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Experimental Biology meets...
Stakeholders on Plastics and Microplastics and Nanoplastics topic

I[st] PLASTAMINATION CONFERENCE

PRIN 2022 PNRR

CUP: D53D23021910001

Salerno, Italy 10-11 October 2024

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ABSTRACT BOOK

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Palazzo della Provincia di Salerno
Università degli Studi di Salerno

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TABLE OF CONTENTS

SESSION I
MARINE PLASTIC POLLUTION: BIOMONITORING AND ECOPHYSIOLOGY

PLENARY LECTURE

FROM LARGE TO SMALL, FROM THE LABORATORY TO THE SEA: UNDERSTANDING THE IMPACT OF NANOPLASTICS IN THE REAL WORLD

Antonio TERLIZZI[1,2,3]

[1]Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Napoli; [2]Department of Life Sciences, University of Trieste; [3]National Biodiversity Future Center, Palermo, Italy

Plastic pollution has become one of the most pressing environmental challenges especially the smaller fraction known as microplastics (1 μm- 5mm) and nanoplastics (<1 μm) (1). Nowadays, there is mounting concern that nanoplastics, due to their tiny size, might be more widespread and potentially more harmful than larger plastic particles (2). As matter of fact, from an ecotoxicological point of view, nanoplastics, thanks to the increased surface/volume ratio show greater chemical, physical and biological reactivity, thus imparting unique properties on a material, which does not happen at other sizes (3). This talk will cover the current knowledge on the real impact of nanoplastics, from release and production to ecotoxicity at various levels of biological organization, with a special focus on the Mediterranean Sea.

In controlled laboratory conditions, the effects of nanoplastics on marine organisms have been studied extensively, revealing toxicological concerns, such as disrupted physiological processes, bioaccumulation, and potential harm to reproduction and growth.

However, to date, translating these laboratory findings into the broader, complex marine environment is challenging due to the lack of proper detection technologies. In the marine environment, nanoplastics interact with different factors like temperature, salinity, and organic matter, potentially altering their behaviour and toxicity. Moreover, nanoplastics can act as vectors for persistent organic compounds, posing an even greater threat to marine life and, by extension, human health through the food chain.

Future efforts are going to be focused on developing new technologies and standardized methods for nanoplastic detection in the environment and studying their interactions with natural systems to devise the effective mitigation strategies.

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CONFERENCE SPEAKERS

AN OVERVIEW OF MICRO- AND NANOPLASTICS FROM SEA TO SPOON: ANY PUBLIC HEALTH IMPLICATIONS?

Michela SALAMONE, Federica CARRATURO, Luisa ALBARANO, Marco GUIDA, Giovanni LIBRALATO

Department of Biology, University of Naples Federico II, Napoli, Italy

Plastic is ubiquitous in our life, and its durability and end of life represents a great problem for public health and the environment. Several studies reported the occurrence of plastic litter in different environmental compartments and, consequently, numerous efforts are currently focused on how improving its recycling process and produce environmentally friendly solutions. The BIOPLAST4SAFE project, funded by the Italian Ministry of Health – PNC, focuses on potential adverse effects of micro- and nanoplastics (MNPs) in a one health perspective. Plastic particles can enter in living organisms, including humans, primarily through ingestion and inhalation. Their biological effects depend on their size, chemical composition, and the characteristics of substances that later absorb these particles. Given the increasing production and use of plastics worldwide and that most MNPs in nature derive from the breakdown of larger sized plastic materials, sometimes several years after their disposal, it is expected that even if plastic production decreases or stops, an increase in their presence in nature will be observed for years and decades, increasing the potential for human exposure. Their effects and consequences of MNPs are not yet fully understood, even though they have been already detected in feces, colon, placenta, scalp hair, hand skin, facial skin, urine, seminal fluid and saliva. An estimate of the ingestion rate is not yet available as well as the identification of the exact route of entrance. Seas and oceans are not only the final sink of MNPs, but also a source of food that can vehiculate MNPs to humans thus increasing public health concerns. An overview of the main potential hot spot of MNPs exposure will be investigated.

This work was realised in the framework of the Project - Biomonitoraggio di micro e nanoplastiche biodegradabili: dall'ambiente all'uomo in una prospettiva one health (BioPlast4Safe) - with the technical and economic support of the Italian Ministry of Health – PNC.

PLASTICS AT SEA: BIO-ECOLOGICAL IMPACTS AND POSSIBLE MITIGATION

Roberto SANDULLI

DiST, Università Parthenope Napoli, Centro Direzionale Is. C4, Napoli, Italy

More than 11 million metric tons of plastics enter our ocean every year, over than the estimated 200 million metric tons that currently circulate in our marine environments. Whether by errant plastic bags or plastic straws winding their way into gutters or large amounts of mismanaged plastic waste streaming from rapidly growing economies, which is like dumping one garbage truck full of plastic into the ocean every minute of every day for an entire year. And that much plastic is bound to have an enormous impact on ocean ecosystems. Plastic touches all of our lives, from the food packaging we

buy to the computers we work with and the cars we drive. But many of the plastics you touch in your daily life are used only once and thrown away. So much of this plastic is ending up in the ocean that in just a few years, we might end up with a pound of plastic for every three pounds of fish in the sea. But the future of plastics in our ocean will be determined by the way we handle plastics on land.
From the tiniest phyto- and zooplankton to the largest whales, plastics is known to impact nearly 700 species in our ocean You've probably seen videos of these impacts first hand, like a sea turtle with a plastic straw embedded in its nose or a whale entangled in a fishing net, approaching divers that release it from harm. Some of these incidents have happy endings, but in reality, many more do not.
Plastic has been found in more than 60% of all seabirds and in 100% of sea turtles species, that mistake plastic for food. And when animals ingest plastic, it can cause life-threatening problems, including reduced fitness, nutrient uptake and feeding efficiency—all vital for survival.
However, some possible idea to mitigate the effects of plastics at sea, might be suggested.

MICROPLASTIC POLLUTION: IDENTIFICATION AND MUTIGATION STRATEGIES

Mariacristina COCCA

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Microplastics, MPs, are ubiquitous pollutants worldwide distributed, derived from different sources. MPs can be produced in sub-millimetric size and used for a specific application, i.e primary microplastics, or released during degradation of large plastic items, i.e secondary microplastics. Due to MP different sources, different shapes and complex chemico-physical characteristics, the identification approaches and the mitigation strategies should be developed *ad hoc*. In this scenario, the set-up of new protocols to identify MPs in environmental matrices represent a growing research field. Promising results were obtained in determining MP pollution in complex matrices such as water, air and soil as well as in biological tissues by combining morphological, thermal and spectroscopic techniques. The results allowed to correlate particle types, sources, occurrence, transport and fate, and their interactions with organisms in different compartments. Due to the different MPs, mitigation strategies were optimized based on the type of MP, the entry point and the complexity of the environmental compartment under investigation. These strategies were aimed at preventing the formation of secondary and primary microplastics through thermomechanical recycling approaches of plastics recovered from the marine environment (1), through the evaluation of the applicability of biodegradable polymers as a replacement for traditional polymers (2) and through the application of degradation techniques for the removal of microplastics from the marine environment (3).

This work was partially conducted in the framework of the Project – Biomonitoraggio di micro e nanoplastiche biodegradabili: dall'ambiente all'uomo in una prospettiva one health (BioPlast4Safe) - with the technical and economic support of the Italian Ministry of Health – PNC.

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(MICRO)PLASTIC POLLUTION: WHAT MARINE ORGANISMS TELL US?

Loredana MANFRA[1,2], Luisa ALBARANO[3], Alice ROTINI[1], Francesca BIANDOLINO[4], Ermelinda PRATO[4], Giovanni LIBRALATO[2,3]

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According to the EU plastic strategy aimed to reduce the environmental impact of the increasing plastic production, the conventional synthetic polymers, of petrochemical origin, should be replaced with recycled and biodegradable polymers and bioplastics. However, the knowledge regarding their short and long-term effects for the environment and human health is still scarce.
Biodegradable plastic - polylactic acid (PLA) - and non-biodegradable plastic - polypropylene (PP), in seven nominal concentrations (1, 5, 10, 12.5, 25, 50, and 100 mg/L) and their leachates (undiluted) were used for testing microplastic (MP) effects through a battery of seven species belonging to different taxonomic and trophic levels and including different life stages and feeding strategies: bacteria (Vibrio fischeri), algae (Phaedactylum tricornutum), rotifers (Brachionus plicatilis), copepoda (Tigriopus fulvus), amphipoda (Corophium insidiorium and Gammarus aequicauda) and brachiopods (Artemia franciscana).
Results highlighted toxic effects of both MPs for three consumers (sensitivity scale: C. insidiosum<G. aequicauda<A. franciscana), but no toxicity for decomposers and primary producers. Instead, leachates did not induce negative effects for five species tested in this study. A dose-dependent toxic effect of PP and PLA on different developmental stages of A. franciscana was observed. The mortality rates indicated that early life stages (*e.g.*, metanauplii) and sub-adults (*e.g.*, juveniles) are more sensitive to the tested MPs. In addition, the molecular analysis showed the increasing of the mRNA levels of Heat shock proteins in A. franciscana (metanauplii and adults), suggesting their role in oxidative stress response, and decreasing in juveniles, indicating potential irreversible damage.
These results indicated that PLA and PP may have comparable ecotoxicological impacts and raise concerns about the effectiveness of biodegradable polymers for plastic pollution mitigation and their potential risks to marine ecosystems and human health. Furthermore, results point out the importance to take into consideration different trophic/taxonomic levels, as

well as different life stages and feeding strategies to better evaluate toxic effects of MPs and potential implications, in a one health perspective.

This work was conducted in the framework of the Project – Biomonitoraggio di micro e nanoplastiche biodegradabili: dall'ambiente all'uomo in una prospettiva one health (BioPlast4Safe) - with the technical and economic support of the Italian Ministry of Health – PNC funds.

