and responsible resource management. In conclusion, it represents a key strategy for environmental management, more effective conservation of natural resources, and increased focus on long-term sustainability.

Pesticide Monitoring Information System (SIMP): data flow and information management

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ISPRA has developed the information system for pesticide monitoring (SIMP). The system stems from the need for a useful tool for the acquisition, processing and dissemination of information on national pesticide monitoring in inland waters, supplied annually by the Regions/Autonomous Provinces. The SIMP was conceived modularly, with a view to its evolution to include other subsystems (e.g. other eco-toxicological databases).

The Regions/Autonomous Provinces enter the results of their monitoring activities for the previous year in a data sheet (in Excel format). The sheet contains information on the master data of the monitoring stations and information on the analytical determinations carried out. ISPRA processes and evaluates monitoring data and provides technical-scientific guidelines for monitoring planning.

The SIMP operates through the following phases:

- checking incoming data and filing them in the database, to verify the completeness and correctness of the information;
- consultation, modification and updating of data in the database. The experts manage the data in the database before moving on to the data processing procedures;
- georeferencing of the monitoring points, creation of the spatial point data of the national cartographic information of the monitoring stations;
- production of statistical outputs (tables, graphs) and contamination maps;
- publication of processing results on the Institute's Pesticide Portal.

The Pesticide Portal is an open tool for the public to consult information on the national monitoring of pesticides in surface and groundwater (www.pesticidi.isprambiente.it).

Specifically, the Portal allows:

- consultation and acquisition of ISPRA publications on pesticide monitoring (National Reports on pesticides in water, guidelines and scientific documents, regional and national statistical tables);
- visualisation of contamination levels of monitoring points on the map. Using selection filters, it is possible to browse maps by year and area. It is also possible to display detailed information on the individual monitoring station on the substances found and their concentrations. The contamination level of each station is obtained by comparison with the legal limits (Environmental Quality Standards);

• search for pesticides on the territory and consultation of substances according to contamination level;

• download of geographical information and contamination levels at monitoring points.

Some video-guides are available in the Portal.

Best Young Italian Scientist Award

Extended Abstracts

1st classified

Detection of ESBL-producing *Klebsiella spp.* adhering plastic debris in Bracciano Lake

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Introduction

Water-related pathogens are considered one of the crucial concerns from one health perspective and challenges to public health across the globe [1]. The world is in a race for human betterment and meticulously relying on medicine, specifically antibiotics, to increase the human lifespan. In the golden era of antibiotics, humans have tipped the scales in their favour; hence this dependency on antibiotics is leading towards an alarming scenario of the post-antibiotic era. The large and often inappropriate use of conventional drugs in human and veterinary medicine has led to a progressive spread of antibiotic-resistant strains. In the European Union, antibiotic resistance bacteria cost €1.5 billion approximately, 2.5 million people contract nosocomial infections, and 25,000 deaths per year are associated with bacterial infections [2]. Pathogens harbouring *extended-spectrum b-lactamase* (ESBL) genes threaten antibiotic therapy's effectiveness and are associated with increased morbidity and mortality rates. Klebsiella spp. is Gram-Negative rod shape bacteria usually known for causing human nosocomial infections, hospital-acquired urinary tract infections, pneumonia, and septicemias. One of these crucial virulence factors of Klebsiella spp. is their ability to form biofilms, decreasing antimicrobials' susceptibility and making infections caused by different species of Klebsiella quite challenging to treat, especially when they colonize medical devices. Although the public health problem related to pathogenic bacteria is not new, in recent years, scientific alarms and reports about the epidemiological risk of biofilm-associated infections have been coming from all continents, especially in the context of spreading Antibiotic Resistance Genes (ARGs).
Since integrating plastic into the consumer arena, this anthropogenic substance has accumulated in the aquatic ecosystem unprecedentedly and has become an emerging pollutant. Plastic debris provides a new surface for the colonization of bacteria and expedites horizontal gene transfer by accelerating biofilm formation [3]. Biofilms formed on plastics have the potential to serve as "hotspots" for exchanging genetic information due to their extensive nutrient availability and high microbial cell densities, which allow for strong interactions between microorganisms. Around the globe, increasing morbidity and mortality rate associated with Multidrug-Resistant (MDR) bacteria or superbug poses a serious threat to humans, animal, and the environment [1]. Antibiotic resistance is a grand challenge that needs to be addressed in this century since its dissemination in the environment eventually contributes at a certain level to antibiotic-resistant infections in humans. The effect of plastic pollution on the dissemination of antibiotic resistance has recently attracted much attention, but very little information is available on their role as vectors for pathogenic bacteria and ARGs [4] . Hence, in-depth follow-up studies are needed to understand the role of plastic in spreading ESBL-producing *Klebsiella spp*. in the aquatic environment. The current study focuses on detecting ESBL-producing Klebsiella spp., in raw water and from plastic debris submerged in a caldera-type lake of central Italy to investigate the potential role of plastic in amplifying and harbouring MDR species.

Materials and Methods

Sampling

In the summer of 2021, Artificial Plastic Substrates (APSs) made of four plastic polymers, i.e., Polypropylene (PP), Polystyrene (PS), Styrene Acrylonitrile Resin (SAN), and Polyethylene terephthalate (PET) were used to sampling potential pathogens spread on to aquatic ecosystem. Artificial Plastic Substrates (APSs)

were submerged deep (1.5 meters) in the euphotic zone at seven geographical sites of the caldera-type volcanic lake of Central Italy. The APSs were kept in the lake for a month to allow bacterial colonization. A comparative analysis of the microbial community residing in raw water and colonizing on APS was performed to investigate plastic's potential role in amplifying and spreading pathogenic bacteria. The APSs and raw water samples were transported to the laboratory under sterile and dark conditions at 4°C, and further analyses were performed within 24 hours.

Biofilm removal and microbiological analysis of samples

In the laboratory, APSs with the grown plastisphere were repeatedly treated with sterile scrapers and swabs; the material was collected and inoculated into pre-enrichment 100 mL Muller-Hinton broth and incubated at a similar temperature recorded in the sampling site at the time of collection, i.e., 20 ± 1°C for 24 hr. Two litres of raw water samples collected from each site were filtered using a cellulose nitrate filter with a 0.45 µm diameter pore size (Sartorius stadium biotech, Germany), and the filters were incubated for pre-enrichment into MH broth, as described above. Aliquots of 100 µl of pre-enriched culture were spread onto a chromogenic medium Chrome Art Extended Spectrum Beta Lactamase agar (ESBL) (Biomerieux, Marcy-l'Étoile, France) and incubated at 36 ± 1°C for 24 hr. Single colonies were isolated onto an MH agar plate at the above-mentioned conditions (Kangda Intercontinental Medical Equipment).

For bacterial identification of all raw water and APS isolates, biochemical identification tests were performed to identify recovered bacterial species up to the genus level accurately. API (Analytical Profile Index) 20-E Kit was used following the manufacturer's manual (API-20E kit Biomerieux, France). A seven-digit unique identification code for each isolate was generated based on positive and negative results following the API colour scale. Isolates were then identified by entering this seven-digit code on APIWEB software.

Antibiotic susceptibility was performed using the Kirby-Bauer disk diffusion assay and interpreted as per Clinical Laboratory Standard Institute (CLSI) guidelines [5]. The isolated strains were evaluated for antimicrobial susceptibility tests against nine commercially available antibiotics (Oxoid, UK): Chloramphenicol (CAM30), Ciprofloxacin (CIP1), Tetracycline (TET 30 µg), Gentamycin (GEN 10 µg), Meropenem (MEM 10 µg), Imipenem (IMP 10 µg), Sulfamethoxazole (SXM 25 µg), Ceftazidime (CAZ 30 µg) and Erythromycin (ERY 15 µg). *Klebsiella pneumoniae* ATCC13883 strain was used as a positive control for antibiotic susceptibility quality check.

19 ARGs and two class 1 integrons (intI1 and intI1-V) were examined in these isolates. The selected ARGs included sulphonamide (sul1, sul2, sul3), tetracycline (tetA, tetB, tetM, tetW), chloramphenicol (cmlA1, cmx(A)), β-lactamase (mecA, blaCTX-M, blaSHV, blaTEM), and multi-drug (acrA, acrB, acrF, acrR, adeA). Genomic DNA isolated from the obtained isolates under investigation was used as a template for PCR amplification using primers and conditions previously described [6].

Results and Discussion

Chrome Art Extended Spectrum Beta Lactamase agar (Biomerieux, Marcy-l'Étoile, France) medium is a chromogenic agar medium used to directly enumerate ESBL-producing strains in environmental and clinical samples. Colonies obtained from raw water and APS samples recovered from seven selected geographical points of the sampling site were analyzed under a microscope to identify typical Klebsiella phenotype on ESBL medium. APSs isolates grown on ESBL agar medium displayed a Blue-Green phenotype; however, bacteria isolated from raw water samples of all lake sites did not display such a phenotype. From seven sampling sites, 9 APSs isolates depicting such phenotype were obtained and processed through a biochemical identification test and *Klebsiella pneumoniae* (n=5) and *Klebsiella oxytoca* (n=4) were identified. The antibiotic susceptibility test against the panel of antibiotics commonly used in animal and human therapeutics demonstrates that all benthic Klebsiella strains were resistant to almost all tested antibiotics. The resistance percentage of isolated strains was as following: Chloramphenicol (84%), Ciprofloxacin (82%), Tetracycline (80%), Meropenem (64%), Gentamycin (88%), Ceftazidime (80%), Erythromycin (96%), and Sulfamethoxazole (92%). Moreover, among all

these isolates, ten clinically significant antibiotic-resistant genes were detected, mainly three tetracycline resistance genes (tetA, tetB, tetW), two sulphonamide resistance genes (sul1, sul2), two β-lactamase resistance genes (mecA, blaCTX-M) and three multi-drug resistant genes were detected. In addition, one gene coding for class 1 integron (intl1), which is involved in the dissemination of ARGs, has been detected.

Conclusions

The environmental fate of plastic debris and its role in trafficking pathogenic microorganisms and antibiotic resistance genes to humans and animals is still an open question. Our findings provide initial information on the pathogenic colonization of Klebsiella on plastic, suggesting it is a hazardous pollutant, a hotspot for MDR bacteria and the vector for spreading antibiotic resistance.

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2nd classified

Physiological and behavioural responses of the sea urchin *Arbacia lixula from volcanic* CO_2 vents

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Introduction

Oceans represent a major carbon sink of the planet, by absorbing about 30% of the atmospheric carbon dioxide [1,2]. However, this absorption occurs through a chemical reaction of CO_2 with water, which results into a gradual reduction of ocean pH worldwide, a phenomenon known as Ocean Acidification (OA) [3]. This phenomenon alters seawater chemistry, leading to important changes on marine life [4].
Calcifying organisms are particularly impacted by acidification, but some of them show a remarkable physiological plasticity [5]. Among them, sea urchins seem resilient to near-future ocean acidification [6], thanks to both acclimation and adaptation mechanisms [7-9]. In the last decade, there has been growing attention towards this topic but scientific literature still suffers of important knowledge gap, regarding some poorly-studied sea urchins' species, such as *Arbacia lixula* [10]. Also, a large part of experiments is based on laboratory conditions and the observation of short-term effects of OA on few species.
In the context of 'global change ecotoxicology', as defined by Byrne in 2012 [11], and the need for a more evolutionary approach to ecotoxicology [12-14], the aim of this work was to analyze physiological and behavioral traits of a population of under-researched sea urchin species *Arbacia lixula*, living along a natural pH gradient generated by the volcanic CO_2 vents of Ischia (Italy) [15]. Using field observation, we intended to highlight potential physiological and behavioral differences among specimens living at different pH conditions, filling the still existing gap of knowledge regarding the effects of ongoing climate changes, such as OA, on natural populations and ecosystems.

Materials and Methods

Arbacia lixula adult specimens were collected from two areas along the natural pH gradient near Castello Aragonese (Ischia, Italy): one (named S1) with a mean pH of 8.1 and another (named S2) with a mean pH of 7.7, respectively the current ocean pH and the forecasted pH in near-future ocean acidification. Individuals collected from each area were then reciprocally exposed to both pH conditions using the water coming from the collection areas. Experiments were repeated three times throughout the year.
Physiological trials regarded the evaluation of respiration rate, by measuring the oxygen consumption in hermetically closed respirometric chambers, and the excretion rate by collecting sample of water from the chambers and analyzing in the lab the ammonia concentration. Behavioral trials tested sea urchins' capacity to rotate from "upside down" to "upside up", using a test called righting time, and their ability to take cover after stimulated by a light source, using a test called "sheltering time".

Results

Physiological results

Respiration rate and excretion rate of animals from the four exprimental treatments performed in three different times during the year are shown in Figure 2.
Respiration rate is significantly affected by factor "pH" during first sampling time and factor "area" during second and third. Excretion rate instead is influenced by pH but only during the first sampling time.

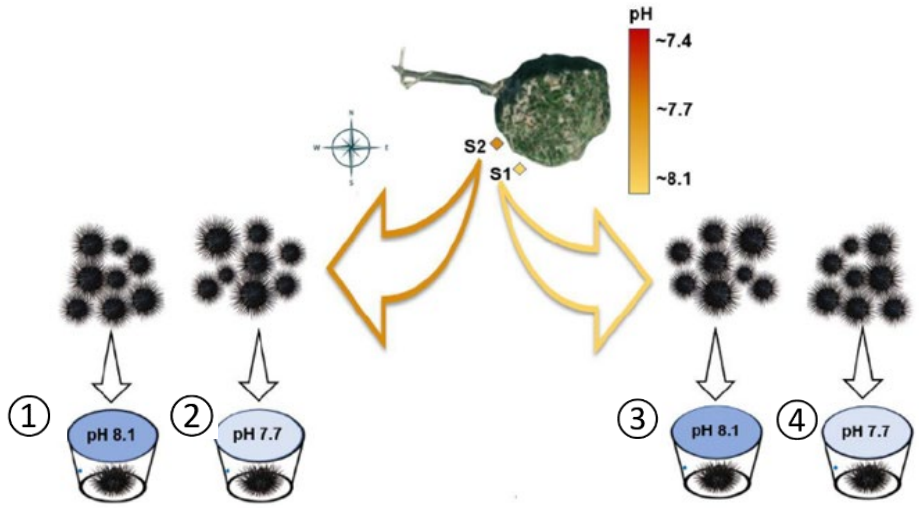

Figure 1. A scheme of the experimental design. Collection of 16 sea urchins from two areas along the gradient and reciprocal exposure to both tested pH conditions. Hence, four experimental conditions were tested: sea urchins coming from S2 population exposed to pH=8.1 ① and pH=7.7 ②, sea urchins coming from S1 population exposed to pH=8.1 ③ and pH=7.7 ④.