MOLECULAR EFFECTS OF MICROPLASTICS ON REPRODUCTIVE HEALTH OF *MYTILUS GALLOPROVINCIALIS*: FOCUS ON THE BINDING BETWEEN PROTAMINE-LIKE PROTEINS AND DNA

Marina PISCOPO[1], Luigi ROSATI[1], Anna DE MAIO[1], Anna Rita BIANCHI[1], Gennaro LETTIERI[1], Carmela MARINARO [1]

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Plastic pollution, particularly from microplastics, is a growing environmental issue that impacts marine and terrestrial ecosystems (1), (2). This study examines the toxicological and reproductive effects of polystyrene microplastics (PS-MPs) on the reproductive health of the marine mussel *Mytilus galloprovincialis*. Mussels were exposed to two doses of PS-MPs (0.5 and 1 µg/L) for 48 hours. The main focus was on the effects of exposure on protamine-like (PL), the major proteins component of the sperm chromatin, which play a critical role in DNA packaging.
Our study indicated that PS-MPs exposure led to significant changes in the SDS-PAGE patterns of PL proteins, particularly at the higher dose exposure. In fact, in the condition of exposure to 0.5 µg/L PS-MPs it was observed comigration of PL-III with PL-II and the presence of an additional protein band with reduced electrophoretic mobility respect to PL-II. Also, in the condition of exposure to 1 µg/L PS-MPs an additional band was detected but, in this case, with lower electrophoretic mobility than that obtained in the condition of exposure to 0.5 µg/L PS-MPs. These additional bands may correspond to PLs aggregates or post-translational modification of PLs or H1 histones. These modifications could affect the DNA-binding properties of PLs, in fact an increased DNA-binding capacity of PLs, especially in the condition of exposure to 1 µg/L PS-MP, was observed by Electrophoretic Mobility Shift Assays (EMSA). In addition, PLs from mussels exposed to PS-MPs showed an enhanced ability to protect DNA from oxidative damage induced by H_2O_2 and $CuCl_2$, with the protection being most pronounced at the highest PS-MPs dose. As a matter of fact, the augmented DNA binding ability and the increased DNA protection coincided with a reduced release of PL proteins from sperm nuclei in response to salt treatment, particularly at 1 µg/L PS-MPs, indicating potential issues during fertilization where DNA needs to be released for successful fusion with oocyte DNA.
Finally, we also registered an increase of poly(ADP)-ribosylation in PL-II proteins after exposure to 1 µg/L PS-MPs. This modification likely contributes to the altered PL DNA-binding observed and suggests a mechanism by which PS-MPs exposure could lead to unusual sperm chromatin structure.
Overall, although it may appear that exposure to the highest dose of PS-MPs induces a kind of gametic plasticity, the results suggest that such exposure could be detrimental to the reproductive health of *Mytilus galloprovincialis*, as it compromises the normal function of PLs and their release during fertilisation. This could have significant implications for the survival of the species and highlights the wider environmental risks posed by microplastic pollution.

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BIOPLASTICS ARE AN ECO-FRIENDLY ALTERNATIVE? EFFECT OF POLYLACTIC ACID ON CHRONIC EXPOSURE OF GAMMARUS AEQUICAUDA

Ermelinda PRATO[1], Isabella PARLAPIANO[1], Francesca BIANDOLINO[1]

Institute of Water Research (IRSA) CNR, Taranto, Italy

Plastic plays an important role in modern life and the modern economy due to its unique functional properties at low cost. For this reason, plastic production has increased twenty-fold in the last fifty years and is expected to double again in the next 20 years. Recently, there has been an increased awareness of environmental pollution by plastics, especially micro- and nanoplastics.
The marine environment is often the final destination of plastic waste. Annual emissions into the oceans are estimated to be between 4.8 and 12.7 million tonnes, with the prediction that without improvements in waste management infrastructure, plastic waste will increase significantly. So, it is inevitable that research will be intensified to solve plastic pollution in the marine environment.
Newly imposed environmental regulations, societal concerns, and growing environmental awareness have triggered the search for new environmentally friendly products. Polylactic acid (PLA) and polylactide plastics are the most important bioplastics on the market today. PLA is based on lactic acid and is mainly produced through the microbial fermentation process of starch obtained from corn, cassava, potatoes, sugar cane, and sugar beet.
Only recently the bioplastics are used, therefore it is necessary to know the potential impacts that could determine when plastic debris come in the marine environment.Therefore, the present paper aimed to investigate chronic sub-lethal effects of polylactic acid (PLA) microplastic (38-120 µm) on growth and reproductive traits (*e.g.* time of first brood, size of first brood, embryo development, mean number of newborns per female, cumulative number of newborns, etc.) of the crustacean amphipod *Gammarus aequicauda*. Amphipod of 400 µm head length were exposed to 5 and 10 mg/L PLA for 55 days. Growth of amphipods, expressed as head length and antennae segments number was negatively affected by PLA, however no significant differences between the two concentrations were observed. A negative impact on some reproductive traits was also observed with a high percentage of abort and reduced number of newborns. However, the pre-copula pairs appeared first in the amphipods exposed to PLA MPs at

both concentrations.The results evidenced the sensitivity of *G. aequicauda* in reproduction and growth to microplastics, highlighting the importance of using the chronic exposure to sublethal concentrations of PLA MPs.

This work was conducted in the framework of the Project – Biomonitoraggio di micro e nanoplastiche biodegradabili: dall'ambiente all'uomo in una prospettiva one health (BioPlast4Safe) - with the technical and economic support of the Italian Ministry of Health – PNC funds.

MONITORING OF MICROPLASTICS IN *POSIDONIA OCEANICA* MEADOWS AND BANQUETTES

Alice ROTINI[1], Laura CIARALLI[1,2], Michela SALAMONE[2], Giovanni LIBRALATO[2,3], Loredana MANFRA[1,3]

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Plastics are distributed in different compartments of the marine environment, from the seabed to the surface. The dynamics and effects of plastic pollution in seagrass are still under-explored, compared to other marine habitats. Considering the last decade (2012–2024), just recent efforts have been dedicated to this topic, with the first study published in 2017. Microplastics seem to affect seagrass plant growth, photosynthesis, nutrient cycling and habitat biodiversity, causing much concern. Out of the 26 studies available in literature only 4 regard the species *Posidonia oceanica* and investigated macro-/microplastics, only in the field (no laboratory experiments), mainly considered the plants alive instead of dead tissues. However, Posidonia beach wracks are potential accumulation hot spots for microplastics and their monitoring can contribute to the plastic pollution assessment in Mediterranean coastal areas. In this framework we investigated the occurrence of microplastics in two well-structured *banquettes* in Favignana Island (Sicily, Italy) and Akrotiri Bay (Cyprus). Preliminary results showed site-specific distribution patterns concerning microplastics frequency and type, identifying in *banquettes* a key point for transferring plastic litter from the underwater Posidonia meadows to the beach ecosystem. Any further investigations may allow to improve the protection measures of *P. oceanica* habitats against the current plastic pollution crisis.

MICROPLASTICS AND BENTHIC INVERTEBRATES INTERACTIONS IN MARINE ENVIRONMENTS

Paolo TOMASSETTI[1], Laura CIARALLI[1,2], Eleonora MONFARDINI[1], Danilo VANI[1], Marco MATIDDI[1]

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The plastic accumulation within the Mediterranean Sea basin is a particularly intense process, so that our sea is considered one of the most plastic-polluted areas worldwide, and exactly the sixth great accumulation zone of marine litter. The main factors driving this pollution are the enclosed structure of basin, the scarce outflow of surface waters, the high population density along coastlines, and the intensive fishing, tourism, shipping and industrial activities. In particular benthic environments are considered sinks for microplastics where they accumulate in considerable quantities, putting at risk the health of benthic communities. Microplastics due to their small size, are a threat to the aquatic biota because can be ingested by a wide range of marine organisms. Indeed, several studies demonstrated that many marine species, both vertebrates and invertebrates, to different trophic levels and with various feeding strategies ingest microplastics, such as marine birds, mammals, reptiles, fish and diverse invertebrates. Ingestion can take place directly, accidentally assimilating throughout filter-feeding or deposit-feeding or confusing for food, indirectly, by ingesting prey of lower trophic levels which contain microplastics. Once ingested, plastic can be excreted in the pseudofeces or accumulate in the body. This event can bring to different biological and ecological effect, as internal physical and chemical damage, trophic transfer and biomagnification, absorption to microplastics surfaces of other contaminants. Several studies have highlighted differences in microplastics ingestion between feeding models, in fact, some studies show a highest microplastic concentration in suspension feeders while others show a greater microplastic concentration in deposit-feeders or predator as compared to other feeding strategies. The taxonomic identity and the feeding mode influence the effects of microplastic contamination in marine animals. It indicates that different taxa can also achieve different particle accumulation rates. Some organisms may be more likely to ingest microplastics due to their food or habitat preferences, while others may reject them. Furthermore, the rate of microplastic ingestion can differ significantly between species. Organisms of different trophic levels can ingest different amounts of microplastics, which makes a direct comparison with the data obtained difficult. More microplastics were detected in organisms at higher trophic levels. Organisms that feed by filtering suspended particles, such as bivalves, may be more likely to ingest microplastics in water than carnivores or detritivores.

POSTERS

DISTRIBUTION AND COMPOSITION OF SEAFLOOR LITTER IN AN UNDER-EXPLORED AREA OF THE CENTRAL MEDITERRANEAN SEA

Giulia LUCIDO[1], Cristina PEDÀ[2], Roberto FIRMAMENTO[1], Francesco LONGO[2], Danilo MALARA[2], Serena SCOZZAFAVA[1], Pietro BATTAGLIA[2], Pierpaolo CONSOLI[2], Silvestro GRECO[1,3], Teresa ROMEO[2,4]

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In recent decades, marine litter (ML) defined as any persistent, manufactured or processed solid material discarded, disposed or abandoned into the sea and on the beaches has emerged as one of the most urgent global environmental issues, representing a growing threat to marine and coastal ecosystems (1). Most ML accumulates on the seafloor due to its non-floatability or as a result of biotic and/or abiotic degradation processes that promote sinking. Although the Mediterranean Sea is one of the most impacted regions due to the intensity of human activities, its semi-enclosed shape and the low water exchange with the open sea, some areas are still poorly explored for seafloor litter. In addition, studies often use different sampling and litter classification methodologies, making it difficult to compare collected data. In this regard, the present study aimed to investigate the distribution and composition of seafloor litter in an under-explored area in the Ionian Sea, off the Calabrian coast using a standardized methodology for ML classification. During experimental trawl surveys carried out in September 2023, litter was collected, measured, photographed, and classified following the Joint List of Litter Categories for Macrolitter Monitoring developed by the Technical Group of the Marine Strategy Framework Directive (MSFD) (2). The categories included in the list are artificial polymer materials, chemicals, cloth/textile, glass/ceramics, metals, organic food waste, paper/cardboard, processed/worked wood, and rubber. Additional subdivisions indicate the use of the litter and, regarding artificial polymer materials, the source of the litter, whether from fishing gear or single-use items. The analysis confirmed that the most prevalent type of litter is plastic, both in terms of number of items (84.8%) and weight (60.1%), in accordance with numerous studies conducted in other Mediterranean areas (3,4,5). Among artificial polymer material litter, the most common item was found to be plastic bags (32%), including both whole bags and fragments. Furthermore, single-use items (SUPs) accounted for 35.2% of the artificial polymer material litter, and items from fishing gear represented 4.8%. Additionally, it was found that most of the litter had an area of ≤ 100 cm². These findings underscore the critical need for continued monitoring and the implementation of effective waste management strategies, highlighting the importance of using standardized methodologies to ensure consistent and comparable data, with the aim of mitigating the impact of ML on the Mediterranean ecosystem and biodiversity.