Behavioural results

Righting and sheltering time of animals from the four exprimental treatments performed in three different times during the year are shown in Figure 3. Behavioral traits are significantly influenced by both factors but in different sampling times. Righting time is affected by area during time one, pH during time two and the interaction of the two factors during time three. Sheltering time in significantly influenced by pH during time one and area during time three.

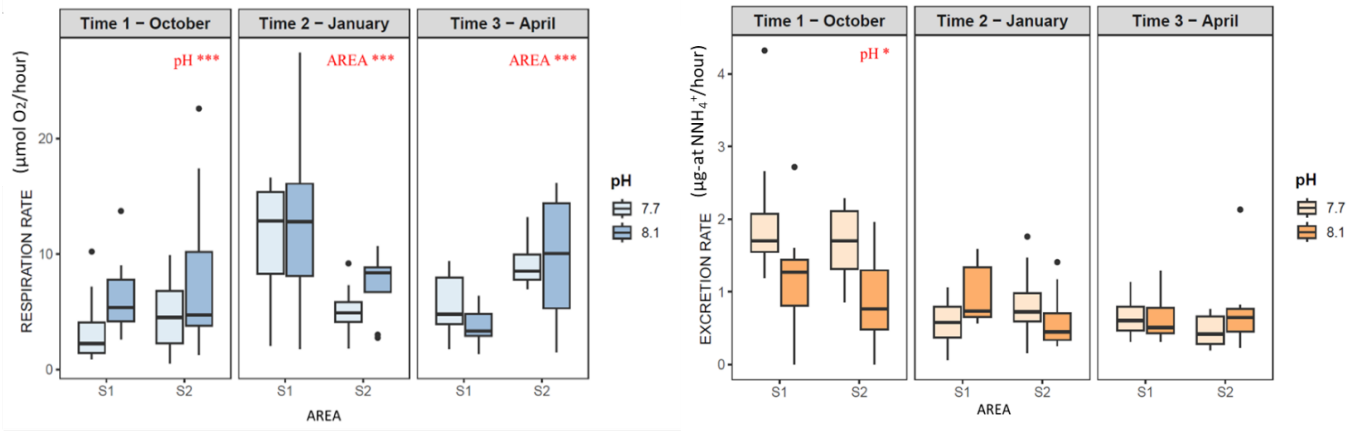

Figure 2. Respiration rate (left) and Excretion rate (right) in *A. lixula. Boxplots show medians and quartiles for each experimental treatment (n=8). Separate boxes refer to different experimental times. At each sampling time, asterisks represent significant variations due to the experimental factors, pH and area: *** p<0.001,* p<0.05. Significant codes from R: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.*

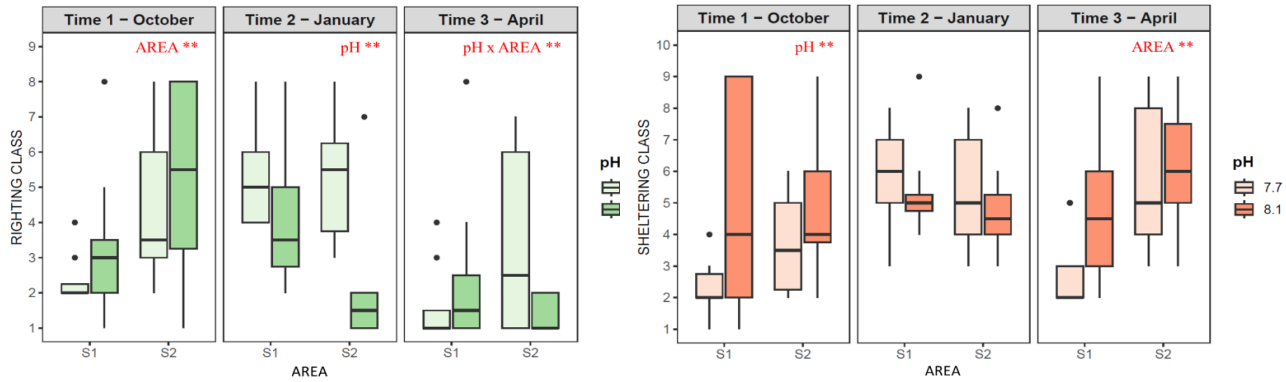

*Figure 3. Righting time (left) and Sheltering time (right) in A. lixula. Boxplots show medians and quartiles (with maximum and minimum values and outliers) for each experimental condition (n=8). Separate boxes refer to different experimental times. At each sampling time, asterisks represent significant variations due to the experimental factors, pH and area: **p<0.01.*

Discussion and Conclusions

Results highlight a complex interplay between area-specific factors, pH levels, and their combined effects on sea urchins, suggesting that both local adaptations and acclimation processes could be involved in determining *A. lixula* tolerance to acidic conditions. A recent study of [8], in fact, has been demonstrated that larvae coming from *A. lixula* specimens living in acified area show a higher resistance to acidic conditions compared to larvae coming from individuals living in ambient pH conditions. Clearly, further analyses are needed to elucidate what are the mechanisms bringing to these responses, especially taking into account the complex interaction of this species with the habitat in which it lives. A perceivable habitat diversification occurs along the investigated pH gradient, with the more acidic area showing a strong decrease in functional diversity and dominance of fleshy algae [16]. Considering the omnivore nature of *A. lixula* [17], differences between S1 and S2 populations might be due not only by direct effects on seaurchins physiology and behavior but, also, to the different availability of food. Also, seasonal variantions could influence evaluated responses in organisms maybe though modifications of the habitat and so, of environmental resources.

These findings contribute to our understanding of the impacts of climate change on an under-researched species by highlighting the effects of acidification on a small-scale level, the species level. By investigating the effects of acidification on the physiology and behavior of *A. lixula*, this research enhances our understanding of the vulnerabilities of marine organisms to environmental stressors.

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3rd classified

Assessing the accumulation of pharmaceuticals and pesticides in *Mytilus galloprovincialis* farmed along the North-western Adriatic Sea: influence on gene transcription

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Introduction

Understanding the biological consequences of pharmaceutical residues in marine ecosystems has become a crucial research focus due to mounting evidence of their occurrence and distribution worldwide. It is increasingly recognized that many pharmaceutical compounds are minimally metabolized by target organisms and not adequately degraded during conventional wastewater treatments, resulting in substantial discharge into receiving systems [1]. Within this context, the Mediterranean Sea, with its unique hydrogeological and climatological features, and high population density along its coasts, has emerged as a hotspot for the presence of pharmaceuticals as well as other contaminants, metals, pesticides, and microplastics [2]. Furthermore, the Mediterranean area is also known as one of the most responsive regions to climate change [3], exacerbating pollutant effects on marine organisms and ecosystems. Marine mussels are a useful tool for monitoring environmental conditions and assessing pollution levels, since they have the ability to accumulate different contaminants, which can exert various biological effects on their physiology [4]. To gain insights into the molecular responses of mussels to contaminant exposure, variations in mRNA expression profiles may provide crucial information at the molecular level and can indentify early-warning molecular markers of physiological stress induced by exposure to pollutants. In this study, we integrate multiresidue chemical analysis with transcriptomics to evaluate the accumulation levels of pharmaceuticals and pesticides in the Mediterranean mussel (*Mytilus galloprovincialis*) collected from commercial farms situated in the Northwestern Adriatic Sea. Specifically, we analyze the presence of the following chemicals: (i) antibiotics, i.e. sulfonamides, macrolides, and tetracyclines; (ii) the psychotropic drug carbamazepine; (iii) the insect repellants N,N-Diethyl-meta- toluamide (DEET); and (iv) herbicides, i.e. chloroacetanilide and atrazine. The selection of these chemicals is based on their widespread use, legal status, and/or known persistence in the environment [5-7]. By examining the accumulation patterns of these chemicals and their impact on various biological processes, including lysosome function, metabolic activity, neuroendocrine control, detoxification, and antioxidant response, we aim to gain informations on the implications of pollutant exposure in mussels in a real-world scenario.

Materials and Methods

Sampling campaigns were conducted from November 2018 to August 2019 at three government-certified farms situated in Goro, Cattolica, and Senigallia. At each location, three sampling campaigns were carried out, representing the autumn, spring, and summer seasons. During each campaign, 43 mussels of commercial size (from 5 to 7 cm in length), were randomly selected, placed in coolers (+4°C), transported to the laboratory, and immediately processed for dissection of the digestive glands (DG) under sterile conditions.
Chemicals accumulation in the mussel DG was assessed according to [8]. The evaluation was based on the average concentration of each pollutant in all samples. The Total Index Pressure (TIP)[9], was calculated for each sample by

counting the number of pollutants with concentrations higher than the mean. TIP values ranged from 0 to 4, indicating the simultaneous presence of four different pollutants with above-average concentrations. Samples with TIP values between 0 and 2 were categorized as low TIP, while samples with TIP values between 3 and 4 were classified as high TIP.

The RNA extraction, cDNA preparation, and assessment of expression profiles for selected transcripts through qPCR were conducted following established protocols and using primer pairs previously reported [10]. The resulting data were subjected analysed using a 2-way permutation multivariate analysis of variance (PERMANOVA) in PRIMER v6 [11] to investigate variations in transcriptional profiles among different sampling sites and seasons. Pairwise comparisons through PRIMER v6 were performed to assess differences at each level of the significant factors. Additionally, to identify discriminating host transcriptional responses between samples with low and high TIP samples, a linear discriminant analysis (LDA) effect size (LEfSe) was conducted [12]. The model was adjusted for seasonality which might influence the tested parameters. Genes with an LDA score threshold of ±2 (on a log10 scale) and a p- value ≤ 0.05 were retained as significant. The online Galaxy Version interface was utilized (https://huttenhower.sph.harvard.edu/galaxy).

Results and Discussion

Accumulation of pharmaceuticals and pesticides in mussel digestive glands

Among the 11 target compounds analyzed, seven were detected above their respective Method Detection Limit (MDL). Our data revealed a site-specific accumulation profile that varied across different seasons. Specifically, summer samples consistently exhibited a higher abundance and diversity of compounds, categorizing them as high TIP regardless of the sampling site. In contrast, spring samples were classified as low TIP, while autumn samples displayed a behavior dependent on the specific site (Figure 1). Our findings align with previous investigations on pharmaceutical accumulation in mussels conducted in the same area [13], showing a heterogeneous distribution of the analysed compounds, with no clear seasonal accumulation trends. However, their study did identify peaks of anti-inflammatories, anxiolytics, and antidepressants during the summer season. These peaks were suggestive of the impact of increasing anthropogenic pressure resulting from tourist activities in the area.

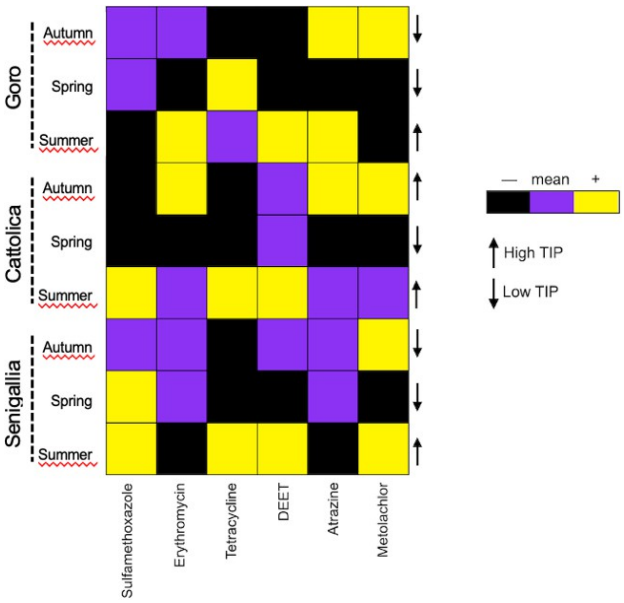

Figure 1. Contaminant accumulation in mussel digestive glands. In purple: pollutant within the mean range. In black and yellow: values below or above one standard error of the mean, respectively. High and low values of Total Index Pressure (TIP) for each site and season are indicated with an arrow next to each row.

Transcriptional profiles

PERMANOVA analyses demonstrated that the single factors "Season" and "Location" had a significant effect on the whole dataset, as well as the interaction between these factors (p-value = 0.001). Further analysis using LEfSe identified notable differences in samples characterized by high and low TIP values., that presented differential regulation of transcripts involved in metabolic (AMYL, PK, LYS), antioxidant and lysosomal responses. Amylase (AMYL) and pyruvate kinase (PK) play crucial roles in glycolitic metabolism [14,15] and serve as robust biomarkers of mussel digestive functions [16]. While lysozymes (LYS) are primarily known for their defensive role in bivalves, there have been suggestions of a potential digestive function in the DG [17]. These findings may indicate an increased utilization of metabolic resources as a requirement for mussel acclimatization at high TIP

conditions, or a more generalized metabolic activation due to chemical biotransformation processes. The relative decrease in lysosomal (CTSL) and antioxidant (MT10) responses in high TIP conditions in contrast to low TIP conditions could be associated with the stimulation of DG metabolic activity. Also, both lysosomes and the antioxidant system are tightly involved in degradative processes [18]. Notably, the up- or down- regulation of CTSL up- or down- regulation may represent a signature for alteration of the lysosomal system due to accumulation of complex contaminant mixtures [19].