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SESSION II
CONTAMINATION BY MICRO- AND NANOPLASTICS AND HEALTH

PLENARY LECTURE

HOW CHEMICALS IN PLASTICS ARE HARMING HUMAN HEALTH, AND WHAT WE CAN DO ABOUT IT

Laura VANDENBERG[1]

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Humans are exposed to hundreds of chemicals from our environments, and plastics are known to be a major source of exposures to many of these chemicals. Some, including bisphenols, are added to plastics to make them rigid and shatter-resistant. Others, like many phthalates, are added to make plastics more flexible. Still others, like PFAS and benzophenones, are added to plastics to covey certain properties like heat resistance, reduce degradation by UV light, or to make production of moulded plastic products easier. Many of the chemicals added to plastics for very specific reasons, including bisphenols, phthalates, PFAS, and benzophenones have received attention because of their properties as endocrine disrupting chemicals (EDCs). Our understanding of EDCs, has expanded significantly in the last twenty-five years, and scientists and the public health community now appreciate several things: 1) hormones are responsible for coordinating biological processes in the body, and have key roles from conception through aging; 2) many EDCs act as agonists or antagonists of endogenous hormones, but others have more complex effects on the endocrine system (*e.g.*, altering hormone synthesis or metabolism); 3) because hormones act at exceptionally low concentrations in the body, even low-level exposures to EDCs can disrupt physiological processes; 4) effects of altered hormone action during vulnerable periods of life (*e.g.*, gestation, puberty, pregnancy) can have lifelong impacts, as well as effects that manifest long after exposures cease. There is now strong evidence that EDCs, including many found in plastics, are contributing to a wide range of human diseases and conditions. Data from both controlled animal studies and human epidemiology studies links EDCs to cancers, neurological conditions, metabolic disease, infertility, thyroid diseases, and immune disorders, among others. Although plastics are all around us, and projections of plastic production anticipate continued production (and thus increasing human exposures), there are actions that individuals, industries, and the broader society can take to reduce their exposures, and thus the harms associated with EDCs in these products. Ultimately, policy is needed to reduce the production of plastics that are contaminating our environment and human bodies.

CONFERENCE SPEAKERS

COMBINED EFFECT OF POLYSTYRENE MICROPLASTICS AND CADMIUM ON RAT TESTICULAR ACTIVITY WITH A SPECIAL FOCUS ON BLOOD-TESTIS BARRIER INTEGRITY, CYTOSKELETAL DYNAMICS, AND SPERM QUALITY

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It is known that microplastics (MP) affect mammalian testicular physiology by inducing inflammation, oxidative stress, impairment of the seminiferous epithelium (SE) cytoarchitecture, and blood-testis barrier (BTB) integrity, ultimately, leading to abnormal differentiation of mature gametes. MP inevitably coexist with other chemicals and, due to their high surface area/volume ratio and hydrophobicity, can adsorb and transport them, thanks the "Trojan horse" effect. This study, other than confirming data obtained by previous apers, expands the knowledge on the combined impact of MP and cadmium (Cd), at cellular and molecular level, on the progression of the differentiative events of germ cells (GC) into mature spermatozoa (SPZ). Forty-two-months-old male Wistar rats were divided into five groups (n = 8 each) and treated for 30 days as follows: (1) control; (2) 0.1 mg fluorescent MP by oral gavage; (3) 0.1 mg MP by oral gavage; (4) 50 mg CdCl2/L in drinking water; (5) Cd+MP (50 mg CdCl2/L + 0.1 mg MPs). At the end of treatment, the animals were sacrificed and, for each rat, left testis was immersed in formol 10% buffer for histological studies (immunofluorescence, TUNEL), while the right was kept at − 80 °C for biomolecular studies (western blot and evaluation of oxidative stress status). Also, epididymal SPZ were collected for the evaluation of conventional parameters (concentration, viability, morphology, and motility) as well as of DNA and acrosome integrity.
Treatment with both substances caused impairment of testicular activity, evidenced by imbalance of antioxidant enzymes' activity, altered steroidogenesis, increased number of apoptotic cells and reduced SPZ parameters. Moreover, the compromission of the BTB physiology was revealed by the downregulated protein levels of occludin, VANGL2, and CX43 and activation status of regulative kinases Src and FAK. Of note, for the first time, MP are reported to activate the autophagy pathway in GC, to reduce damaged organelles and molecules, probably to avoid apoptosis. Surprisingly, the results obtained with the simultaneous Cd+MP treatment showed more harmful effects than those produced by MPs alone but less severe than with Cd alone.
Considering that the two substances were administered separately (oral gavage for MP and drinking water for Cd), we hypothesized that MP, by exploiting a sort of "positive-Trojan horse effect", may reduce the bioavailability of Cd, explaining the minor consequences observed by concomitant treatment with Cd+MP as compared with Cd alone.

POLY(LACTIC ACID) PLASTICS CONTAMINATION (PLASTINATION): ORGAN INJURIES AND UNDERLINING MOLECULAR MECHANISMS – *IN VIVO* STUDY ON ZEBRAFISH MODEL

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One of the main concerns of plastic pollution is its degradation into microplastics (MPs) and nanoplastics (NPs). MPs -ending up in water bodies - may be ingested by aquatic organisms, determining several kinds of toxicity and posing high health risk also for humans, considering the food chain. To limit petroleum-based plastic pollution, biodegradable plastics, namely bioplastics, have been introduced with the positive connotation of "environmentally sustainable". However, in sub-optimal conditions, they undergo the degradation process, similarly to fossil-based plastics, producing MPs and NPs. Nowadays, very little is known about the exposure risk to biodegradable plastic debris such as the ones deriving from widely used polylactic acid (PLA). Therefore, the overall aim of the PLASTAMINATION project was to evaluate the biological effects of PLA NPs *in vitro* and *in vivo*. Since ingestion and contact are the main exposure routes, the *in vitro* studies were conducted on human dermal fibroblasts (HDF) in order to evaluate the PLA-NPs uptake potential (UNISA), while *in vivo* studies were conducted on zebrafish (*Danio rerio*) (UNIPA). In particular, zebrafish embryos exposed to PLA NPs were used to investigate their acute toxicity on organ development. In order to resemble more real exposure conditions, PLA NPs suspensions are also produced for biological *in vitro* and *in vivo* studies, ensuring all researchers use NPs with similar physical characteristics (UNISA). An antioxidant aquatic plant, *Lemna minor* frequently used in ecotoxicological tests will be also used in the zebrafish culture water system to assess its potential to act as an anti-toxic agent (UNICAMPANIA). The results obtained until now, demonstrate that PLA-NPs internalize within HDF and zebrafish in a size- and concentration-dependent manner. Curiously, PLA-NPs were demonstrated to induce heartbeat deceleration in zebrafish at 120hpf, suggesting cardiotoxicity. Moreover, stress-related markers gene expression was altered in zebrafish exposed to PLA-NPs, suggesting oxidative stress and inflammation induction. Since toxic effects of PLA-NPs have been observed in zebrafish embryos, juvenile zebrafish exposed to PLA NPs will be soon used to better investigate their chronic toxicity. To this scope, molecular mechanisms will be investigated with a focus on gut and reproductive organs (UNINA), brain (UNISA), heart and liver biomarkers (UNIPA).

The experiments were conducted as part of the PRIN project "Plastic Contamination by Poly(Lactic Acid) (PLASTAMINATION): organ lesions and underlying molecular mechanisms", MUR, PRIN-PNRR2022 CODE NUMBER: P2022AA47Y-CUP: B53D2303206000.

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FROM PLASTIC TO BODY: THE IMPACT OF BPA ON THE ENDOCRIN SYSTEM AND BEYOND

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Bisphenol A (BPA), widely utilized in the manufacturing of polycarbonate plastics and epoxy resins, is a recognized endocrine disruptor with well-documented adverse effects on human health. A growing body of evidence reveals severe implications especially in reproductive health and developmental processes. Here we analyze findings from various research studies investigating the biological impact of BPA, particularly focusing on placental function, fetal development, neurodegeneration, and hormone-related carcinogenesis.

Animal studies on rats reveal that low-dose BPA exposure during pregnancy disrupts placental vascularization, particularly reducing micro-vessel density, which impairs fetal growth and may lead to intrauterine growth restriction (IUGR). Unlike apoptosis-driven cell death, these effects seem to result from disrupted angiogenesis, as apoptosis markers like caspase-9 remain largely unchanged. Histological analyses further show abnormalities in the placenta, such as enlarged and irregular maternal blood spaces, which may impair nutrient exchange between the mother and fetus.

Human studies corroborate these findings, linking higher placental BPA levels to reduced birth weights, indicating that BPA crosses the placental barrier and interferes with fetal development by altering nutrient and hormone exchange.

Beyond developmental concerns, BPA has been associated with neurodegenerative diseases like Parkinson's, primarily due to its impact on dopaminergic neurons. Nevertheless, as observed in several studies, the variability in individual BPA metabolization plays a crucial role in modulating these effects, with slow metabolizers exhibiting higher active BPA levels and an increased susceptibility to neurotoxic effects, explaining some inconsistencies in the literature regarding BPA's role in neurodegeneration.

Furthermore, other human studies suggest a link between BPA exposure and hormone-dependent cancers, such as endometrial carcinoma. A case-control study demonstrated significantly higher concentrations of BPA in the blood and urine of women with endometrial cancer compared to controls with benign uterine conditions. These findings suggest that BPA's estrogen-mimicking properties may contribute to the pathogenesis of hormone-dependent cancers by promoting abnormal cell proliferation.BPA's broader effects extend to altering gene expression related to glucose metabolism, angiogenesis, and inflammation. These findings highlight the systemic risks of chronic BPA exposure, emphasizing the need for stricter regulatory measures for this chemical as well as for its analogues. Moreover, additional research is needed to investigate the long-term health consequences, particularly concerning reproductive health, neurodegeneration, and developmental outcomes.