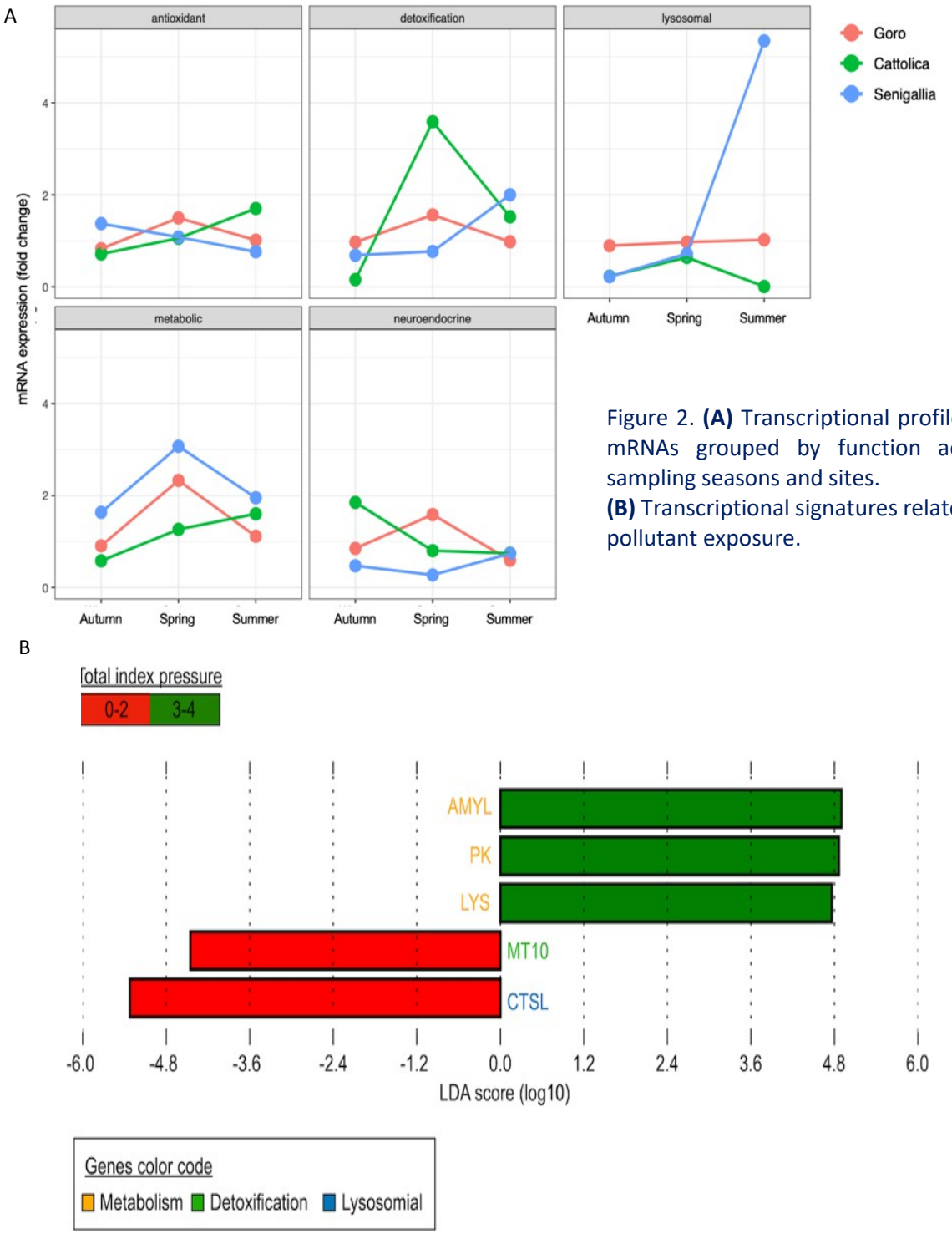

Figure 2. **(A)** Transcriptional profiles of mRNAs grouped by function across sampling seasons and sites.
(B) Transcriptional signatures related to pollutant exposure.

Conclusions

Our study sheds light on the exposure of farmed mussels in the Northwestern Adriatic Sea to a diverse and ever-changing mixture of emerging contaminants, with distinct variations specific to each site and season. Additionally, we have identified the transcriptional responses of mussels to these contaminants. The data presented in this study serve as valuable baseline information regarding the molecular response of farmed mussels exposed to real-world contaminant scenarios. This information may improve current monitoring strategies for farmed mussels and aid in forecasting potential detrimental impacts arising from the increasing of contaminants levels in coastal waters, supporting the development of effective management and mitigation strategies to protect both the mussels and the surrounding marine ecosystem.

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Presence of pharmaceuticals and other emerging contaminants in different substrates used in agriculture as fertilizer

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Introduction

Sewage sludge is a typical by-product of our society, and its reuse in agriculture is potentially an ideal solution for its disposal. The application of sludge and other biosolids on overexploited agricultural land can bring great benefits due to their content of organic carbon, nitrogen, phosporus and micronutrients. However, great attention must be paid to their potential adverse effects due to the presence of several classes of emerging contaminants (ECs), such as pharmaceuticals, including antibiotics, personal care products and perfluoroalkyl compounds. Several ECs coming from people's daily life, agricultural and industrial activities are detected in wastewater and sludge.

The aim of this study was to investigate the presence of about 40 ECs in different substrates (sewage sludge, compost, digestate, pig and cow manure) and to assess whether their application in agriculture poses a threat to the terrestrial ecosystem and human health. This was done by analysing ECs presence in earthworms (*Eisenia andrei*) and lettuce *(Lactuca sativa*) grown in soil mixtures containing the different substrates.

Materials and Methods

The samples were initially freeze-dried and stored at -20°C. Extraction was performed by accelerated solvent extraction (ASE) for sewage sludge and the other biosolids and by ultrasonic-assisted extraction (UAE) for *Lactuca sativa* and *Eisenia andrei*. The samples were then purified by solid-phase extraction (SPE) using specific cartridges, and analysed by liquid chromatography tandem-mass spectrometry (HPLC-MS/MS) (Figure 1).

Results and Discussion

The selection of ECs to be studied in the different substrates was done following several specific criteria, such as their reported presence in wastewater and sludge, the potential for adsorption in sludge based on properties such as solid-liquid partition coefficient (K_d) and octanol-water partition coefficient (K_{ow}), the potential for persistence in sludge and developing of toxicological effect to humans and the ecosystem. The results showed different profiles of contamination in the different substrates investigated, with prevalence of plasticizers and fluoroquinolones, antibiotics for human use, in sewage sludge (S) and of plasticizers, veterinary

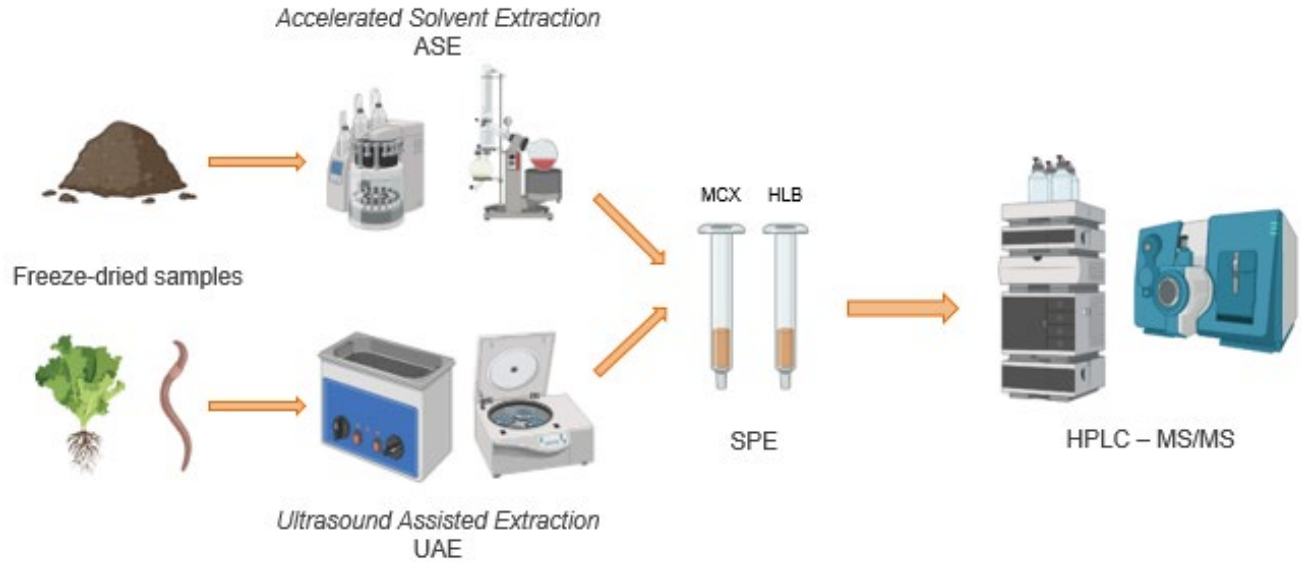

Figure 1. Protocol of extraction and analysis of ECs from different biosolids, lettuce and earthworms.

antibiotics, such as lincosamides, and estrogens in pig and cow manure (M1-M2). (Table 1). ECs were also present in compost and digestate samples. The presence of ECs and especially antibiotics in substrates that may be used in agriculture indicate a potential contamination of soil with risks for the terretrial ecosystems and human health related to the potential uptake of edible plants. Traces of ECs were also found in lettuce.

Table 1: Concentration (ng/g) of the selected classes of ECs investigated in sewage sludge (S), pig and cow manure (M1-M2), compost (C) and digestate (D). For S the represented concentration is the mean of three sewage sludge samples.

Classes of Emerging Contaminants	S	M1	M2	C	D
Antibiotics					
Fluoroquinolones	3867.5	283.8	13.0	12.4	10.7
Sulphonamides	5.7	310.2	880.4	0.0	31.5
Macrolides	60.3	0.0	0.0	4.5	1.1
Lincosamides	0.0	8392.4	23.5	62.0	19.0
Amphenicols	0.0	0.0	0.0	0.0	0.0
Diaminopyrimidines	1.6	0.0	0.0	0.0	0.0
Tetracyclines	1.8	100.8	1614.2	0.0	207.6
Other Pharmaceuticals					
Antihypertensives	84.3	0.0	0.0	0.0	0.0
Anti-inflammatory	59.8	0.0	0.0	663.2	0.0
Antidepressants	21.4	0.0	0.0	9.8	0.0
Anti-asthmatic	0.0	0.0	0.0	0.0	0.0
Diuretics	73.8	0.0	0.0	0.0	0.0
Estrogens	11.2	3205.8	216.5	5.5	930.6
Disinfectant					
Antimycotic	1957.6	0.0	0.0	0.0	0,0
Antibacterial	349.9	0.0	0.0	9.5	0,0
Other ECs					
Plasticizer	4759.0	4256.3	68.8	1363.2	76.0
Perfluoroalkyl phenols	9.5	0.0	0.0	0.0	0.0

Conclusions

The input of ECs into agricultural lands through the application of these substrates can create a stressful condition to the ecosystems. Particularly, the presence of antibiotics promotes the spread of antibiotic resistance, a serious threat for human health. This study improved information on ECs presence in sludge and manure and on uptake in lettuce with the overall goal to convert a potentially risky practice such as the application of biosolids in agriculture into a safe process of circular economy.

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Glyphosate activation of Estrogen Receptors a and b in human cell line PNT1A

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Introduction

Although many harmful effects of the herbicide Glyphosate (Gly, N-(phosphonomethyl) glycine) on non-target organisms are known, it is still used in many countries, especially in Monsanto branded formulation, i.e. Roundup. Given the ever-increasing demand for food closely related to the increase in human population, it is widely used in agriculture and horticulture, to maximize and speed up harvests. As a result, Gly has become an emerging environmental pollutant, causing contamination of soil, water and crops. Unfortunately, Gly does not remain confined to ecosystems but reaches animals and humans through the food chain, endangering the health of the population [1]. Recent studies carried out on the field lizard aimed at verifying the impairment of reproduction induced by Gly exposure on terrestrial vertebrates, demonstrated that Gly acts as an endocrine disruptor by altering the expression and localization of estrogen receptors (ERα and ERβ) in the tissues (liver and gonads) [2-4]. Both upregolation and activation of ERα have also been demonstrated in vivo and in vitro models of the mammalian blood-testis barrier [5]. Finally, the ability of Gly to mimic 17β-estradiol effects promoting ERa activity has been found in breast cancer cells [6].
This study aimed to verify the ability of Glyphosate and Roundup to exert the endocrine disruption effect through ERs activation, promoting their nuclear translocation.

Materials and Methods

Human immortalizated prostate epithelial cell line (PNT1A) was cultured in RPMI-1640 medium supplemented with 10% dextran-coated charcoal FBS, 2 mM l-glutamine, 100 U/mL penicillin/streptomycin, 10 µg/mL gentamicin, in a humidified incubator at 37°C and 5% CO_2. Cells were treated with different concentrations of Gly and Roundup (from 0.006 to 6 mg/mL for 30', 2h and 4h), 10^{-5} M E2 (as positive control) and Tamoxifen (as inhibitor). Cell viability and proliferation were evaluated using the MTT and LDH assays. Cellular localization of ERa and ERb was determined by immunofluorescence, in presence or absence of inhibitor, added at different time-intervals. Western blotting was performed to assess the levels of the pro-apoptotic proteins Bak and Bax.

Results and Discussion

MTT and LDH assay

MTT and LDH assays were used to test the effects of the different concentrations of Gly and Roundup. As expected, cells respond to treatment in a dose-dependent manner: as concentrations increased, viability decreased and cytotoxicity increased. No significant differences were found between pure Gly and Roundup. The tests allowed us to determine the adequate concentrations to use in all further experiments (6 and 60 µg/ml).

Localization of ERα and ERβ by Immunofluorescence

Fluorescence microscopy demonstrated that ERα was translocated to the nucleus after 30' of treatment, remained in the nucleus at 2h and returned in the cytoplasm after 4h; after estrogen treatment, ERα slowly entered in the nucleus reaching maximal nuclear translocation at 4h. When cells are pre-treated with the inhibitor tamoxifen, the receptor always remained in the cytoplasm, regardless of the time or type of treatment.
For ERβ, fluorescence analysis demonstrated that the nuclear translocation reached its maximum after 2h of treatment; the receptor was back in the cytoplasm after 4h. ERβ activation by estrogen treatment was slower, as for ERα, reaching a maximum at 4h of treatment. Again, the pretreatment with the specific inhibitor tamoxifen blocked the receptor in the cytoplasm, regardless of treatment.
No differences in ERs levels were found for both substances (Gly and Roundup) and concentrations (6 and 60 µg/mL) tested (Figure 1).