SWIMMING IN PLASTAMINATION: THE ROLE OF NANOPLASTICS ON SPERM FUNCTION

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Plastic materials, introduced in the early 20th century, have progressively and significantly integrated into daily life due to their cost-effectiveness, lightweight nature, and durability. The limited degradability of many of these materials has led to a significant contamination problem in environments. Over time, these plastics tend to fragment into microplastics (less than 5 mm) or nanoplastics (from 1 to 1000 nm) (1). The severe environmental plastic pollution (PLASTAMINATION), driven by both massive use and considerable durability and poor degradation, has prompted the development of new materials that, while possessing similar physical properties, are more easily degradable in the environment. Among these, polylactic acid (PLA) has gained considerable use in recent years. However, the effects of this nanoplastic on biological systems remain unknown (2). To investigate whether PLA affects sperm function, seminal material from 10 bulls was collected, diluted, and incubated with increasing concentrations of PLA (0, 50, 100, 200, and 300 μg/ml). The impact of various PLA concentrations on sperm kinetics was evaluated using a computerized semen analyzer. Additionally, flow cytometry was used to determine if the nanoplastic could increase cytoplasmic membrane permeability (using Yo-Pro1), alter membrane organization (using Merocianin 540), and whether PLA affects mitochondrial function by assessing the bioenergetic state (using Mitotracker Deep Red). Analyses of cellular function and structure were conducted immediately after exposure to different PLA concentrations and after co-incubation periods of 60 and 120 minutes. Statistical analysis revealed that bovine spermatozoa exhibit considerable resilience to PLA contamination: no differences in sperm kinetics were observed at any of the tested concentrations. The minimal effect on sperm kinetics was also confirmed by the cellular bioenergetics results, suggesting that PLA, at the tested sizes, does not penetrate the cytoplasmic membrane or does not interact with mitochondria. Similarly, there was no significant increase in early membrane permeability or membrane disorganization, indicating that PLA is inert with respect to the cytoplasmic membrane. Moreover, the data suggest that, under the concentrations and conditions used in the study, which are compatible with sperm manipulation in cattle, PLA does not negatively impact the environment where the spermatozoa are located. A previous study has demonstrated that PLA degrades much more rapidly in liquid media compared to other environmental conditions. However, the durations (2 hours) and temperatures tested in this study (37°C) appear insufficient to induce the rapid hydrolysis reported during incubation in liquid at 65°C (3). Therefore, under physiological and sperm processing conditions, PLA appears to induce negligible effects on ejaculated bovine spermatozoa.

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POSTERS

INVESTIGATING MICROPLASTIC-INDUCED BIOLOGICAL EFFECTS THROUGH METABOLOMICS: EXPLORING METHODOLOGIES AND THEIR POTENTIAL

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Over the past century, the exponential rise in global plastic production has led to a dramatic increase in the release of micro- and nanoplastics into the environment. These particles pose a growing environmental and biological threat due to their persistence in ecosystems, where they gradually accumulate in living organisms and biomagnify through food chains, increasing the potential for harmful effects on human health. Their presence has been documented in a variety of food products, including seafood, sea salt, and drinking water, just to cite a few. A growing body of evidence links microplastics to harmful effects on the human organism, ranging from chronic inflammation to tissue damage. However, their precise impact on cellular and tissue metabolism remains largely unclear. Therefore, to gain a deeper understanding of the mechanisms underlying these effects it is essential to investigate how these particles are metabolized and which metabolic pathways they disrupt. Metabolomics is a high-throughput technology that comprehensively studies the entire set of metabolites, or the metabolome, within a biological system. This technology captures dynamic changes in metabolic profiles, providing a holistic view of metabolism and insights into how organisms respond to environmental changes, diseases, and other perturbations. In the context of microplastics exposure, metabolomics could offer significant contributions to both preclinical and clinical studies, holding the potential to allow the development and the testing of novel mechanistic hypotheses. Specifically, three complementary approaches can be employed to yield different and complementary results: - Untargeted metabolomics: an exploratory analysis of metabolic profiles aimed at identifying pathways altered by the uptake of exogenous particles. - Targeted Metabolomics: focused on the identification and quantification of key metabolites, for instance those involved in oxidative stress processes and energy metabolism.- Fluxomics: a dynamic and advanced approach that enables the tracking and monitoring of exogenous particles by applying techniques such as isotope labeling and high-throughput analytical methods. Thus, fluxomics provides a detailed understanding of how external molecules are processed and transformed within cellular metabolism. These methodologies are highly versatile and can be applied to a wide range of biological samples, including cell cultures, tissues, and biofluids, offering the potential to advance our understanding of microplastic-related health effects and develop strategies to mitigate their impact.

MICROPLASTIC TOXICITY IN PLANTS: A PENDING ALARM

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Plastics are lightweight, durable, and convenient materials that are widely used around the world. The production and consumption of plastics has reached 300 million metric tons per year, and it is predicted that nearly 10% of plastic chips will eventually accumulate in the water environment after weathering. It is common for multiple pollutants to coexist in polluted water, and such coexistence can lead to changes in the migration of pollutants and their toxicity to plants. Several research articles highlighted the impact of microplastics/nanoplastics (M/N-Ps) on the aquatic food chain; however, very little has been done in the terrestrial ecosystem.

Apart from aquatic ecosystems that are directly and daily exposed to plastic debris with different outcomes on the health of aquatic microorganisms, the exposure risk for terrestrial plants and animals' species also appeared. The toxic impact of M/N-Ps in plants and the food chain has recently become a top priority. Studies of uptake of microplastics are few, not to mention phytotoxicity of microplastics. It has been shown that both particles smaller than 50 nanometers and those larger than about 40 times can penetrate plant roots. Increasing evidence has proved that M/N-Ps can alter soil properties, plant performance, soil biota, and soil processes and functions.

M/N-Ps have been found to decrease soil water holding ability, disrupt soil nutrient cycling, increase the emission of greenhouse gases, and cause the loss of soil fertility and health, which may pose risks to soil functioning and the sustainability of agroecosystems. Microplastics accumulate on pores in seed capsule and delay germination and root growth of the terrestrial vascular plant.

New research has studied lettuce and wheat crops and found that microplastics can penetrate the roots of the plants and pass into the edible parts of the crop. Furthermore, they can change plant growth and physiological metabolism via direct and indirect pathways. In Cucurbita plants cultivated in pots with increasing concentrations of different microplastics, all the pollutants impaired root and, especially, shoot growth. However, the impacts of M/N-Ps on soil-plant systems vary dependent on their characteristics, plant species, exposure duration, and soil conditions.

Plant leaves are one of the main targets of air pollution and their large and irregular surfaces play a key role in intercepting a large portion of particles suspended in the air and, among these, microplastics.

Understanding what the effects of these pollutants on plant leaves important implications for an assessment of the risk have that these materials can bring to the safety of our ecosystem.

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THE CHRONIC EXPOSURE TO THE PLASTICIZER BISPHENOL A (BPA) AFFECTS THE SERINE PROTEASE PREP DURING THE FIRST ROUND OF SPERMATOGENESIS IN RATS

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Introduction. Spermatogenesis is controlled by endocrine, paracrine, and autocrine communications along the hypothalamus-pituitary-testis (HPG) axis and is highly sensitive to environmental pollutants, including *plast*ic cont*amination* (PLASTAMINATION). Bisphenol A (BPA), an estrogen-mimic endocrine disrupting chemical, is an environmental contaminant commonly used to manufacture polycarbonate plastics and epoxy resins. Since its toxic effects on male reproduction, here we investigated the effects of the chronic exposure to BPA on the prolyl endopeptidase (PREP), an upcoming markers in the regulation of spermatogenesis and spermiogenesis in mammals, during the first round of spermatogenesis.
Methods. Six pregnant female (200–250 g) Wistar rats were given BPA, or vehicle (n = 3/group) in the drinking water (0.1 mg/l BPA) to mimick the most common exposure route in humans. The vehicle consisted of 0.1 ml/l ethanol. Dams received the treatment all over lactation and at weaning each male newborn received the same treatment of the mother *via* drinking water. To evaluate possible effects of BPA on the first round of spermatogenesis, the male newborns were sacrificed at 17 PND (late infantile), 45 PND (pubertal), or 60 PND (young adult), randomly choosing a total of five animals/treatment/time point from different litters. Morphofunctional and molecular investigations were carried out to analyse the effects of BPA exposure on spermatogenesis progression, PREP expression and localization in rat testis and spermatozoa (SPZ).
Results and Discussion. During the first round of spermatogenesis, BPA treatment deregulated the structure of the blood testis barrier due to oxidative/nitrosative stress, particularly at 45 PND, with partial rescue at 60 PND. At molecular level, it significantly affected both the endocannabinoid and kisspeptin systems, two signaling systems respectively critical for puberty onset, spermatogenesis progression and spermiogenesis. In parallel, BPA induced a significant decrease in testicular PREP protein levels, at both 45 and 60 PND. The immunofluorescent analysis confirmed these results: indeed, in the control testis at 45 or 60 PND, PREP localized mainly in spermatocytes and Sertoli cells cytoplasmic protrusion, where a clear colocalization with tubulin was evident; in addition, a positive signal in the interstitial Leydig cells was also observed. Although in BPA-treated animals PREP retained the abovementioned localization, its signal intensity was significantly decreased. Finally, as expected, PREP localized in the tail and the acrosome region of epididymal spermatozoa, collected at 60 PND; however, in the BPA group, the signal intensity drastically decreased.
Taken together, these findings confirm that BPA impacts the post-natal testis development and suggest that PREP protein in testis and SPZ could be critical for the progression of spermatogenesis and the acquisition of SPZ competence for fertilization. Due to the reduced expression of PREP in both the testis and SPZ of BPA treated animals, PREP may represent a marker for assessing the outcomes of plastic contamination in male reproduction functions.