promote nuclear translocation of ERs, with a

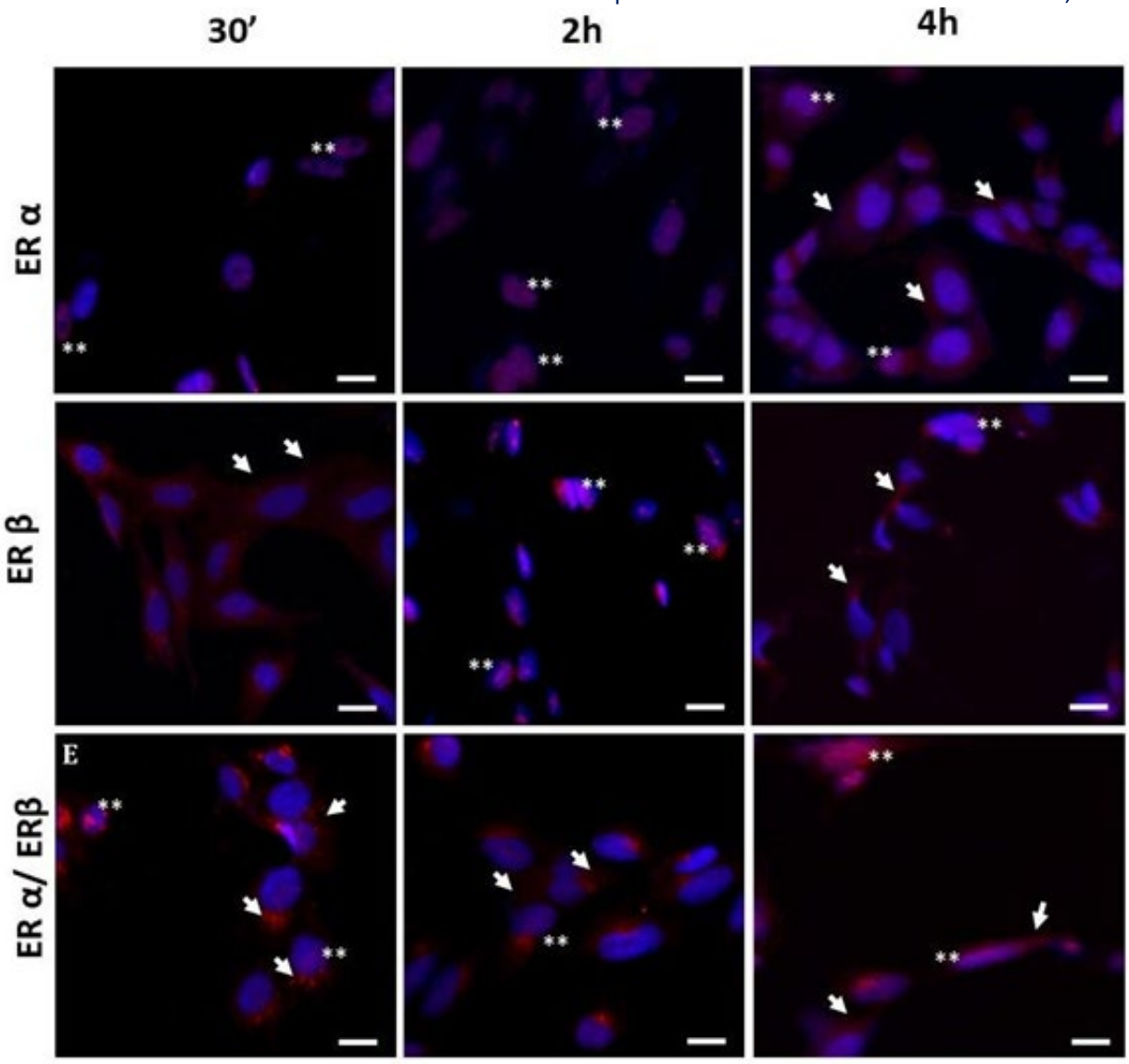

Figure 1. Immunolocalization of ERα and Erβ: red staining= ER positivity, blue staining= nucleus. Arrows: signal evident in cytoplasm; double asterisks: positivity evident in nucleus. Scale bars correspond to 10µm.

Determination of Bak and Bax levels by Western blotting

A statistically significant increase in the levels of the pro-apoptotic proteins Bax and Bak was detected in all treated cells.

Conclusions

Data described in this work leads to the conclusion that Gly, as well as the commercial formulation Roundup, acts as endocrine disruptor by activation of the estrogen receptors. The two substance, already at low concentrations, timing different comparing to E2, that induces a slower but protracted response over time.

The data also demonstrate that Glyphosate and Roundup are cytotoxic; the increase in the expression of the pro-apoptotic proteins Bak and Bax observed in treated cells suggests that the latter respond to the induced damage by activating the apoptotic pathway.

Further studies are needed to elucidate the molecular mechanisms underlying Gly-induced estrogen receptor activation and Gly cytotoxicity.

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The ABC response of *Halomicronema metazoicum* (Cyanobacteria) to ocean acidification scenarios

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The rising carbon dioxide atmospheric concentration due to anthropogenic emissions led to an increase in ocean absorption and in ocean acidification (OA) by lowering the surface pH. Despite the seriousness of such changes certain species can deal with and adapt to naturally acidified conditions in their habitats. Resistance mechanisms played by ATP-binding cassette (ABC) transporters have been investigated in few studies on Cyanobacteria and suggest the involvement of specific ABC-like proteins in acid stress tolerance. The marine cyanobacterium *Halomicronema metazoicum* has been recently isolated from seagrass meadows of *Posidonia oceanica* populating low-pH seawaters around the Castello Aragonese of Ischia Island (Italy). Here, we investigated the potential involvement of ABC transporters by qPCR in *H. metazoicum* tolerance to low seawater pH values (7.7, 7.2, 6.5) for 7 and 30 days in controlled laboratory conditions. Mats viability was assessed qualitatively based on observations of filaments' morphology and colour and quantitatively by measuring chlorophyll *a* and carotenoid content. Primers of ABC-like gene *slr2019* belonging to the ABCB subfamily and isolated from *Synechocystis* sp. PCC6803 were used. *H. metazoicum* did not show any sign of distress upon exposure to all three low pH values since all mattes resulted still green and alive and chlorophyll a and carotenoids were measured in each group. An upregulation of *slr2019* gene was observed in mattes exposed to pH of 7.7, while no gene modulation was evident at lower pH values of 7.2 and 6.5. Preliminary results show that *H. metazoicum* can face low seawater pHs and the potential involvement of ABC proteins in acid stress tolerance. Unravel the role of ABC in acid stress resistance could be a first step to highlight cellular and molecular mechanisms behind adaptive resistance to OA.

Examining of persistent organic pollutants (POPs) in stranded *Grampus griseus* specimens on Italian coasts

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Introduction

In literature, there are scarce information on biology and toxicology of Risso's dolphin (Grampus griseus, Cuvier, 1812). According to the IUCN the Mediterranean subpopulation is classified "Data Deficient" [1]. The biggest threats to this species are noise pollution, bycatch, and pollution from toxic substances [1,2]. This latter can affect reproduction and health at the individual and population level [3]. Moreover, cetaceans are sensitives to environmental contaminants, especially organochlorine compounds (OCs) [4]. The aim of this study is to expand knowledge on OCs, known as endocrine-immune disruptors, including dichlorodiphenyl trichloroethane (DDTs) and its metabolites, polychlorinated biphenyls (PCBs) and hexachlorobenzene (HCB) [5,6] which induce adverse health effects such as reproductive dysfunctions [3,7] and immunosuppression [8,9]. The main objective was to determine the levels of OCs in Grampus griseus' biological samples stranded along the Italian coast between 1998 and 2021, extending the interest in this species still scarcely studied as well as the knowledge of contamination impact on them.

Materials and Methods

The samples (blubber, liver, muscle and brain) were collected from 20 stranded *Grampus griseus* specimens along the Italian coast between 1998 and 2021. Determination of HCB, *DDT*s and *PCB*s were performed according to the modified USEPA 8081/8082 method [10]. Briefly, samples were freeze-dried and extracted in a Soxhlet apparatus. Then purified with sulfuric acid and then they underwent liquid chromatography on a column containing Florisil. High resolution capillary gas chromatography was performed with an Agilent 6890N coupled to 63Ni ECD. Statistical analyses were carried out with the STATA 14 software [11].

Results and Discussion

OCs analysis for each tissue

Blubber was analysed in 20 individuals while, liver and muscle in 10 and brain in 6. The toxicological analyses revealed the presence of HCB, 30 congeners of PCBs and the op' and pp' forms of DDT, DDE and DDD in all the animals. In addition, the percentage of extracted organic material (EOM%) has been calculated. HCB was the compound with the lowest levels in every tissue, while PCBs were the class of contaminants with the highest mean followed by DDTs (Figure 1). The Kruskal-Wallis test showed statistically significant differences ($p < 0.0553$) between tissues for HCB. The results of multiple pairwise comparison tests were significant for brain-blubber, liver-blubber, and muscle-blubber. The same was performed for DDTs, highlighting comparison for blubber-brain, blubber-liver, and blubber-muscle.
Using also for PCBs the multiple pairwise comparison, it was suggested comparison for blubber-brain, liver- blubber, and blubber-muscle. Using the tissue variable and considering the levels of the 3 contaminants, we went statistically elaborate the results with the non-parametric ANOVA test for Kruskal-Wallis ranks, noting that there were statistically significant differences for HCB ($p = 0.0552$), PCBs ($p = 0.0572$) and DDTs ($p = 0.0798$).

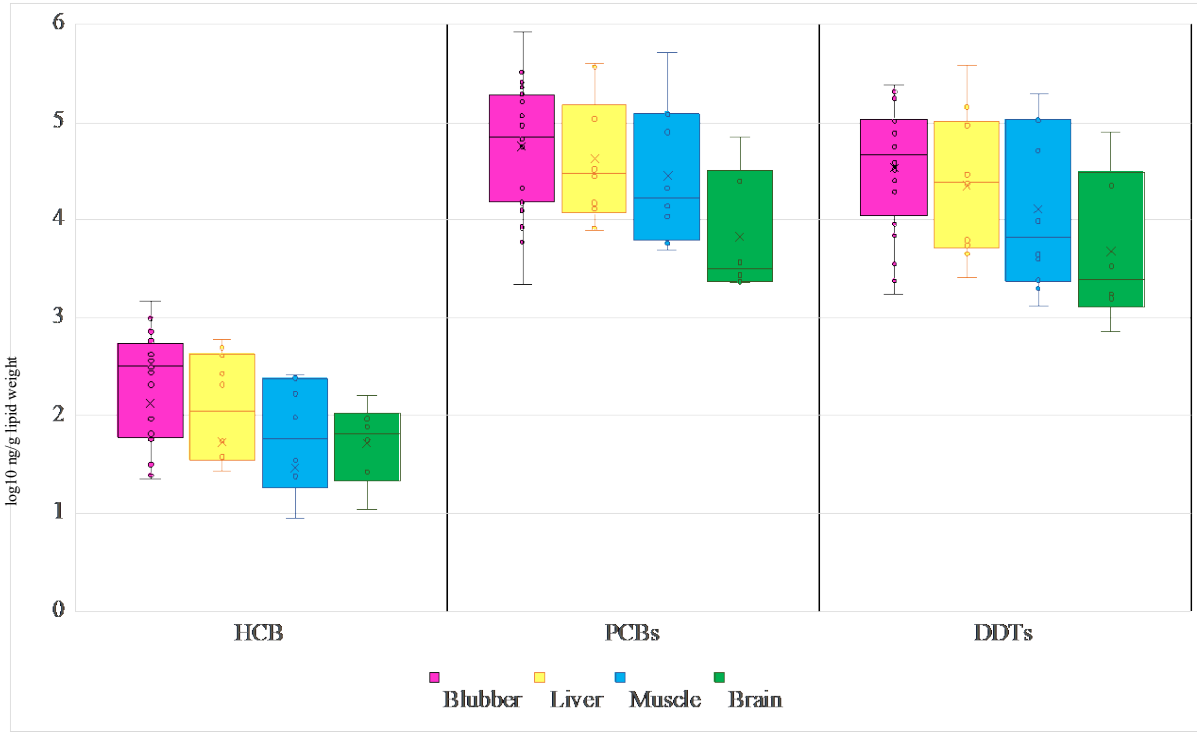

Figure 1. Boxplots of the three organochlorine compounds (HCB, DDTs and PCBs) expressed in log10 ng/g l.w by the four tissues (Blubber, Brain, Liver and Muscle).

Conclusions

The results showed the presence of all the three *OC*s classes (*HCB*<*DDT*s<*PCB*s) which continue to be priority contaminants in the Mediterranean basin despite their regulation in production and use in most areas of the world.

In the literature until now there is little information, not only on the species' ecotoxicology and biology, but also on the distribution and use of the habitat, which still have not allowed to fully understand the ecological and biological aspects of the species.

Considering that Risso's dolphin has been ranked on the IUCN Red List as "*Data Deficient*" in the Mediterranean Sea, it would be essential to expand knowledge regarding the toxicological status and encourage conservation measures that also take this threat into account.

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Plastic contamination in water and sediments of four Po River tributaries (Northern Italy)

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Introduction

Plastic pollution represents an emerging global issue for the aquatic environments, and rivers are one of the main sources of plastic toward marine ecosystems [1]. In this context, plastics do not affect only the aqueous matrix, but also heavily impact the bottom of water bodies, with different types of ecotoxicological consequences (e.g. oxidative stress, protein modulation, inflammation) [2]. In particular, floating plastics are dangerous materials for nekton and plankton [3,4], while high-density polymers, being able to settle on the water body bottom, affect benthic organisms [5]. Considering that the majority of studies on plastic pollution concern the marine environment, this research aimed on quantification and characterization of plastic contamination in water and sediments from four tributaries of Po River, in Lombardy Region (Italy).

Materials and Methods

The study focused on sampling areas in four North-Italian rivers, namely Ticino, Adda, Oglio, and Mincio, which are tributaries of Po River. These areas were chosen because previous studies highlighted plastic pollution in lakes Maggiore, Como, Iseo and Garda, from which the abovementioned rivers are emissaries [6]. The sampling sites were located near the entrance of each water-course into the Po. The sampling was conducted between June 1st and June 8th 2022, with consistent weather conditions and no precipitations. Sediments were collected using a Van Veen grab, and floating plastics by water filtering using plankton nets with a mesh size of 100 µm. Sediments and floating plastics were collected in the same day from each sampling site and stored at 4 °C before the analysis.
The sediments were characterized in terms of grain size, water and organic matter (OM) content, all aspects potentially involved in plastic retention on the bottom of water courses. In particular, the grain size analysis was performed using sieves with decreasing mesh sizes. Sediments were dried, and a portion of each sample was placed in a muffle furnace to remove organic particles and determine the total OM content. Plastic extraction from sediments was done using a hypersaline solution of zinc chloride ($ZnCl_2$; density of 1.6 g/cm^3), which allows the separation of high-density plastics (potentially present in sediments). On the hoter hand, plastics in the water samples were extracted using a hypersaline solution of sodium chloride (NaCl; density of 1.2 g/cm^3). The collected plastics were filtered on membrane filters (8 µm) and digested with hydrogen peroxide (15 % v/v) to remove organic matter. The filters were then dried, and the plastics were quantified and characterized, using the Fourier Transform Infrared Microscopy (µFT-IR), in terms of shape, size, and polymer.

Results and Discussion

Sediment characterization

The sediment characterization showed that Ticino River had a different sediment composition compared to Oglio, Adda, and Mincio. Indeed, the Ticino River sediments presented a higher percentage of particles larger than 8 mm (49 %) and 1 mm (28 %), while the other three rivers showed a main particle distribution in the range > 0.25 mm and > 1 mm. Lastly, the Mincio River sediments had the highest percentage (7%) of silt material compared to the other rivers.
The water content in the sediments reflected the grain size results; Ticino River sediments presented the lowest water content (7 %) and the other three rivers showed values higher than 17 %. The organic matter (OM) content was highest in the Mincio River sediments (about 2 %) compared to the other rivers.