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INTEGRATED METABOLOMICS AND FLUXOMICS APPROACH TO STUDY THE EFFECTS OF PLA EXPOSURE ON *IN VITRO* MODELS: A STUDY DESIGN

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With the increasing global demand for sustainable materials, bioplastics such as polylactic acid (PLA) are emerging as promising alternatives to conventional plastics. PLA, derived from renewable resources like corn starch or sugarcane, stands out due to its biodegradability and reduced carbon footprint, making it a key player in the shift toward greener materials. However, as the use of PLA continues to grow, so do questions about its long-term impact on human health. Throughout their lifecycle, indeed, these materials can release micro- and nano-plastics, whose metabolic effects and fate within the human body are yet to be described. When internalized, they may represent additional sources of glucose and lactic acid, potentially leading to significant metabolic imbalances, especially in organs and tissues that primarily rely on carbohydrate and lipid metabolism, such as the liver, central nervous system, intestinal epithelium, and immune system.
This ongoing project aims to investigate the effects of PLA exposure on cellular metabolism, using an integrated approach that combines metabolomics and fluxomics. *In vitro* models of various cell lines, mimicking the major contamination routes for humans including intestinal cells (CaCo2 and HT29), CNS (C6 and PC12) and endothelial cells, will be exposed to PLA nanoplastics.
Untargeted metabolomic analyses will be initially performed using gas chromatography-mass spectrometry (GC-MS) to identify both intracellular and extracellular metabolites. These analyses will be conducted on the cellular component, to investigate the metabolic alterations induced by PLA exposure, as well as on the culture medium, to study possible changes in the secretome.
Subsequent analyses will focus on key metabolic pathways through a targeted metabolomics approach, providing detailed insights into specific metabolites and their roles in the metabolic processes under investigation. Finally, fluxomics, using labeled glucose analogs will allow for the evaluation of changes in metabolic fluxes and the metabolic fate of exogenous particles, offering a comprehensive understanding of the metabolic perturbations caused by PLA exposure. By integrating metabolomics and fluxomics, these results have the

potential to reveal critical metabolic pathways affected by PLA exposure, potentially paving the way for future research exploring the broader implications of PLA in human health. This could ultimately inform future research directions, contribute to the development of safety guidelines, and influence regulatory policies related to the use of PLA.

UNRAVELING THE INTERPLAY BETWEEN NANOPLASTICS AND IMMUNE SYSTEM: THE ROLE OF ECM IN MACROPHAGE RESPONSE

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The use of plastics is common all over the world. Their biodegradability is often limited, and for this reason, plastics can cause a significant level of environmental pollution. They are classified based on their size: the smallest ones are recognized as nanoplastics (NPs) - sized from 1 nm to 1000 nm - while microplastics include those with sizes from 1 μm to 5 mm. Nano- and microplastics can enter the human body through different ways: the respiratory system via inhalation; the digestive tract via consumption of contaminated food and water; penetration through the skin via cosmetics and clothes contact. Plastics can be accumulated in the human body and can potentially lead to a range of health issues, including respiratory disorders like lung cancer, asthma and hypersensitivity pneumonitis, neurological symptoms such as fatigue and inflammatory bowel disease.
In most *in vitro* studies, cells were exposed to NPs dispersed in culture medium. The results confirmed that nano- and microplastics can induce apoptosis in cells and have genotoxic and cytotoxic effects. In human tissues, NPs are not only diffusible in the biological fluids, but they may be entrapped/adsorbed by the ECM components. The ECM-NP interaction could change the ECM properties and the way the cells meet NPs. The controlled exposure of NP to cells, mediated by the ECM, could influence cell behavior and response to NPs. In the present work funded by the PRIN 2022 PNRR program, our main goal is to study the role played by the microenvironment on the macrophage response to nanoplastics embedded in the ECM. We exposed macrophage-like cells, derived from the human monocytic THP-1 cell line, to ECM-like substrates previously loaded with polystyrene NPs. We used ECM-like substrates that resemble the structural, chemical, and physical characteristics of the original ECM. The matrices are based on gelatin and hyaluronic acid (HA) and processed via the electrospinning technique. Preliminary experiments demonstrated that macrophages adhere to all substrates, in particular to matrices with gelatin and cell viability is not affected up to 48 hours after cell seeding. The presence of gelatin on matrices also influenced the adsorption of NPs: a higher percentage of NPs was adsorbed by substrates with gelatin compared to controls without gelatin. Macrophages can remodel the ECM-like substrates, making NPs bioavailable to be internalized, as indicated by fluorescence microscopy imaging.
This research highlights the need for further research to fully understand the cellular and molecular mechanisms of immune cells exposed to nanoplastics, which may help to understand the impact of plastics on human health.

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SESSION III PLASTICS AND MICROPLASTICS: MANAGEMENT ASPECTS AND ENVIRONMENTAL IMPACT

CONFERENCE SPEAKERS

GENERATION OF MICROPLASTICS FROM BIODEGRADABLE PLA-BASED FILMS IN AQUATIC ENVIRONMENTS

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The environmental pollution generated by the accumulation of synthetic plastic wastes in terrestrial and aquatic ecosystems has raised numerous concerns in the last years and has been recently the subject of numerous studies. In particular, plastics represent the largest and most persistent fraction of aquatic litter, entailing the production of large amounts if microplastics (MPs), with harmful consequences for living organisms and the human health.
Currently, the replacement of fossil-fuel-based plastics by biodegradable polymers, such as polylactic acid (PLA), polybutylene succinate (PBS), polyhydroxyalkanoates (PHA), poly (butylene adipate-co-terephthalate) (PBAT) etc. has been encouraged as a possible solution to reduce environmental issues. However, even if theoretically most biodegradable polymers decompose into environmentally acceptable products such as water, carbon dioxide, and biomass, this is only achievable under physiological conditions or controlled industrial composting conditions. When biodegradable polymers enter the natural environment, their behavior becomes similar to that of non-biodegradable materials: this entails long-term decomposition through photodegradation, biodegradation and hydrolysis mechanisms, and generation of large amounts of bio-microplastics (BioMPs, ≤ 5 mm) which can be persistent and remain for decades, posing a real ecological pollution risk.
In this scenario, this study investigates the formation of biomicroplastics and the changes in the physico-chemical properties of biodegradable packaging films based on polylactic acid (PLA) and its blends after degradation in aquatic environments. The tests were carried out in both natural sea water and fresh water, while the chosen environmental conditions of temperature and light allowed to simulate both degradation in warm water, under sunlight exposure, and in cold deep water. The tests were carried out up to 12 weeks. The pH changes in the aqueous environments were evaluated, while the morphology, size, wettability, crystallinity, and mechanical properties of the formed microplastics were studied, analyzing the changes induced by polymer blending in the degradation behavior with respect to the neat polymers.
The results highlighted that all the films underwent hydrolytic degradation, which led to a reorganization of the polymer crystalline phases, along with a strong embrittlement and an increase in hydrophilicity. The formation of Bio-MPs was observed already after 5 weeks exposure; however, the extent of degradation was found to be highly dependent on the nature of the biopolymer. Moreover, results pointed out that temperature and light exposure severely affect degradation rate of bioplastics, which can be highly persistent in the cold deep water behaving like non-degradable polymers.

FLUORESCENT COPOLYMERS FOR THE MICRO- AND NANOPLASTIC DETECTION

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In the last few decades, the indiscriminate disposal of plastic waste has strongly damaged and contaminated both the terrestrial and aquatic habitats. Although most plastics are recyclable, only a small amount of them is truly recycled, while the majority is diverted either to landfills or improperly littered in the environment. As a result, most of plastic wastes undergo slow degradation due to abiotic and biotic factors and are converted to micro- and nano-plastics (MPs and NPs) which then ends up in the environment causing tremendous damage. Both MPs and NPs are very detrimental to the aquatic ecosystem since they may enter the food chain via biomagnification and bioaccumulation within aquatic organisms (1). The agroecosystem is also highly contaminated by both MPs and NPs, which negatively affects plant growth and development (2). The effect of MPs and NPs on the biota can be evaluated by identifying their absorption on organic tissues of living species. For this purpose, methods based on fluorescent tagging have been proposed. These innovative strategies consist of incorporating into the plastic material a small concentration of a substance with specific luminescence properties that can be then recognized by UV fluorescence spectrometric analysis (3) (4). Herein, we present the synthesis and the microstructural, chemico-physical and optical characterization of new fluorescent carbazole-containing copolymers derived from common plastic materials such as polyethylene, polypropylene, polystyrene and polymethylmetacrylate. All synthesized fluorescent copolymers contain an amount of fluorescent units to make them optically active and, at the same time to ensure that their chemical and physical properties do not differ from those of their homologous homopolymers. Due to their peculiar features, they could behave as fluorescent markers and be used to mimic the behavior of their homologous homopolymers after their conversion to MPs and NPs. Preliminary data show that copolymer samples are easily identifiable within both animal and plant tissues through confocal microscopy, paving the way for the use of labeled copolymers to study the fate and effect of MP and NP pollutants on the biota.

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PLASTIVORE INSECTS: A NEW SOURCE OF TOOLS FOR MICROPLASTICS BIOREMEDIATION

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The world plastics production has surpassed 400 million metric tons in 2022, and their excessive consumption had raised concerns about their impact on the environment. Indeed, discarded plastic litter can break down into microplastics (MPs < 5 mm), which could be both easily consumed by living organisms and transferred along the food chain, and accumulate in the environment. Among the strategies to manage the recalcitrant MPs, biodegradation represents the most ecofriendly and affordable technique. Several bacterial strains capable to degrade plastic polymers has been isolated, but plastics microbial biodegradation has limitations because of the high molecular weight and hydrophobicity of MPs. Efforts to improve plastic biodegradation have revealed that insects gut environment can support a rapid plastic degradation. Insect have developed many strong defense strategies to survive in a hostile environment and could represent a source for new MPs bioremediation strategies. In general, after entering the insects, the xenobiotic starts to be degraded by several enzymes which catalyze oxidation, reduction, and chemical cleavage of toxins (phase I). Then, conjugation of the by-products with endogenous molecules to enhance their solubility (phase II) and, finally, the metabolites are secreted from the cell through transmembrane transporters (phase III). The first phase is catalyzed by enzymes such as P450s (CYPs) and carboxylesterases, the second by Glutathione S- transferase (GSTs) and the third by ABC transporters. It is also known that the adaptability of insects, including their resistance to xenobiotics, may be directly or indirectly influenced by their symbiotic microbes. Thus, degradation abilities could depend solely on microorganisms present in the insect's gut, or on the cooperation between enzymes secreted by gut microorganisms and those produced by the host. To date, plastic degradation has been demonstrated in several holometabolous insects such as *Tenebrio molitor* (yellow mealworms), *Tenebrio obscurus* (dark mealworms), *Zophobas atratus* (superworms), *Plodia interpunctella* (Indian meal moth), *Achroia grisella* (lesser wax moths), and *Galleria mellonella* (greater wax moths). Studies have shown that plastivore insects degrade plastics through the synergistic action of gut microorganisms and enzymes from their digestive tract and salivary glands.