Quantitative results

In terms of plastic monitoring, the study analyzed water and sediment samples from each river. In Ticino, the water samples presented 15 plastics out of 75 particles analyzed (mainly represented by cellulose fibers), while the sediments had 9

plastics out of 152 particles. The concentration of plastics ranged from 0.86 plastics/m^3 to 6.75 plastics/kg sediment dry weigh (d.w.). The main polymer type of plastics detected in Ticino River was polyester (PEST) fibers and microplastics were the main size category in both water and sediments. A similar pattern was observed in Adda River, with 19 plastics out of 75 particles in water samples and 22 plastics out of 88 particles in sediments. The concentration of plastics ranged from 3.61 plastics/m^3 to 17.38 plastics/kg sediment d.w. Once again, PEST fibers were the main type of plastics detected. In Oglio River, 14 plastics were found in water samples out of 67 particles, while 21 plastics were found in sediments out of 111 particles. The concentration of plastics ranged from 4.99 plastics/m^3 to 17.38 plastics/kg sediment d.w. Again, PEST fibers, which reflected a domestic contamination, were the most common polymers. Mincio River had the highest number of particles, resulting the main contaminated river, with 221 plastics out of 300 particles in water samples and 31 plastics out of 113 particles in sediments. The concentration of plastics ranged from 62.89 plastics/m^3 to 26.50 plastics/kg sediment d.w. The main type of plastics detected in Mincio River was polystyrene (PS) pellets, which represented an industrial source of plastic pollution. Overall, microplastics were the most prevalent size category in both water and sediments of all rivers. The obtained results highlighted a higher contamination in sediments than in water for all considered rivers, with significant differences ($p < 0.05$), in terms of plastic content, between water and sediments of Adda and Oglio River. However, no significant covariation was found between the number of plastics in sediments and the measured grain size.

Conclusions

The results of this study confirm that the selected four rivers contribute with a high ammount of plastics to Po River contamination, as well as, indirectly, to the Mediterranean Sea pollution. Rivers can also stored plastics in sediment for longer times and release this contaminant slowly into the hidrographic network. To evaluate, in a holistic way, the real plastic pollution in river ecosystems, our results suggest to monitor both sediments and waters, since these matrices present a different contamination in terms of both ammount and plastic types.

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Development of a holistic approach for river health assessment: from bioindicators to the ecosystem

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Freshwater ecosystems are essential for the planet. They can help to protect and restore biodiversity, mitigate pollution through water filtration and purification and contribute to climate stability by providing both mitigation and adaptation benefits. Despite their importance, anthropogenic pressure decreases water quality and affects the ecological health of river basins. The Water Framework Directive (WFD) has the main goal ofprotecting the aquatic environment in its entirety, for the achievement of "*good ecological status*" in all European rivers by 2027 (2000/60/CE). The Directive establishes some principles such as the importance of defining the ecological status of rivers with an interdisciplinary e multiparametric approach. In this study, we propose an integrated and holistic methodology to evaluate the river's health status taking into account chemical, ecological and ecotoxicological parameters. We used the Elsa River as a case study. It is a tributary of the Arno River, in Tuscany (Italy). The area is characterised by agricultural and industrial activities and by the presence of some cities. Chemical parameters of water (concentrations of nitrates, phosphates, ammonium and pH) were measured thanks to a citizen science project, inscribed to "Fresh Water Watch", carried on by the population and high schools of the area. The involvement of the community through experimental educational activities has allowed the formation of an intergenerational group trained in monitoring and a wide diffusion of the project. For the ecological analysis, the EBI (Extended Biotic Index) and the FFI (Fluvial Functionally Index) were evaluated. We used as bioindicators the freshwater fish Italian chubs (*Squalius squalus*), for the chemical and ecotoxicological analyses. In the tissues of this bioindicator, we assessed the microplastics ingestion, heavy metals and organochlorine concentration and the biomarkers AChE, EROD, PAHs metabolites, LPO, GST, LDH, IDH and ENA Assay. The results showed a general negative trend going from upstream to downstream, with many sources of macronutrients and contaminants, mainly in correspondence with the presence of inhabited centres. The ecotoxicological analysis showed a genotoxic effect correlated to Hg, PCBs and B(a)P metabolites in bile. The contamination is probably due to agricultural and industrial activities, vehicular traffic, polluted tributaries and inappropriate disposal of waste. The greatest abundance of microplastics was found in chubs caught in the *Parco Fluviale dell'Alta Valdelsa*, due to the presence of the urban area, the regular release of waste along the river in the past, and the increase of tourism in the last years. This holistic approach has permitted us to define the health status of the river and identify the contamination and the sources of contaminants. This tool is helpful in promoting mitigation actions to block the sources of impact and bring the river to a good ecological state.

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Lake Avernus has turned red: bioindicator monitoring unveils the secrets of "Gates of Hades"

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Introduction

Cyanobacteria are ubiquitous organisms, living in almost all phototrophic aquatic environments, including recreational water bodies, fisheries, and reservoirs. Over the past two decades, worldwide attention has been given to the ecotoxicological aspects of cyanobacteria blooms and their exploitation as a source of bioactive compounds. Indeed, they represent a not yet fully explored source of new lead compounds for drug discovery. Eutrophic conditions allow cyanobacteria to bloom, producing large green mats covering water surfaces and producing cyanotoxins giving rise to a serious problem for public health.
Our studies allowed us to set up a new advanced multidisciplinary 'Fast Detection Strategy' (FDS) protocol [1] for cyanobacterial blooms and associated cyanotoxins, combining proximal remote sensing (satellite and drone) data and analysis [2], in situ sampling and MS-based molecular networking [3].
In this communication, the red colouring event that occurred on Lake Avernus, a volcanic lake in southern Italy, at the end of March 2022 will be discussed.
As a result, we determined that the red colour was attributable to a bloom of *Planktothrix rubescens*, a toxin-producing cyanobacterium. We detected and identificated 14 anabenopeptins of this P. rubescens strain, of which 7 are known and 7 unknown. In addition, we explained possible causes for this recurring phenomenon, confirming the effectiveness of cyanobacteria as bioindicators of pollution.

Materials and Methods

In recent years, Lake Averno and its recurring cyanobacterial blooms have been the 'test bed' for the of the FDS protocol [1]. This was developed and optimised by our research groups with the hypothesis that cyanobacteria can be used as bio-indicators of environmental pollution phenomena. This strategy is based on a multidisciplinary approach that involving several stages and multiple levels of data acquisition and analysis [2]. The following data phases/levels were used for the Lake Avernus monitoring programme: satellite remote sensing and analysis of the related multispectral data; proximal remote sensing with droneand analysis of related multispectral data; in situ sampling and taxonomic identification in the laboratory; metabolomic profiling via HRMS/MS-based molecular network [3].

Results and Discussion

In March 2022, a sudden red bloom occurred in Lake Avernus. This bloom event was detected and characterised as a result of our FDS approach [1]. Our multidisciplinary strategy allowed not only the early detection of the bloom, but also the identification of the main species of the bloom (Planktohrix rubescens) and the identification of 14 variants of the toxin anabenopeptin.

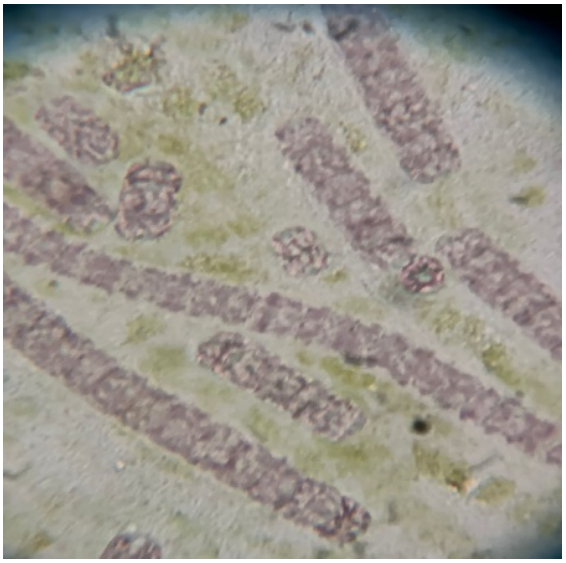

Figure 1. Microscopic view of Planktohrix rubescens.

The integration of remote sensing and proximal sensing data within the FDS approach revealed the onset of the cyanobacian bloom and its periodic recurrence. The use of molecular MS-based

molecular networks enabled a comprehensive profile of the *P. rubescens* (AvL22) metabolome. These MS-based molecular identifications confirm the usefulness of this strategy for the rapid dereplication of complex natural mixtures, without the need for complex and time-consuming chromatography purifications.

The causes of the red bloom were also investigated. There are two broad areas of causality that potentially lead to the recurring phenomenon of cyanobacteria blooms on Lake Avernus: the favourable natural conditions associated with the volcanic origin of the lake and, in particular, those relating to its physical-chemical state, and the specific anthropogenic activities that release pollutants/nutrients into the lake through surface runoff of rainwater and non-rainwater.

The identification of the catchment area of Lake Avernus made it possible to delimit the area from which surface waters are collected and conveyed into the lake. The thematic maps of land cover and land use, provided by the Copernicus Programme and defined by catchment basin closure section, highlighted the main anthropogenic activities releasing pollutants/nutrients such as: intensive agriculture (vineyards, fruit trees, etc.), natural and semi-natural deciduous forests, commercial units and medium and low density urban areas.

Conclusions

The abundance of the main anabaenopeptin found in the cyanobacterial extract from Lake Avernus, anabaenopeptin B, was estimated to be 4% of the total extract.

Due to the well-known toxicity of anabaenopeptin [4], there are fears of a serious risk to humans and other organisms.

In addition to being a popular recreational site, the lake has an outlet to the sea that is close to mussel farms, infact the same toxins were detected in water sea and bivalve samples collected from the outlet of the channel of Lake Avernus in the sea.

The result of this research should then confirm the usefulness of cyanobacterial blooms and their toxic secondary metabolites as bio-indicators of the degree of water quality in coastal ecosystems and the effectiveness of our multidisciplinary monitoring protocol to provide early warning that a bloom event is occurring and to detect cyanotoxins without the need for long and complex chromatographic purification.

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A preliminary assessment of the potential adverse effects of organochlorines on the success of *Caretta caretta* (Linnaeus,1758) hatchling in Tuscan nesting events (Italy)

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Introduction

Among the three species of sea turtles that regularly live in the Mediterranean Sea, the loggerhead turtle (*Caretta caretta,* Linnaeus, 1758) is the most abundant and the only one nesting along the Italian coasts [1]. Nesting events are reported in Tuscany only from 2013 where they are monitored from the deposition to the hatching. After hatching, in order to collect biological material such as unhatched eggs, pipped and hatchlings, the chamber nest is opened. The samples collected during the digging can give valuable information about genetic, pathological, parasitological, toxicological aspects [2,3]. In this study, for the first time in Italy, we reported the concentrations and the fingerprints of some chlorinated xenobiotics analysed in the unhatched eggs sampled from four Tuscan nests: Marina di Cecina (LI), Rimigliano (LI), Le Marze (GR) and Riva del Sole (GR). Persistent, organic, bioaccumulative, and old generation contaminants such as HCB, DDTs, and PCBs are still causes of concern for *C. caretta* and other sea turtle species [4-6].

Materials and Methods

The samples were collected from 4 nesting sites laid along the Tuscan coast (Italy) in summer 2019. Determination of HCB, DDTs and PCBs were performed according to the modified USEPA 8081/8082 method [7]. Briefly, samples were first freeze-dried and extracted in a Soxhlet apparatus. They were then purified with sulfuric acid and subsequently they underwent liquid chromatography on a column containing Florisil. High resolution capillary gas chromatography was performed with an Agilent 6890N coupled to 63Ni ECD. Statistical analyses were carried out with the RStudio software [8].

Results and Discussion

The toxicological analyses revealed the presence of HCB, 29 congeners of *PCBs* and the *op'* and *pp'* forms of DDT, DDE and DDD in all 52 samples (13 eggs for each nest). In addition, the percentage of extracted organic material (EOM%) has been calculated. Even if the mean values of contaminant class in each nest are different (Table 1), all samples present the same pattern as follows: PCBs>DDTs>HCB.
Parametric statistical tests have shown significant differences between the two southern nests (Riva del Soleand Le Marze) and the two notrthern nests (Rimigliano and Marina di Cecina) for DDTs and PCBs. The DDTs and PCBs fingerprints have revealed a homogenous pattern with the only single execption of few samples. These small variabilites can be explained by a different nutrient trasnfer by the mother during the ovogenesis.

Table 1: Mean values and standard deviations of HCB, DDTs and PCBs expressed (ng/g lipid weight) for each nest.

	Riva Del Sole (*n*=13)	Le Marze (*n*=13)	Rimigliano (*n*=13)	Marina di Cecina (*n*=13)
HCB	0.73 ± 0.24	1.01 ± 0.63	1.01 ± 0.52	1.31 ± 1.20
DDTs	84.77 ± 13.73	81.96 ± 21.54	65.61 ± 16.48	53.80 ± 13.90
PCBs	158.94 ± 21.57	147.60 ± 31.70	125.18 ± 23.61	111.20 ± 23.25

Conclusions

The incubation period (42–91 days) embryonic development, decomposition, bacterial metabolism, dehydration, exposure to sand and water infiltration from tides or disturbances, are all factors that could potentially alter the concentrations of contaminants originally contained in the egg [6]. Thus, future studies should investigate the toxicokinetic of chemical compounds through maternal and offspring tissues. Levels ofcontaminants found in our study are lower than those found in the few available studies worldwide and they cannot be correlated to the hatching failure. Despite this, the research of this contaminants is still a priority especially in the Mediterranean basin, because even if they were banned many years ago their levels are still high in marine biota. The reptile, a very long living species, can be considered an indicator of pollution of the Mediterranean basin and, and for this reason, the scientific community offered *C. caretta* as bioindicator in the Good Environmental Status (GES) of Marine Strategy Framework Directive, not only for Descriptor 1 (Biodiversity) and 10 (Marine Litter), but also for Descriptor 8 named Contaminants.

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A complementary approach based on contaminant analysis, biomarker responses and behavioural performances to investigate the toxicological status of *Parus major* from Veneto region

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Pesticides and other classes of anthropogenic contaminants can cause reversible alterations or permanent damage to avian species. Great tit (*Parus majoj*) is an important model species for environmental studies. Its aptitude for nesting in artificial boxes and territoriality can provide precise toxicological information on a given territory. In the last six years, several nest boxes for great tit were installed in different areas of Veneto region, characterised by different anthropogenic impacts. This study aimed to investigate the effects of environmental contaminants on great tit populations by a non-lethal approach integrating chemical analysis, biochemical/cellular responses, breeding and behavioural traits. In 2021 and 2022, biological materials such as blood, feathers, excreta and unhatched or abandoned eggs were collected from nestlings living in nest boxes installed in different areas of Veneto region. The study areas, subjected to different anthropogenic pressures, were wooded, agricultural and urban environments. Per- and polyfluoroalkyl substances (PFAS) and heavy metal levels were determined on eggs and feathers, respectively and porphyrin concentrations were evaluated on excreta. A set of non-lethal biomarkers capable of providing different toxicological responses was performed on blood. Behavioural traits such as reactivity performances and fitness were also assessed. PFOS was the most abundant compound, and the long-chain perfluorinated carboxylic acids levels were agricultural<urban<wood. Cu was the most abundant metal in all areas and Hg had higher values in the urban area. Nestlings from urban and agricultural areas had higher genotoxic effects than this from wood. The H/L ratio was more severe in urban birds, which showed a physiological stress condition. Complement system activity was higher in nestlings from an agricultural area suggesting that these birds might be exposed to substances that act on innate immune system. The applied approach was proved to be a valuable tool for the ecotoxicological monitoring of great tit. It is fundamental to obtain a complete picture of the health status of this species and to investigate the environmental quality in which the studied species lives.