POSTERS

CHARACTERIZATION OF TRANSCRIPTOMIC RESPONSE OF A *D. MELANOGASTER* RESISTANT STRAIN TO PENTACHLOROPHENOL

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Microplastics (1 µm~5 mm) have caught global attention due to the potential threat they represent for the environment. They are known for being resistant to degradation and water-repellent. Given their hydrophobic properties microplastics can carry organic pollutants, release them in the environment, aggravate their toxicity or enhance their bioaccumulation. Among organic pollutants that interacts with microplastics, chlorinated aromatic compounds, such as Pentachlorophenol (PCP), represent a problem for human and environmental health. Because of the presence of the five chlorine atoms on the aromatic ring, PCP degradation is slow and only few bacteria are known to be capable of its degradation. Under this perspective it is crucial to find alternative organisms and new active molecules for PCP detoxification. Insects could represent a promising source of tools for bioremediation because of the chemical pression they usually undergo and their genetic plasticity. The aim of this work is to find new enzymes for PCP degradation starting from a *Drosophila melanogaster* resistant strain. The resistant strain selected is able to live on a growth medium supplied with 2000 ppm of PCP. HPLC conducted on growth medium where resistant larvae fed reported a reduction of 38% in PCP content compared to control (growth medium without larvae). To identify genes involved in PCP degradation, we conducted an RNAseq analysis, comparing the resistant strain (rD) grown with and without PCP and the susceptible strain (sD) grown without PCP. These comparisons aimed at determining whether the resistance genes are constitutive or induced.

Differential expression analysis identified 206 genes significantly (Fold change ≥1.5; $p_{adj} \leq 0.05$) upregulated and 150 downregulated by the presence of PCP. Among the overexpressed genes, we found 10 cytochrome P450s (CYP450), 5 Glutathione S- transferase (GSTs) and 1 UDP- glycosyltransferase (UGTs). All these genes are known to be involved in detoxification process in insects. This trend is confirmed by the Gene ontology (GO) analysis, which reveals a significant enrichment in biological process ($p<0.001$) related to the response to toxic substances (GO:0009636), lipid metabolic process (GO:0006629) and transmembrane transport (GO:0055085). Transcriptome and GO analyses suggest a general response to PCP in the resistant strain. Next step of this works will be focused on selecting the most promising upregulated genes involved in PCP degradation, clone and express them in *Escherichia coli* to isolate the enzyme they code for. The characterization of these enzyme and the by- product they produce will be the start point for a new bioremediation strategy for Pentachlorophenol.

NHC-Zn COMPLEXES FOR THE SYNTHESIS AND CHEMICAL UPCYCLING OF SUSTAINABLE POLYESTERS AND POLYCARBONATES

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Over the past century, our lives have been revolutionized by using cheap, durable, and adaptable plastics, mainly manufactured from petroleum-derived sources.[1] On the other hand, problems related to the disposal of these materials, such as the uncontrolled release into the terrestrial and marine environment as microplastics, has given rise to great interest in the development of more sustainable alternative polymers.[2] Among them, aliphatic polyesters and polycarbonates have attracted significant attention as they are biodegradable, obtainable from renewable resources; moreover their diverse and tunable mechanical properties make them the election candidates as sustainable plastics.[3] Different synthetic methods can be exploited to obtain these categories of polymers: among them, the Ring-Opening Polymerization (ROP) of lactones and cyclic carbonates has emerged as one of the most efficient ways to produce aliphatic polyesters and polycarbonates, respectively, under mild conditions, with good control over molecular weight. To ensure that the sustainability criteria are respected throughout the entire life cycle of the polymer, the use of high performance, selective and biocompatible catalysts is required: a possible strategy involves the use of "biometals", which eliminates the difficulty of removing trace amounts of metal residues from the produced polymers. In this work, we report N-Heterocyclic carbene (NHC) ligands used as coordinative environments for a biocompatible, inexpensive and environmentally benign metal, such as zinc. The main advantages of using NHC ligands are related to their attractive characteristics, including easy synthesis, easy tunability of steric and electronic properties. After synthesis and characterization, the complexes have been tested as catalysts in the ROP of cyclic esters and cyclic carbonates, obtained from biorenewable feedstocks. These complexes showed good control of the polymerization processes, furnishing polymers with the desired molecular masses and chain end groups.[4-5] In terms of end-of-life treatments for plastic wastes, chemical recycling has attracted increasing interest. Currently, available options are the depolymerization, with reformation of the monomer unit, and the degradation to produce chemical feedstocks[6]. In this context, the NHC-Zinc synthetized complexes have been used in methanolysis reactions of poly(L-lactide), showing good activity in the upcycling of the polymer to methyl lactate, which can be used as a green solvent.

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WORK IN PROGRESS ON PLASTICS RESEARCH

ORAL COMMUNICATIONS

COMPARATIVE STUDY OF POLYLACTIC ACID NANOPLASTICS (PLA-NPS) AND POLYSTYRENE MICROPLASTICS (PS-MPS) IN ZEBRAFISH MODEL

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At the dawn of its discovery, the plastic polymer represented a revolution in human daily life, finding application in various sectors. The durability and resistance properties of plastic together with improper disposal, have led to an increasing accumulation of plastic in the environment. Plastics, exposed to environmental agents, progressively fragment up to microplastics (MPs) and nanoplastics (NPs), which, through biomagnification phenomena, can reach humans. Biodegradable plastics, such as polylactic acid (PLA), have been introduced as a green alternative to conventional plastics (1), such as polystyrene (PS).

In the last decade, the zebrafish (*Danio rerio*) has been widely used as a model in biomedical (2) and toxicological research due to its many advantages. Several studies have reported that exposure of zebrafish larvae to conventional NPs/MPs determines behavioural and morphological alterations and high toxicity in different tissues (4). Instead, few studies have investigated the effects of exposure to biodegradable NPs/MPs on zebrafish.

For this reason, here, we report the comparative data relating to the morphological and molecular alterations on zebrafish embryos and larvae up to 120 hours post-fertilization (hpf) exposed to biodegradable rhodamine-labeled PLA-NPs (250 nm) and to fluorescent PS-MPs (1 µm), at concentrations of 100 and 1000 µg/L. The toxicity of plastics was assessed in accordance with OECD test no. 236: Zebrafish Embryo Acute Toxicity Test (ZFET). The effects of plastics were investigated by histological analysis and the expression of inflammatory and cellular stress markers was assessed by gene expression analysis. The results suggest that any kind of plastic may be harmful for animals and, likely also for human beings, however, further investigations are needed to confirm the evidence, to extend the investigation to other aquatic models, and to understand the physiological effects of biodegradable plastics on human health.

The experiments were conducted as part of the PRIN project "Plastic Contamination by Poly(Lactic Acid) (PLASTAMINATION): organ lesions and underlying molecular mechanisms", MUR, PRIN-PNRR2022 CODE NUMBER: P2022AA47Y-CUP MASTER B53D23032060001.

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POLYSTYRENE MICROPLASTICS IMPAIR MITOCHONDRIA FUNCTIONALITY AND MITOCHONDRIAL-ENDOPLASMIC RETICULUM INTERACTION IN TESTIS SOMATIC CELLS

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Leydig and Sertoli cells are testis somatic cells playing an essential role in gametogenesis. Leydig cells are the testosterone-producing testicular cells; Sertoli cells provide physical support and energy sources to germ cells. In this study, we investigated the effects of polystyrene microplastics (PS-MP) on the functionality of the mitochondrial compartment and its association with the endoplasmic reticulum (ER) in cultured mouse Leydig (TM3) and Sertoli (TM4) cells. Mitochondria are the main energy producers in cells; changes in the mitochondrial membrane potential (MMP) can affect ATP production. Furthermore, they are essential for maintaining intracellular homeostasis in the male reproductive tract. In this study, we found a decrease in MMP, a typical marker of mitochondrial defects, as well as a decrease in ATP content and mitochondrial SOD2 protein levels in PS-MP-treated TM3 and TM4 cells, indicating that a mitochondrial disruption and an impairment of antioxidant defense occurred. The inhibition of the mitochondrial functionality by PS-MP was also evidenced by the decreased biogenesis and fusion/fission processes in TM3 and TM4 cells. Mitochondria are closely associated with the ER to form the Mitochondrial-Associated Endoplasmic Reticulum Membranes (MAM), which are the site of calcium ions transfer from the ER to the mitochondria. In this regard, our results showed that PS-MP induced MAMs dysregulation, as evidenced by reduction in GRP75, VDAC and GRP78 protein levels, with consequent loss of calcium homeostasis and an increase in ER stress in both TM3 and TM4 cells. Furthermore, the decrease in protein expression of FACL4 and ATAD3 in these cells suggested an impairment in lipid biosynthesis and trafficking functions of MAMs by PS-MPs.

This study elucidates the intracellular mechanisms underlying the effects of PS-MP on somatic testicular cells, providing new knowledge on their reprotoxicity.