Combined effects of long-term exposure to copper and heatwaves on the reef-forming serpulid *Ficopomatus enigmaticus* through a biomarker approach

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Introduction

Heatwaves (HWs) are periods of anomalous ocean warming which are happening at the regional level [1]. These events are rapidly increasing in frequency, magnitude, and severity worldwide [2-4]. Furthermore, in the Mediterranean Sea the situation could be worse than expected, as warming here occurs very quickly [5,6].
Plus, in addition to global climatic changes, sessile benthic invertebrates have been threatened also by local pressures such as chemical pollutants, including metals such as copper (Cu) [7, 8]. Cu is a trace metal essential for cell and organism physiology [9,10] but also an extremely toxic cation, a well-known aquatic contaminant, and a relevant physiological stressor [11, 12].
Shallow-water communities, such as biogenic reefs, are severely impacted by anthropogenic impacts. Several episodes of mass mortality of these organisms in the Mediterranean have already been reported since the 90s [13-18]. The studies of combined effects of global and local impacts on aquatic organisms are undergoing increased interest recently [19] since organisms are exposed to numerous stressors in their natural environments [20-23]. Thus, the effects of HWs alone and in combination with exposure to copper were evaluated on the reef-former brackish alien and invasive tubeworm *Ficopomatus enigmaticus* using a multi-biomarkers approach.

Materials and Methods

The effects of HWs and Cu contamination (alone and in combination) on adults of F. enigmaticus chronically exposed were tested on a set of 10 biomarkers. Portions of the reefs were exposed for 28 days to 4 experimental conditions in triplicates as described in Figure 1, after an acclimation period of 7 days. *F. enigmaticus* adults were exposed in 1L tanks, with photoperiod of 10:14 hours light:dark, constant aeration, pH 8.1 ± 0.1, food supply twice a week with an *Isochrysis galbana* suspension. Complete water changes were performed weekly.

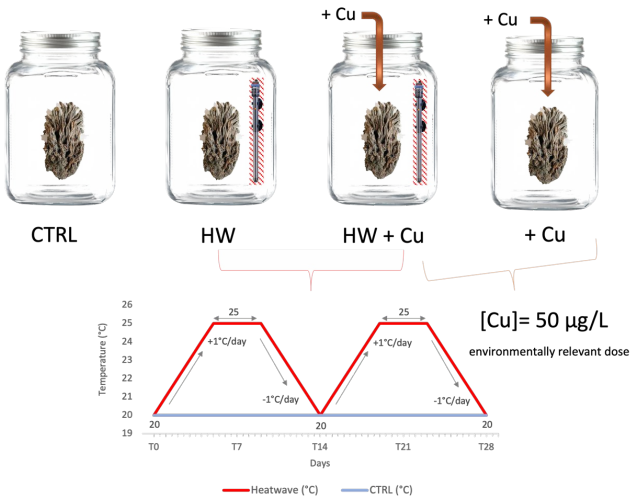


Figure 1. Experimental design.

Endpoints tested were cellular damage in terms of proteins and lipid damages [protein carbonylation content (PC) and lipid peroxidation (LPO)], antioxidant status [Glutathione peroxidases (GPx) and superoxide dismutase (SOD), biotransformation [glutathione-S-transferase (GST) and carboxyl-esterase (CE)], neurotoxicity and DNA integrity [acetylcholine esterase (AChE) and DNA single strand breaks (DNAssb)], and cellular reserves [protein content (PROT) and carbohydrate content (CARB)] responses.
The Principal Coordinates (PCO) analysis were performed and the Integrated Biomarker Response (IBR index) were achieved as multivariate graphic method which allows visual integration of a set of early warning responses measured with biomarkers.

Results

As regards the results of the cell membrane damage, a statistically significant increase of 2-fold was observed in PC values when exposed to a combination of HWs and Cu contamination compared to the control, while no significant variation in LPO levels was observed among conditions tested (Figure 2a).

For the enzimatic antioxidant defences, no statistically significant differences were found in the values of both SOD and GPx.

Regarding GST activity, *F. enigmaticus* samples exposed to HWs alone and in combination with Cu showed a significantly higher activity, in comparison to control, and Cu exposure conditions. In terms of CE activity, significantly lower values were observed in the HW and Cu combined condition in comparison to all the other conditions (Figure 2a).

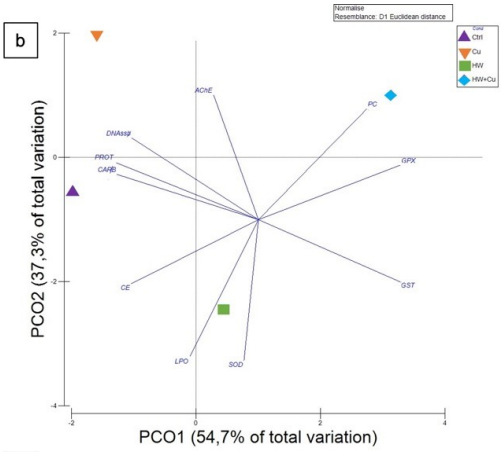
A decrease was also detected in the PROT content, which showed a significantly lower amount when exposed to HWs alone or combined with Cu compared to control and to Cu contamination conditions alone. Exposure at HWs combined with Cu produced a significant effect with a decrease in CARB content in comparison with the control and the increased Cu condition, while HWs alone showed a significant decrease compared to the Cu contamination condition (Figure 2a).

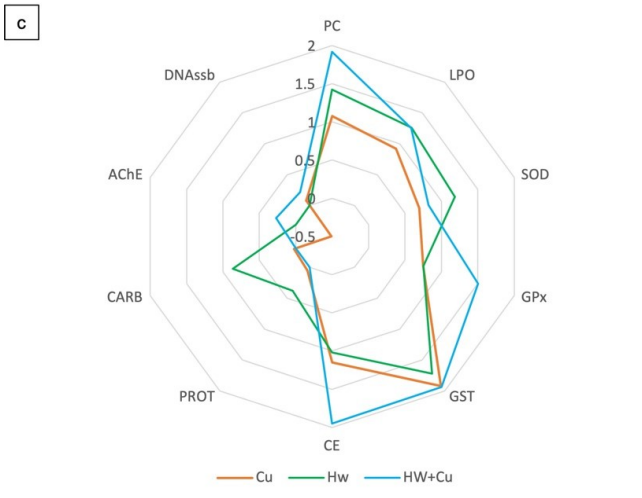
Respecting the neurotoxicity and DNA integrity, AChE levels did not show any significant differences for the conditions assessed, while DNAssb values were statistically different between control and HW along condition, while no differences resulted among other conditions (Figure 2a).

The results from PCO analyses, based on biochemical responses in all tested conditions, were reported in Figure 2b. The first principal component (PCO1), which accounted for 54.7% of the total variability, clearly separated HW and HW+Cu conditions (on positive side), from CTRL

a

Biomarkers		Significance	Direction	Condition (respect to CTRL)
Cell membrane damage	PC	✓	↑	HW+Cu
	LPO	×		
Enzymatic antioxidant defences	SOD	×		
	GPx	×		
Metabolic activity	GST	✓	↑	HW, HW+Cu
	CE	✓	↓	HW+Cu
Energy reserves	PROT	✓	↓	HW, HW+Cu
	CARB	✓	↓	HW+Cu, HW respect to Cu
Neurotoxicity and DNA integrity	AChE	×		
	DNAssb	×		





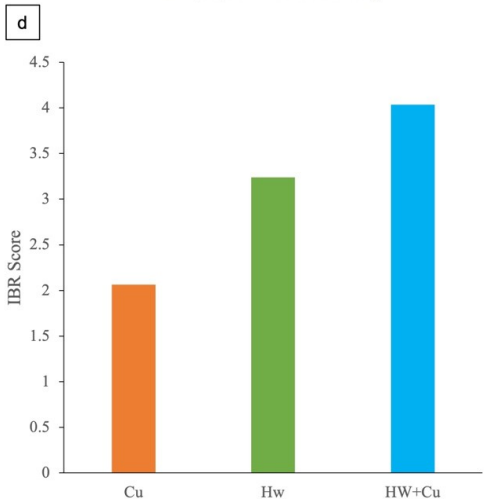


Figure 2. Summary of the results obtained. a) Results, with respectively significance and direction, of the set of 10 biomarkers tested on the tubeworm. b) PCO analysis results measured in CTRL and Cu, HW, HW+Cu -treated tubeworms. (c) radar diagram (d) IBR score for all assessed biomarkers (ten) after exposure to Cu, HW and HW+Cu conditions.

and Cu conditions (on the negative side). Considering the IBR, the radar diagram was presented in Figure 2c, with the global scores of the measured parameters (bar graphs, Figure 2d). Bar graphs showed that HW+Cu condition scored the highest IBR index respect to the other conditions.

Discussion

This work is the first to describe how F. enigmaticus responds biochemically to copper exposure and temperature increase, alone or in combination. The interaction between the two stress factors considered in this study demonstrated synergistic effects in the biochemical response in the Atlantic coral Mussismilia harttii [24-27]. The increase in temperature, with respect to exposure to Cu, was the factor that most influenced the trophic behavior of this species [27]. These findings agree with our results, in which the exposure to Cu alone was never significant, while the temperature was more significant. However, the condition that resulted significantly different more frequently compared to the control was the condition of combined exposure to temperature and copper, for 5 of the biomarkers analyzed (PC, GST, CE, PROT, and CARB, Figure 2a) and for the IBR output (Figure 2d). Plus, the results of the present study agree with the results found previously in terms of the reduction of cellular reserves under stress conditions [28-35].

Conclusions

The most threatened habitats globally are biogenic temperate coral reefs, despite playing a fundamental role in maintaining biodiversity as ecosystem engineers [36,37]. One of the most widespread threats to these ecosystems is the increase in temperature [21], which has caused several mass mortality events also in the Mediterranean. In addition to the effects of climate change, sessile organisms are also impacted by local threats, such as pollutants. Although *F. enigmaticus* has been described as a model organism [38], there is a lack of knowledge on the biochemical response of this species in response to stressors related to climate change. Thus, this scarcity and sometimes conflicting results of biomarker responses in Mediterranean reef- formers to different climate change-related stressors, single or in combination, should be addressed, thus further efforts are needed for the conservation of these species.

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A comparative study of acute and delayed toxicity of perfluoroalkyl substances (PFAS) and their binary mixtures in *D. magna* and *R. subcapitata*

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Introduction

Among the emerging contaminants of high interest, Per- and Poly-fluoroalkyl substances (PFAS) are in the spotlight. These substances are highly used in industry due to their special chemical-physical properties; however, they are extremely stable and difficult to degrade, a characteristic that has earned them the name of 'forever chemicals'. Once released into the environment, they tend to distribute in various matrices, including water and soil, and to accumulate in biota. Possible associations have been indicated between PFAS presence in human body and increased occurrence of various diseases, including liver, pancreas, and kidney cancer, endocrine and immune system disorders, male reproductive system disorder and foetal development impairments [1]. Moreover, PFAS have recently been shown to cause neurotoxicity [2]. Despite this, the effects of PFAS on natural environment and trophic chains are still unknown. To fill this gap of knowledge, the aim of this research was to assess and compare the effects of PFAS in two model aquatic organisms of two different trophic levels, *Daphnia magna* and *Raphidocelis subcapitata*. Acute and delayed toxicity were evaluated by mimicking an environmental exposure occurring in the nearby of an industrial plant site. Besides the assessment of single PFAS toxicity, for the most toxic compounds their interaction in binary mixtures was evaluated.

Materials and Methods

Chemicals

AFPO (CAS No. 3825-26-1, 98%) and GenX (CAS No. 62037-80-3, 95%) were purchased from abcr GmbH, while PFDA (CAS No. 335-76-2, ≥98%), PFNA (CAS No. 375-95-1, 97%), PFOS (CAS No. 1763-23-1, ≥98%), PFHpA (CAS No. 375-85-9, 99%), PFHxA (CAS No. 307-24-4, ≥97%), PFPeA (CAS No. 2706-90-3, ≥97%), PFBS (CAS No. 375-73-5, 97%) and PFBA (CAS No. 375-22-4, 98%) were from Sigma-Aldrich. For the execution of OECD 202 and US-EPA OCSPP 850.4500 tests, 1.0 g/L of each compound was individually dissolved in Rocchetta® water or in Bold's Basal Medium (BBM), respectively. The pH of the solution was then neutralized with 1M NaOH, and serial dilutions were set up with a dilution factor of 2.0. The concentration ranges used in the single-substance toxicity tests for daphnids and algae were 1000 mg/L to 62.5 mg/L and 1000 mg/L to 31.25 mg/L, respectively. For the toxicity assessment of binary mixtures in algae, the maximum exposure concentrations were based on the sum of the two individual half maximal effective concentration (EC50) values; then, a dilution factor of 2.0 and six exposure concentrations were applied.

Acute immobilization test on D. magna and Delayed toxicity evaluation

The *D. magna* acute toxicity test was performed according to the OECD 202 guideline [3]. However, a follow-up in pure medium was added to the standard procedure in order to detect any possible delayed toxicity [4]. The endpoints assessed at the end of the 12 days observation were the immobilization rate and the average daily growth.

Algal toxicity test on R. subcapitata

The Algal toxicity tests were performed according to the US-EPA 96h Acute Toxicity Test - OCSPP 850.4500 [5], with minor modifications. These latter ones basically consisted in test miniaturization (10 mL instead of 50 mL exposure solution). Each replicate (n=4) was placed in one well of a flat-bottomed P6 plate. To keep the algae in suspension a 150 cycles/min shaking was adopted. At the end of test (96 hrs), to assess the potential algicidal or algistatic effect, an aliquot taken from the lowest concentration at which 100% growth inhibition was recorded was adequately diluted in pure medium and incubated for additional 9 days. If no algal growth was detected after these nine days of incubation in pure medium, an algicidal effect was attributed

to the substance. The evaluation of the interaction between compounds in binary mixtures was carried out according to the method proposed by Tallarida [6].