POLYLACTIC ACID NANOPLASTICS: A POTENTIAL THREAT FOR CNS

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The increasing use of petroleum-based plastics has raised concerns about their environmental and biological impacts, prompting a shift toward eco-friendly alternatives like polylactic acid (PLA). PLA is currently widely used in biomedicine as well as food packaging, however, despite PLA being valued for its sustainability and biocompatibility, it can degrade into microplastics and nanoplastics (MP/NPs), which may affect human health in ways that are not yet understood. On the other hand, evidence from studies on conventional MP/NPs demonstrated that they could cross the blood-brain barrier (BBB), potentially causing neurotoxicity, disrupting neurons, and altering memory and behavior [1,2,3]. To this end, the present research aims to investigate the harmful effects of PLA nanoparticles (PLA-NPs) on two *in vitro* cell models: rat adrenal pheochromocytoma PC-12 cells (neuronal model) and rat glioma C6 cells (astrocyte model). For the experimental setup, PLA-NPs conjugated with Rhodamine (180±58 nm size) were synthesized through a microfluidic-assisted nanoprecipitation technique and used for cell treatment at different concentrations (10 - 300 µg/mL) and exposure times (24 – 48hs). Afterward, cell viability and proliferation were assessed using MTT and BrdU assays respectively, while uptake ability was evaluated through flow cytometry and immunofluorescence (IF) analysis. Experiments were performed on undifferentiated PC12 cells and PC12 Nerve Growth factor (NGF)-induced differentiation under two different conditions: early differentiation (T0), in which cells were simultaneously treated with PLA and NGF, and late differentiation (T72), in which PLA treatment was performed after three days from NGF addition. Results demonstrated that PLA-NPs increase cell viability in both types (T0 and T72) of differentiated PC12 cells, while in undifferentiated cells did not. Furthermore, the increased viability observed in T0 differentiated PC12 cells is likely attributable to increased proliferation, as confirmed by the BrdU assay. Flow cytometric analysis revealed that PC12 cells (undifferentiated and differentiated) are able to internalize the PLA-NPs in a dose-dependent manner. IF analysis showed that PLA-NPs localize predominantly into the cytoplasm corroborating cytofluorimetric data. Interestingly, PLA-NPs appeared to negatively affect PC12 cell differentiation, as evidenced by a reduction in both the number and length of neurites. Concerning C6 cells, PLA-NPs show no impact on their viability even though almost 100% of C6 cells internalize efficiently PLA-NPs. The nanoplastics are mainly localized near the nucleus and into the cytoplasm. Altogether these findings suggest that PLA-NPs could interfere with CNS cell function and neuronal development, suggesting the need for further investigation.

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POLYLACTIC ACID EXPOSURE AND CELLULAR METABOLISM: AN UNTARGETED METABOLOMIC INVESTIGATION IN CNS AND HUMAN INTESTINAL CELL LINES

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Polylactic acid (PLA) has emerged as a frontrunner in the bioplastics industry due to its sustainable origins and biodegradability. As a prominent alternative to traditional petroleum-based plastics, PLA is increasingly integrated into eco-friendly packaging and medical devices.

Nonetheless, while degrading throughout its life cycle, PLA fragments into micro and nano plastics (MPs/NPs), which are raising growing concerns about their potential impacts on human health. Indeed, the current understanding on how these particles affect cellular functions and metabolism remains limited.

In this preliminary investigation, we sought to explore the metabolomic alterations induced by PLA exposure in two distinct cell lines: astrocyte-like model of rat glioma C6 cells and human colon adenocarcinoma CaCo-2 cells. By employing an untargeted metabolomic approach, we aimed to reveal the metabolic perturbations triggered by PLA and assess their biological relevance.

Both cell lines were incubated with PLA-NPs at concentrations of 100 and 300 µg/mL, with metabolic effects evaluated after 24, 48, and 72 hours of exposure. In order to arrest the metabolic activity at the studied time-points, cells were subjected to cold methanol quenching. After extraction, purification and derivatization, the metabolomic profiling was performed using Gas Chromatography-Mass Spectrometry (GC-MS), a technique well-suited for high-resolution detection of a wide array of metabolites, allowing for an unbiased and comprehensive assessment of the intracellular metabolic shifts.

Results revealed distinct, cell-specific metabolic perturbations following PLA exposure. In C6 cells, significant disruptions were observed in pathways related to galactose metabolism, starch and sucrose metabolism, fructose and mannose metabolism and cysteine and methionine metabolism, suggesting interference with carbohydrate processing and oxidative stress mechanisms. Moreover, sphingolipid metabolism was notably altered, indicating potential alterations in membrane dynamics and cell signaling. Conversely, in CaCo-2 cells, significant alterations were observed in butanoate metabolism, alanine, aspartate, and glutamate metabolism, arginine and proline metabolism, phenylalanine, tryptophan, and tyrosine metabolism, nitrogen metabolism and arginine

biosynthesis, indicating broader metabolic reprogramming involving amino acid synthesis, energy production, and nitrogen balance.
These preliminary findings demonstrate that exposure to PLA-NPs can cause significant alterations in cellular metabolism. Ongoing investigations are expanding the analysis to other cell lines to further elucidate the nature and the extent of the metabolic changes in different cell types.

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THE TRANSPORT AND FATE OF NANOPLASTICS THROUGH THE BLOOD BRAIN BARRIER: THE ROLE OF INTRACELLULAR LOCALIZATION

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Nanoplastics (NPs), synthetic polymers ranging from 1 nm to 1 μm, are widely detected in the environment and the food chain, raising concerns about their potential health risks. Indeed, they may cause health problems like inflammation and immune system disruptions following exposure via different routes such as inhalation or ingestion. Critically, once inside human body, the interactions between nanoplastics and biological molecules, such as proteins, can significantly influence their cellular uptake and subsequent behavior. The formation of a «protein corona» around NPs due to adsorption of proteins from body fluids has been shown to affect their characteristics and properties. This places particular emphasis on the understanding of the mechanisms and pathways involved in their accumulation and translocation within the human body, being directly related to their fate and persistence within organs and tissues. This study investigates the capability of 100 nm carboxylated polystyrene nanoparticles, used as a nanoplastic model, to cross the human brain endothelial hCMEC/D3 cell layer and be internalized by human brain tumor U87 cells, focusing on the role of intracellular localization. We compared NPs confined in the endo-lysosomal compartment, delivered through endocytosis, with free NPs in the cytoplasm, delivered by the gene gun method. The results indicate that the intracellular behavior of NPs changed as a function of their entrance mechanism. Notably, by bypassing endo-lysosomal accumulation, free NPs were released from cells more efficiently than endocytosed NPs. Furthermore, once excreted by the endothelial cells, free NPs were released as smaller aggregates compared to endocytosed NPs, and consequently, they entered the human glioblastoma U87 cells more efficiently. These findings demonstrate that the intracellular localization of nanoplastics can significantly impact their long-term fate, including their cellular release and subsequent cellular uptake in the brain parenchyma. The study opens new questions about their potential long-term effects on human health, the biological mechanisms underlying their excretion and the evolution of the protein corona around NPs that experience different intracellular environments. The insights gained from this research contribute to a better understanding of the fate and behavior of nanoplastics in the brain, which is crucial for assessing their potential toxicological implications.

POLYAMIDE MICROPLASTICS EFFECTS ON DIGESTIVE SYSTEM OF LAMBS

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Microplastics (MPs) are an emerging pollutant also in the livestock sector (1). Originating from the fragmentation of various plastic polymers (2), which are extensively used on farms (such as covers for horizontal silos, silage bags, bale nets and wraps, veterinary drug packaging, as reported by Borreani and Tabacco (3), and from atmospheric agents, MPs became easily ingestible via diet and water (4). Once inside the animal's digestive system, MPs may potentially impair ruminal-gastro-intestinal functionality (5), interfere with gastro-intestinal epithelium (6) and cause toxic effects due to the adhesion of harmful molecules, such as persistent organic pollutants, heavy metals, pesticides and additives used in the plastic production, as bisphenol (7). This study investigated the effects of concentrate contamination with polyamide (PA) MPs at different dosages (0, 0.6, and 1.8% dry matter) on *in vitro* lamb rumen activity. Samples of 200 mg of concentrate, fortified with PA MPs (dimensions < 2 mm) were inoculated with 30 mL of buffered rumen fluid and incubated in triplicate into serum bottles under anaerobic conditions for 96 h in a sharing water bath at 39°C and 120 rpm. The rumen fermentation profile and gas production at 2,4,6,8,12,24,48,72, and 96 h were measured. Compared to the control, ammonia-nitrogen concentration increased linearly with rising MPs concentration, showing a 5.7% increase at the highest dose. Dry matter degradability was reduced by 3% only at the highest level of contamination. Microbial efficiency decreased linearly with increasing PA MPs concentration, while the ruminal protozoa population dropped by 13% at the highest dose. No effect was observed on pH. The presence of PA MPs in the concentrate at the highest dose, compared to the control, increased total gas production by 5%, with an average fermentation rate increase of 20% mL/hour. Additionally, it accelerated the initial gas formation, reducing the lag time of 0.68 h, thereby shortening the time required to initiate the gas production process.
The presence of PA MPs in concentrate negatively affected lamb rumen activity. It reduced nitrogen utilization, lowered ruminant performance, and posed ecological challenges, impairing environmental sustainability. The negative effects on rumen degradability could reduce feed intake, animal performance, and feed efficiency, leading to higher feed costs and economic losses (Oba & Allen, 1999). Moreover, it could lead to digestive and metabolic disorders in ruminants. Incomplete feed fermentation in the rumen, could increase gas emissions (mainly greenhouse gases) per unit of degraded feed and escalate nutrient waste in feces, contributing to environmental pollution (Blümmel *et al.*, 2003).
In conclusion, the adverse effects of PA MPs on lamb rumen activity and concentrate degradability were dose-dependent. Increasing the dose of PA MPs ingested led to a significant linear decrease in the efficiency of rumen microbiota.

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CAN *CHLORELLA VULGARIS* ALLEVIATE HISTOLOGICAL ALTERATIONS INDUCED BY MICROPLASTICS ON THE GILLS OF *MYTILUS GALLOPROVINCIALIS*?

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Plastic is one of the most widely used materials of modern times, with over half of all plastic ever produced being made since 2000. The production of plastics worldwide has seen a rapid and significant rise, reaching around 400.3 million tons of plastic goods in 2022.

Once released into the environment, plastic waste enters the aquatic environment through different pathways, including wastewater treatment plants and atmospheric transport. Microplastics (MPs), with a size of < 5 mm, represent about 93% of the plastic waste in the marine environment and polyethylene (PE), in particular, has been recognized among the MPs with the higher concentration in marine environments.

Due to their sedentary and filter-feeding behaviour, mussels can accumulate and concentrate dissolved contaminants in their tissues and have been widely employed as good bioindicators of pollution in coastal waters. By fulfilling almost all required criteria for bioindicator species, they are also recognized as valuable model organisms for revealing the detrimental effects of plastic pollution. Numerous studies demonstrated that the exposure of mussels to PE particles causes changes in the antioxidant enzyme system, increased reactive oxygen species (ROS) concentration and induces histological alterations (1) (2) (3). However, available information does not refer to realistic MP concentrations, representative of the marine environments, and often assesses the effects of MPs in combination with other stressors.

The protective abilities of various natural products against MP toxicity in fish have been examined in recent literature (4) (5). Among these natural products, the microalga *Chlorella* has been successfully used in aquaculture to improve nutrition and alleviate damages caused by microplastics (4) (5).