Results and Discussion

Acute and delayed toxicity in D. magna

D. magna was shown to have a wide range of sensitivity to the compounds analysed. With the exception of PFDA, the toxicity data obtained at 48 hrs agreed with the "PFAS rules" written by Buck and colleagues in 2011, i.e. that toxicity is directly proportional to the carbon chain length of PFAS and that sulphonic acids are more toxic than carboxylic acids [7]. Nevertheless, six out of the ten substances tested showed no toxicity at the concentrations we investigated; thus, it can be assumed that their toxicity in *D. magna* is negligible. Not surprisingly, the substance showing the highest toxicity was PFOS (EC_{50} = 97.66 mg/L), followed by PFNA, AFPO and GenX (Table 1).

It should be noted that for each compound that showed toxicity towards *D.magna*, the follow-up to the acute immobilization test evidenced delayed toxic effects. Indeed, the EC50s calculated after the supplemental 10 days in pure medium were noticeably lower than those obtained after the standard 48 hrs test (Table 1). As previously considered [4], an overstatement of the EC50 implies an underestimation of the Risk Quotient. Therefore, it is advisable to assess delayed toxic effects when evaluating the acute toxicity of PFAS.

Table 1: EC_{50} values of PFAS in Daphnia magna after 48 hours of exposure and after 10 days of follow-up.

Chemical Compound	Carbon atoms	CAS n.	EC_{50} (mg L^{-1}) 48h	EC_{50} (mg L^{-1}) 12day
PFDA	10	335-76-2	≥ 1000.00	≥ 1000.00
PFNA	9	375-95-1	187.5 ± 4.68	75.89 ± 14.69
PFOS	8	1763-23-1	97.66 ± 2.45	68.18 ± 10.92
AFPO	8	3825-26-1	397.06 ± 11.98	326.92 ± 53.66
PFHpA	7	375-85-9	≥ 1000.00	≥ 1000.00
PFHxA	6	307-24-4	≥ 1000.00	≥ 1000.00
GenX	6	62037-80-3	785.71 ± 31.46	486.11 ± 71.62
PFPeA	5	2706-90-3	≥ 1000.00	≥ 1000.00
PFBA	4	375-22-4	≥ 1000.00	≥ 1000.00
PFBS	4	375-73-5	≥ 1000.00	≥ 1000.00

Acute toxicity in R. subcapitata

The unicellular alga *R. subcapitata* proved to be significantly more sensitive to PFAS than *D magna*. The least toxic compound was PFHxA, followed by GenX. Notably, AFPO, whose trade and supply have long been banned, was among the least toxic compounds. Interestingly, PFBA, a short-chain replacement compound exhibited the lowest EC_{50} (*i.e.*, 30.03 mg L^{-1}) among the tested compounds, thus resulting even more toxic than PFOS (EC_{50}: 44.85 mg L^{-1}), the first PFAS to be banned due to its proven high toxicity (Table2). Furthermore, it was observed that nine out of the ten target substances showed an algistatic effect, while PFNA demonstrated an algicidal effect (Table 2).

The toxicity evaluation of mixtures showed that PFOS has a tendency to additive interactions. However, PFNA seems to give mainly antagonistic interactions when combined with PFBA and PFBS. Likewise, the mixture composed of the two short-chain substances (PFBA + PFBS) showed an antagonistic effect (Table 3).

Table 2: EC_{50} values of PFAS in R. subcapitata after 96 hrs of exposure.

Chemical Compound	Carbon atoms	CAS n.	EC50 (mg L^{-1}) 96h	Effect type
PFDA	10	335-76-2	34.25 ± 1.32	Algistatic
PFNA	9	375-95-1	90.47 ± 11.14	**Algicidal**
PFOS	8	1763-23-1	44.85 ± 1.36	Algistatic
AFPO	8	3825-26-1	423.20 ± 47.80	Algistatic
PFHpA	7	375-85-9	385.98 ± 56.33	Algistatic
PFHxA	6	307-24-4	957.42 ± 64.80	Algistatic
GenX	6	62037-80-3	737.98 ± 132.38	Algistatic
PFPeA	5	2706-90-3	288.50 ± 107.00	Algistatic
PFBA	4	375-22-4	30.03 ± 3.70	Algistatic
PFBS	4	375-73-5	105.80 ± 43.22	Algistatic

Table 3: EC_{50} mixtures values and PFAS interaction mode in R. subcapitata after 96 hrs of exposure.

Mixture Composition (A+B)	EC50 (mg L^{-1}) 96h	A (mg L^{-1}) at EC50	B (mg L^{-1}) at EC50	Effect type
PFOS + PFBS	56.98 ± 13.80	17.61	39.37	Additive
PFOS + PFBA	44.48 ± 7.58	27.21	17.27	Additive
PFNA + PFBS	212.50 ± 82.40	97.95	114.55	Antagonistic
PFNA + PFBA	116.60 ± 16.15	87.54	29.06	Antagonistic
PFBA + PFBS	121.40 ± 32.38	26.84	94.56	Antagonistic

Conclusions

Based on these results, we can state that the unicellular algae *R. subcapitata* is considerably more sensitive to PFAS than *D. magna*, although some delayed toxic effects were observed in the crustacean. PFBA, despite still being freely sold and traded, and not even being considered a PFAS substance of primary concern by EFSA, was the most toxic compound to *R. subcapitata*. However, it showed an algistatic effect, only temporarily inhibiting algal growth. On the other hand, PFNA showed an algicidal effect, whereby its leakage into the environment could cause bottom-up effects in aquatic ecosystems. Lastly, the assessment of mixture toxicity revealed no synergistic interactions, suggesting that the Concentration Addition (CA) model is sufficiently conservative in estimating the toxicity of PFAS mixtures.

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Abstracts

Posters

Preliminary assessment of biological effects of contaminants in *Mullus barbatus* within the scope of Marine Strategy and IMAP-UNEP monitoring

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Both in Europe and in the Mediterranean Countries (DM 2008/56/CE, Marine Strategy Framework Directive-MSFD and the Barcellona Convention, 1976, IMAP-UNEP) the evaluation of the state of environmental quality and, in particular, the effects of contaminants are among the most pressing needs. To this end, the analysis of biomarkers in bioindicator organisms has been envisaged in the Italian Environmental Monitoring Programme. The target species and the set of biomarkers to analyze were selected in accordance with the IMAP-UNEP indications and the Italian National Monitoring Plan defined according to the requests of MSFD. In the red mullet (*Mullus barbatus*) from 20 sites in the Adriatic Sea (AS), Ionian and Central Mediterranean Sea (ISCMS) and Western Mediterranean Sea (WMS), the micronuclei frequency, the inhibition of acetylcholinesterase activity and the stability of lysosomal membranes, as well as the EROD activity and the metallothioneins level, were analyzed. This study represents an attempt to apply a new qualitative method (C-BEI) for the integrated assessment of the effects of contaminants by means of biomarker analysis, performed by ISPRA during 4 years of National Environmental Monitoring (2019-2022). This method seems to give a suitable assessment criterion for the target species, however further developments are still needed before the most suitable method is defined for the determination of the good environmental status (GES) according to the Marine Strategy in the Mediterranean Region.

Ecotoxicological health status of honey bee colonies using a complementary approach based on biomarker responses, proteomics and honey analysis

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Apis mellifera is a widespread species and the most valuable for its pollination service and for honey production. Honey bees foraging in a wide range can come in contact with food and water contaminated by pesticides; the routes of exposure are pollen and nectar stored in the hive, surface water, air contamination and extra floral secretion from some plants. The uptake and accumulation of pesticides in honey bees can influence their health and the colony's ones. The aim of this work was to evaluate the health status of honey bee colonies, using a complementary approach based on biomarker responses, proteomic analysis and the quality and origin of honey. Honey bee specimens were exposed to two environmentally realistic doses of the commercial fungicides Sakura®, of the commercial herbicide Elegant 2FD, and to their mix. Their effects were assessed using neurotoxicity, metabolic, immune system and genotoxicity biomarkers and proteomic analysis. The toxicological status of honey bees from 10 areas with different anthropic impacts in Tuscany in 2020 and 2021 was also assessed by the application of the set of biomarkers used on laboratory studies. The melissopalynological profile, humidity, carbohydrates, and amino acids contents of the honey, collected in the monitoring study, was carried out. Both pesticides modulated the detoxification process. The fungicide alone had also effects on the metabolism, while the herbicide demonstrated to be neurotoxic and to influence the mixture treatments results. The proteomic approach revealed that the two pesticides were able to affect the energy metabolism, the immune system and the protein synthesis. The monitoring study revealed the presence of neurotoxic effects, oxidative stress and DNA damage higher in 2021 compared to 2020, probably due not only to contaminants but also to changing climatic conditions. The scarcity

of water and the high temperatures in 2021 could have caused a concentration in the presence of contaminants that were not washed away from the plants where bees went to forage, leading to a weakening of the colonies. The melissopalynological analysis confirmed that bees collected pollen in sites different from where beehives were located, suggesting that bees were exposed to contaminants through plant guttation water or topic contact during the treatments of the fields. The use of a complementary approach was proven to be useful to understand exposure and effects of xenobiotics on bees.

Genome-wide identification of ATP-binding cassette (ABC) transporters and future applications in studies on adaptation to ocean acidification in the marine polychaete *Platynereis dumerilii*

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ATP-binding cassette (ABC) transporters represent one of the largest protein family in animals. Due to their ability to transport a wide range of substrates across membranes using ATP cleavage-derived energy, they are involved in various biological processes, including stress responses. A relevant role recognized for ABC proteins is their involvement in tolerance to low-pH/high-pCO_2 environments in bacteria. This is an important finding in the framework of ocean acidification (OA) (the global surface pH decrease caused by anthropogenic CO_2 emissions) and needs to be further investigated in marine species since the understanding of molecular mechanisms of adaptation to acid stress conditions will permit predictions on the impacts of future OA scenarios for marine life. *Platynereis dumerilii* is a marine annelid used as model in a wide variety of fields, such as genetics, single-cell genomics and ecotoxicology. Due to its abundance in naturally acidified areas, as the CO_2 vents off Castello Aragonese (Ischia Island, Italy), it has been proven to be an excellent model to highlight mechanisms for acclimation/adaptation to OA. Since its genome and proteome has been completely sequenced, we carried out bioinformatics analyses to identify *P. dumerilii* ABC-like genes. A total of 81 ABCs were found out in the genome of *P. dumerilii*, belonging to seven distinct subfamilies (A–G) based on phylogenetic analysis. ABC transporters were comprised of 11 ABCA genes, 14 ABCB genes, 34 ABCC genes, 4 ABCD genes, 1 ABCE gene, 3 ABCF genes and 13 ABCG genes. Furthermore, single-cell analyses on a subset of ABC transporters have been performed to determine their distribution in *P. dumerilii* body. Most of ABC transporters can be divided into two groups, the first one seems midgut specific and the other one appears epidermis and ectodermal sensory cell specific. The other examined ABCs did not show any specific cell clade being present almost in the hole body. Selected ABC transporters from ABCB, ABCC and ABCG families identified in *P. dumerilii* genome will be used in studies on adaptive mechanisms to OA. ABC genes transcription will be analyzed in *Platynereis* spp. populations living along Ischia coast, in areas characterized by normal (8.2) and low pH (from 7.4 to 6.9) values, to assess their basal expression and test differences between the two populations. Additionally, transcriptional analyses will be performed in samples subjected to a reciprocal transplant experiment to better elucidate the role of specific mechanism of adaptation involving ABC.

Plastic contamination in water and sediments of four Po River tributaries (Northern Italy)

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The demand of plastics follows a positive trend worldwide, resulting in an increase of plastic production from 365 million tons (Mt) in 2018 to 391 Mt in 2021. Consequently, a continuous release of these materials into the marine and freshwater environments is observed. For these reasons, this study aimed to monitor the presence of plastics in both water and sediments of four

tributaries of Po River (Ticino, Adda, Oglio and Mincio), also investigating the role of sediment composition, as grain size, water content and organic matter, in plastic retention.
Sediment samples were obtained with a Van Veen grab, while the floating plastics were filtered using a plankton net (100 µm mesh) and, after a density extraction process, the particles found were quantified and characterized in terms of shape, size, colour and polymer, using a Fourier Transform Infrared Microscope System (µFT-IR).
The main quantitative results showed a higher amount of plastics in sediments than in water for all analyzed rivers, with significant differences ($p < 0.05$) between water and sediments of Adda and Oglio Rivers. From a qualitative point of view, in both water and sediments of Ticino and Adda, as well as in sediments of Oglio River, we observed principally a domestic contamination by polyester fibers. A different situation was obtained in both water and sediments of Mincio and Oglio water, where polystyrene pellets represented the highest percentage of plastics, highlighting an industrial contamination. Despite this, no significant differences ($p > 0.05$) among rivers in terms of plastic content was observed, neither covariation between the number of plastics and the grain size of river sediments. Our data highlight that plastic monitoring in both water and sediments provides complementary information for a more holistic risk assessment of these emerging contaminants.

Phenolic endocrine-disrupting compounds (PEDCs) and polycyclic aromatic hydrocarbons (PAHs) in Arctic marine sediments (Svalbard Islands, Norway)

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Climate change (CC) is altering the global atmospheric and oceanic circulations, causing sea-ice reduction, glaciers retreat, permafrost thawing, and increased seawater temperatures. Arctic regions are very sensitive to CC and are also affected by the increase in the human footprint, such as tourism, resource extraction and fishing activities, which is negatively impacting this fragile ecosystem. All these aspects, directly or indirectly affect contaminant transport, distribution and fate within the polar regions. In this context, the present study aims to investigate the occurrence of phenolic endocrine-disrupting compounds (PEDCs) and polycyclic aromatic hydrocarbons (PAHs) in Arctic marine sediments, which represent the final sink of many hydrophobic contaminants. The study area was the Arctic fjord Kongsfjorden (KF- 79°N, 12°E, Svalbard, Norway), extensively investigated for many years thanks to the presence of international scientific bases (including the Italian Station “Dirigibile Italia"). This small fjord is directly influenced by the inflow of warm Atlantic waters, various terrestrial and tidal glaciers as well as the increasing human presence. It is, therefore, a highly sensitive marine system to CC and represents one of the most comprehensive environmental monitoring locations to evaluate the CC impacts on new emission sources, remobilization, transport and circulation of contaminants. Sampling was performed along a transect of the fjord during the melting season of 2018 and 2019. The occurrence of selected organic micropollutants in sediment samples and different sources' contributions to contaminant distribution was assessed. Finally, the potential toxicity of target compounds for the KF benthic community was evaluated. The results indicated that local anthropic activities were the major contributor to PEDCs and PAHs within KF, although the role of cryosphere melting as a secondary pollution source was recognized. A comparison among the measured concentrations of contaminants and empirical and mechanistic indices derived from sediment quality guidelines suggested that the occurrence of PEDCs and PAHs in Arctic sediments does not currently pose a risk for this ecosystem, but further investigations on the spread of hazardous contaminants and their effects on these fragile environments are needed.

Environmental safety of nanocellulose: an *in vivo* acute study with marine mussels *Mytilus galloprovincialis*

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Nanocellulose (NC) is a biodegradable and renewable material steadily occupying a larger

portion of the market and with an increasing higher environmental load that requires a proper safety evaluation. Through sewages and wastewaters, NC will likely end up in the aquatic environment for which limited information in terms of ecotoxicity is currently available. Here investigated the environmental safety of two types of cellulose nanofibers, non-oxidized (CNF) and TEMPO-oxidized (TOCNF), by means of an acute *in vivo* study (48h) with the marine bivalve *Mytilus galloprovincialis* exposed to 1 µg L^{-1} and 1 mgL^{-1} as respectively resembling realistic and acute exposure scenarios. Behavior and changes of NC fibers in waters of different salinities were investigated. Mussel's uptake and sub-lethal biological responses (lysosomal membrane stability, neurotoxicity, oxidative stress and biotransformation) were analyzed in mussels. TOCNF resulted more dispersed than CNF in natural sea water (NSW), probably due to higher repulsion among fibers driven by their negative surface charges and colloidal organic material. Both CNF and TOCNF were found in mussel's tissues (gills and hemolymph) using labelled stocks thus confirming uptake from seawater. Destabilization of lysosomal membranes of hemocytes, more significant at the lowest concentration tested (1 µg L^{-1}) was observed and likewise an inhibition of P-gp efflux activities in gills being stronger for CNF at the highest concentration (1 mg L^{-1}). Cholinergic enzymes (ASCh-ChE activities) resulted inhibited in hemocytes, gills and digestive glands regardless of CNFs oxidation and concentration tested. By contrast, neither oxidative stress nor biotransformation resulted affected in mussel's digestive glands and gills. Overall findings showed CNF uptake by marine mussels and disruption in gills functionality and immune cells by mechanical interaction even at environmentally realistic exposure scenarios. Recent evidence on the presence of textile fibers, mostly made of cellulose, in mussels populating marine coastal areas worldwide raises questions on potential hazard posed by NC and consequences on marine biodiversity. The paucity of data on hazard posed by NC for marine species demands for safety aspects to be considered in future risk assessment scenarios.

Antibody validation through Immunohistochemistry and Western Blot in affected marine vertebrates and invertebrates of the Mediterranea Sea

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The Mediterranean region is a dynamic climate change hotspot where episodes of morbidity and mortality have been impacting populations of more than 30 species from different phyla along the French, Greek, Spanish and Italian coasts. The need of sensitive predictors markers of animal disease is needed at lower and higher trophic scale level. Tissue biomarkers can be as useful diagnostic and prognostic indicator of environmental quality and to describe the effect on its biological components. Immunohistochemistry is an important tool for scientific research for human and veterinary medicine, used as complementary technique to explain etiopathogenetic features in course of disease outcome not determinable by conventional haematoxylin and eosin. In the last couple of decades, there has been an exponential increase in publications on immunohistochemistry in marine animals, in particular in teleost fish, while few antibodies have been used and validated in invertebrates belonging to different phyla involved in episode of morbidity and mortality. A limited availability of specific antibodies targeting proteins of interest are present for invertebrates because of their sequence difference compared to vertebrates. In this study we tested through both Western Blot and immunohistochemistry (IHC) different highly conserved antibodies involved in stress response heat shock (Hsp70), genotoxic stress (p53), inflammation (TNF-alpha) and apoptotic cell death (Caspases). We performed our study on four different species: two endangered species, such as the penshell *Pinna nobilis* and the purple sea urchin *Paracentrotus lividus*, and two species usually used as sentinel organisms of environmental state, such as the Mediterranean mussel *Mytilus galloprovincialis* and the red mullet *Mullus barbatus,* the latter selected as

sentinel species in the National Environmental Monitoring Programme (2021-26) of the Mediterranean Sea. Other markers must be tested in the future to provide a broader understanding of the biology and health status of fish and marine invertebrates.

Factors influencing the distribution of antibiotic-resistant genes in honey bee beneficial bacterial symbionts

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The spread of antibiotic resistance genes (ARGs) in environmental microorganisms is an issue of public concern worldwide. Honey bees (*Apis mellifera*) have long been considered bioindicators of environmental pollution and, more recently, also of antimicrobial resistance in the environment. These pollinators live in close association with bacterial species with a beneficial role towards colony health, which indicate the presence of ARG. Nevertheless, the knowledge of the factors contributing to the spread of ARGs is limited, and few studies have been performed worldwide. In this study, fifty-three bacterial strains belonging to the Firmicutes, Proteobacteria and Actinobacteria phyla isolated from the surface of honey bees at different growth stages, collected from 10 different apiaries in Italy in a previous study, were tested for the presence of genes encoding for resistance to some of the most widely used antibiotics in veterinary and human medicine: tetracycline, streptomycin, quinolone, vancomycin, carbapenemase, sulfonamides colistin. This study aimed to test whether the presence of ARGs was influenced by geographic location, bacterial species and/or the growth stage of the bees. The presence of ARGs in bacterial symbionts indicates that they can act as collectors and disseminators of these genes in the environment visited by honey bees.

Tigriopus fulvus as a bioassays target species: deepening of the acute toxicity response

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Tigriopus fulvus (Fisher, 1860) is a well-distributed copepod species in the shallow supratidal rock pools of the Mediterranean Sea, used in marine ecotoxicological studies because of its culturing easiness, sensitivity and adaptability to laboratory conditions. The species has a characteristic propriety: its released moults persist for a long time.
The study examines the release of moults natural variability in artificial or natural sea water, so that a toxicity threshold could be estimated for the 96h exposure. Results showed a natural oscillation up to 10% of the observed moults release, and this percentage can be used as an effect threshold for such endpoint. Acute toxicity bioassays performed with field samples showed a moults release oscillation up to 60%. This effect was not usually followed by mortality; therefore it can be considered as a valid sub-lethal endpoint particularly useful in low toxicity-highly diluted environmental samples, when mortality cannot be used as an endpoint even with nauplii, known to be more sensitive than adults. Such sub-lethal endpoint allows the extension of 96h acute toxicity bioassays application.

Evaluations on the feasibility of bioremediation applied to securing of natural occurring asbestos (NOA)

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The amiantiferous rocks in Italy are widespread in the western Alpine arc, along the Apennines and limited to the Etna massif (Biancavilla's fluoro-edenite). The environmental risk of NOA can be determined both by erosive and gravitational phenomena in natural outcrops, and attributable to human activities (extractive areas, stone dumps). Various scientific studies have shown that

the main agents responsible for the toxicity of asbestos fibers are attributable to iron and other metals. Other works attest that bacteria, lichens and the production of natural acids of plant species, are able to remove large quantities of heavy elements by reducing the dispersion of fibres. For securing of NOA's, fitoremediation and micoremediation could be combined, with the same scheme of intervention often adopted in sites polluted by heavy metals and organic substances. First of all, plant species capable of establishing mutualistic interactions with fungal species (mycorrhizae) on the ground should be chosen, to favor the activity of the latter in the inertization process of the asbestos fibers. Then employ fast-growing tree species (poplars), herbaceous plants or shrubs, capable of forming mycorrhizae of fungal species suitable for speeding up and favoring their interaction in amiantiferous soils. To test the potential of the consortia in transforming asbestos into less toxic forms, pilot trials in the mesocosm should be carried out, consisting in the identification and development of fungal vegetable consortia in asbestiferous soils in pots, followed by in situ tests. In areas characterized by significant gravitational phenomena, the planting of plant species would also make it possible to mitigate situations of hydrogeological instability in the presence of "green stones", as achieved in the Balangero mine. Other relevant technical aspects to consider are: the characterization of the asbestiferous species, the amphiboles have different capacities for alteration and consequent removal of metals compared to chrysotile [5], the conduct of analytical campaigns aimed at verifying the effectiveness of the treatments, assisted by periodic environmental. It should be highlighted that these technologies, if applied, would present a high degree of sustainability due to minimal environmental impacts and above all free from the production and handling of waste.

Plant-assisted bioremediation for recovering a multi-contaminated soil by using *Brassica napus*

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The Plant-Assisted Bioremediation (PABR) for the recovery of areas contaminated by organic and inorganic pollutants represents an effective and eco-sustainable technological strategy. In recent decades, the potential of some plant species to promote the processes of transformation and extraction of different types of contaminants from the soil has been investigated. The Brassicaceae family is one of the most studied in the application of PABR techniques. Brassica napus L. is a species particularly suitable for this purpose, thanks to its high tolerance to high concentrations of contaminants and the ability to phyto-extract heavy metals. In this study, the potential of *Brassica napus* in the plant-assisted bioremediation of a soil of Southern Italy, historically multi-contaminated by heavy metals and polychlorinated biphenyls (PCBs), was investigated. Furthermore, the use of two different organic soil amendments (compost and biochar) was tested to evaluate the effectiveness of biostimulation treatments in increasing the decontamination efficiency of PABR technology.
Specifically, microcosms of soil maintained in a greenhouse were set up by creating six experimental conditions: contaminated soil (A), contaminated soil and compost (B), contaminated soil and biochar (C) with and without plant; the preparations were carried out in triplicate for each investigation time (0, 80 days).
A multi-disciplinary analysis was performed using chemical, biochemical, microbiological and molecular methodologies on soil samples taken at different experimental times from microcosms set up in different experimental conditions.
The results observed after 80 days of experimentation highlighted: an increase in

microbial abundance and dehydrogenase activity in all microcosms set up with the plant species; a greater reduction in heavy metal concentrations in microcosms set up with organic soil improvers; a re-arrangement of PCB congener groups highlighting a decrease in dioxin-like congeners and an increase in marker and non-dioxin-like ones.
Overall, the joint use of Brassica Napus and organic soil amendments (compost and biochar) has revealed interesting potential for recovering the quality of historically multi-contaminated soil in southern Italy using PABR technology.

Ecological multidisciplinary studies for bioremediation of contaminated water and soils

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Anthropogenic activities have been continuously influencing natural ecosystems through the input of various types of legacy (e.g. pesticides, heavy metals and surfactants) and emerging contaminants (e.g. pharmaceuticals, micro-nanoplastics). It is very crucial to assess the environmental impact of a single chemical or a mixture with an ecological and holistic approach in order to plan a suitable recovery strategy.
Nature-based solutions (NBS) are alternative methodologies inspired and supported by nature (e.g. natural microorganisms, plants), in line with the environmental sustainability and One-Health concepts. Examples of bioremediation and phyto-assisted remediation as NBS for restoring soil and water from various contaminants derived from agricultural or industrial practices will be reported, showing microcosm studies. Microcosm experiments coupled with microbial, molecular and ecotoxicological analyses make it possible to evaluate how microorganisms can respond to various scenarios of contamination in different environmental matrices. More recently, electroactive bacteria have been also identified and tested in microbial fuel cells (MFCs) for their potential not only to produce electricity, but also to promote contaminant degradation.

Scientific and Organizing Committee of 2nd SETAC ILB Workshop: The Team

Ilaria Corsi – President of SETAC ILB

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). Expert in ecotoxicology of contaminants of emerging concern including nanomaterials and nanplastics. Co-chair of the Plastic 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. FULBRIGHT fellow at the Stanford Univ.in 2002-2003 and two times fellow of the King's College of Cambridge in 1994 and 2017. Co-chief

Silvia Casini – Past President of SETAC ILB Council

Past President of SETAC ILB (from May 2021 to May 2023), Member of the SETAC ILB Council Member since 1996, member of SETAC Wildlife Toxicology group. She is Associate Professor of Ecology at the Univ. of Siena. Expert in the study of the responses of organisms and natural populations to anthropogenic stressors, in particular contaminants; development and application of non-destructive ecotoxicological biomarkers in endangered species (particularly reptiles and birds); sustainable agriculture, evaluation of sub-lethal effects of contaminants in Apis mellifera and other insect pollinators. Member of the Union for Mediterranean (UfM) Environment Task Force. Member of the Steering Committee of Interreg Med and ENI CBC projects. Editorial board member of various peer-review Journals

Stefania Marcheggiani – Vice President of SETAC ILB

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.

Paola Grenni – External Advisor of SETAC ILB

Member of SETAC Europe since 2004 (Chair of different sessions in EU meetings), co-Chair of the SETAC EU Annual Meeting 2018 (Rome); from 2020, part of the 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 Consiglio Nazionale delle Ricerche, Istituto di Ricerca Sulle Acque (CNR-IRSA) 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.

Claudia Vaj – External Advisor of SETAC ILB

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.

Federica Cacciatore – Member of SETAC ILB Council

Council member since 2016 and 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. Expert at WG Ecological Status (ECOSTAT) CIS WFD 2000/60/CE; and at WG SO VI/09-06 Eutrophication – classification criteria. Participation to 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.

Ginevra Moltedo – Member of SETAC ILB Council

Council member of SETAC ILB since 2018 (Chair of session of the last meeting in Rome). Researcher at ISPRA, Rome. Expert in ecotoxicology, mainly in biomarker analyses, and in marine environmental monitoring. Member of the Supporting Group to the Italian Ministry of the Environment (MASE) within the EcAp activities and expert on the effects of contaminants within the technical group CorMon (Ecosystem Approach Correspondence Group on Pollution Monitoring) for "Contaminants" of UNEP-MAP/MEDPOL Programme. Coordinator of the ISPRA WG on Monitoring of the Effects of Contaminants in biota within the Marine Strategy Framework Directive, and of the Italian National WG for the Technical Manual for "Referencing methodologies in biomarker analyses on marine organisms".

Camilla Puccinelli – Member of SETAC ILB Council

Council member of SETAC ILB from 2022. She is a Researcher at Environment and Health Dept. of Istituto Superiore di Sanità. She is a diatom taxonomy expert, including teratological forms due to environmental stressors, in aquatic ecosystems. She has proven year experience on: environmental water quality assessment based on biological communities; application of GIS system in the study of pollutant sources, in the assessment of the non-point pollution impacts on aquatic ecosystems; quality assurance and accreditation of ecological and ecotoxicological tests including sampling phase; scientific and technical support to the Italian Ministry of Environment and Energy Security.

Massimiliano Scalici – Member of SETAC ILB Council

Council member of SETAC ILB since 2021. He is a hydrobiologist expert on ecology of inland and sea waters, working in the Dept. of Sciences of Univ. Roma Tre as Associate Professor in Ecology. Major skills in: permanent and temporary waters; monitoring and assessment of contamination in biota, water and sediments; environmental quality assessment; morphological biomarkers; multilevel approach; statistical data analyses; scientific and technical support to public and private institutions.

Silvia Franzellitti – Member of SETAC ILB Council

Council member since 2022 and 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.

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