Based on this background, we conducted a study to examine, for the first time, the histological alterations in the gills of *Mytilus galloprovincialis* caused by PE and to explore the potential protective effects of *Chlorella vulgaris*. In this experiment, the mussels were exposed to a 100 µg/L concentration of PE for 7, 14 and 21 days in the presence or absence of *C. vulgaris*, and the changes were compared to the unexposed control group.

Our observation revealed that exposure to environmentally relevant concentrations of PE induced severe alteration in the gills of *Mytilus galloprovincialis*, including thickening of the gill filaments, increased mucous-secreting cells, disarrangement of epithelial cells, granulocyte proliferation and epithelial detachment. Furthermore, we found that co-exposure with *C. vulgaris* partially mitigates the histopathological damage to the gills, although it did not completely prevent the onset of some alterations, such as epithelial detachment. In conclusion, our findings suggest that the use of natural agents could be a promising avenue to mitigate the impact of microplastic pollution in the marine environment.

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MORPHOLOGICAL AND MOLECULAR EFFECTS OF BIODEGRADAABLE PLASTICS ON SEA URCHIN *PARACENTROTUS LIVIDUS*

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Plastics are widely used materials in our daily lives, but their long-lasting nature poses significant environmental issues. Many studies showed that plastic wastes can be found in various environments, both on land and in water, where they break down into smaller fragments known as micro- and nanoplastics. As a result, there is a growing interest in improving the recycling techniques for traditional micro(nano)plastics and promoting eco-friendly alternatives, like biodegradable plastics/polymers (BPs). The past two decades were characterized by extensive research on the destiny and toxicity of traditional petroleum-based non-biodegradable micro(nano)plastics in marine environments. However, the need of additional studies on BPs is still evident (1). In this scenario, the impacts of five microplastics, poly(butylene succinate) (PBS), poly(butylene succinate-co-adipate) (PBSA), polycaprolactone (PCL), polyhydroxy butyrate (PHB) and polylactic acid (PLA) obtained from biodegradable polymers (BPs), were analysed on the sea urchin *Paracentrotus lividus* (Lmk) embryos, comparing: i. embryos deriving from wild adults; ii. embryos deriving from experimental BP-fed adults (in an *ad hoc* set mesocosm). Morphological analyses performed on *P. lividus* embryos exposed to the polymers showed developmental delay and malformations. These effects corresponded, at the molecular level, to variation in the expression levels of eighty-seven genes involved in key cellular processes, such as skeletogenesis, differentiation and development, stress, as well as detoxification responses (2) (3). Histological analyses on gonadic tissues of BP-fed adults showed empty follicles and atretic gametes in various phases of maturation, some in a spent stage, with a few developing oocytes exhibiting an atypical shape. Our findings permit us to hypothesize that

BMPs might accumulate inside various organs in adult sea urchins. Furthermore, this research demonstrated that BPs, generally considered a good alternative to other polymers to reduce marine plastic pollution, may affect sea urchin physiology and, for extension, impact the life of marine invertebrates when present in marine environments in microscopic sizes. These effects could represent potential risks to human health, considering that sea urchins are worldwide consumed.

This work was realised in the framework of the Project - Biomonitoraggio di micro e nanoplastiche biodegradabili: dall'ambiente all'uomo in una prospettiva one health (BioPlast4Safe) - with the technical and economic support of the Italian Ministry of Health – PNC.

1. Manfra L *et al.* (2021) *Journal of Hazardous Materials*, *416*, 125763.
2. Viel T *et al.* (2023) *Environmental Pollution*, *334*, 122129.
3. Viel T *et al.* (2024) *Science of the Total Environment*, *929*, 172586.

HISTOPATHOLOGICAL EVALUATION OF BIODEGRADABLE MICROPLASTICS: A PRELIMINARY STUDY ON *PARACENTROTUS LIVIDUS*

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Biodegradable plastics (BPs) are considered a sustainable alternative to conventional plastics for mitigating marine pollution. However, their effects on marine organisms, particularly at the histological level, remain insufficiently understood. This study explored the impacts of two sets of microplastics on the gonadal morphology of the sea urchin *Paracentrotus lividus*. In the first experiment, adult sea urchins were exposed to biodegradable microplastics, including polylactic acid (PLA), poly(butylene succinate) (PBS), poly(butylene succinate-co-adipate) (PBSA), polycaprolactone (PCL), and polyhydroxybutyrate (PHB). In the second experiment, the effects of commercial PLA (derived from disposable dishes) and conventional non-biodegradable polypropylene (PP) were evaluated. In both experiments, sea urchins were fed weekly with agar blocks (0.84 g microplastic/Kg of agar) for five weeks (1), and the gonads were collected and fixed in Davidson's solution for histopathological analysis. The histopathological index was developed based on the presence of lipofuscin accumulation, detachment of acinal borders, and enlargement of interstitial spaces among the gametes.
The results indicated some alterations in gonadal morphology due to biodegradable microplastic exposure. Sea urchins exposed to BPs showed occasional empty follicles, atretic gametes at different stages of maturation, lipofuscin accumulation, and tissue detachments. In particular, PHB induced a higher histopathological index compared to control, PLA, and PCL. In contrast, sea urchins exposed to PP microplastics showed no significant alterations compared to both the control and commercial PLA. Overall, this study highlights the potential risks posed by biodegradable plastics on marine reproductive health. These findings underscore the need for further research into the potential long-term consequences of microplastic exposure on marine ecosystems and their entry into the food chain.

This work was realised in the framework of the Project - Biomonitoraggio di micro e nanoplastiche biodegradabili: dall'ambiente all'uomo in una prospettiva one health (BioPlast4Safe) - with the technical and economic support of the Italian Ministry of Health – PNC, and of the project CRIMAC (Centro Ricerche ed Infrastrutture Marine Avanzate in Calabria) "Blue Economy of Calabrian coasts: biocoenotic characterization, analyses and exploitation of innovative aquaculture productions" (BluCaProd).

1. Viel *et al.* (2024) *Science of the Total Environment*, *929*, 172586.

CRUSTACEANS ARE MODELS TO STUDY THE EFFECTS OF BIODEGRADABLE PLASTICS: A POLYPHASIC APPROACH

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Biodegradable polymers (BPs) are promising materials widely used to replace conventional synthetic plastics to reduce environmental impact. However, since the biodegradation processes depend on various biotic and abiotic factors, they need to be correctly used and, above all, managed adequately at the end of life. In fact, they are gradually broken down into smaller pieces and remain suspended in the seawater, forming biodegradable microplastics (BMPs). To date, poor data are available in the literature on BPs fate, as well as on their environmental impacts and their long-term effects (1). Crustaceans play fundamental roles in aquatic food webs, representing important connections between primary producers and consumers, both in benthic and in planktonic communities, and they are excellent models for evaluating the toxic effects of BMPs. In this work, for the first time we aimed at determining the toxicity of five BMPs on two crustaceans: the isopod *Idotea balthica basteri* Audouin, 1826 and the decapod *Hippolyte inermis* Leach, 1816. The microplastics were obtained from *poly (butylene succinate)* (PBS), *poly (butylene succinate-co-butylene adipate)* (PBSA), *poly (ε-caprolactone)* (PLC), *poly (3-hydroxy-butyrate)* (PHB) and *poly (L-lactic acid)* (PLA), whose size ranges from 0.30 to 335.0 μm. Adult individuals of the isopod *I. balthica basteri* were fed twenty-seven days with the five BMPs at two concentrations (0.84 g/kg of food and 8.4 g/kg of food), in agreement with previous research on benthic invertebrates performed by Viel *et al.* (2). In addition, larvae of the decapod *H. inermis* were exposed ten days at three concentrations (1-5-10 mg/L) of BMPs, in agreement with tests performed on planktonic organisms by Viel *et al.* (2023). The mortality rates recorded at the end of the experiment were evaluated for both organisms and, in addition, molecular investigations were conducted using *Real Time qPCR* to determine the variation in the expression levels of eighteen orthologous genes exhibited by both crustaceans and involved in several functional classes, including stress responses and detoxification processes. Our results show the broad spectrum of effects that BPs induce in marine organisms, demonstrating that *I. balthica basteri* and *H. inermis* are convenient models to investigate the responses to biodegradable plastics and, more in general, to environmental pollution and emerging contaminants.

This work was conducted in the framework of the Project – Biomonitoraggio di micro e nanoplastiche biodegradabili: dall'ambiente all'uomo in una prospettiva one health (BioPlast4Safe) - with the technical and economic support of the Italian Ministry of Health – PNC.

1. Manfra L *et al.* (2021) *Journal of Hazardous Materials*, *416*, 125763.
2. Viel T *et al.* (2023) *Environmental Pollution*, *334*, 122129.

IMPACT OF MICROPLASTICS AND ASSOCIATED CONTAMINANTS ON MYTILUS GALLOPROVINCIALIS REPRODUCTION: A COMPARATIVE AND MULTIDISCIPLINARY STUDY

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Microplastics (MPs) and its derivatives are stressors able to exert single as well as combined effects on biological processes, with synergistic or antagonistic interactions. Our research group studies the effect of multiple stressors, such as polystyrene microplastics (MP) and Bisphenol A (BPA), from a molecular, physiological and histological point of view. We focus on the reproductive cycle of *Mytilus galloprovincialis* to assay the alterations induced by different concentrations of MPs, BPA and Natural Organic Matter (NOM) alone and in complexation. The team has set up a methodological protocol to evaluate BPA bioaccumulation in bivalve tissues. By histological analyses and by evaluation of specific genes and proteins altered expression we aim to understand the molecular mechanisms activated under stress conditions and the ability of NOM to prevent severe tissue damages. We tested and assayed the alterations induced by different concentrations of BPA and Natural Organic Matter (NOM) alone and in complexation and demonstrated a protective effect induced by the NOM which prevents the availability of BPA and its absorption by tissues.

By comparing our results with those obtained in other model organisms with different phylogenetic positions, ecological niches, gonadal structure and reproductive strategies, we aim to investigate contaminant fate and tissue specific responses. We will try to identify new protection strategies to prevent or reverse the damage exerted by these endocrine/metabolic disruptors.

NANOCARRIERS FABRICATED VIA MICROFLUIDICS-ASSISTED NANOPRECIPITATION OF PLA LOADED WITH RHODAMINE PROVIDE AN EFFICIENT APPROACH FOR TRACKING THEIR CELLULAR UPTAKE AND DISTRIBUTION

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The study explores for the first time how microfluidic technology can effectively manufacture Rhodamine-loaded PLA nanocarriers by co-precipitation, focusing on optimizing key parameters like total flow rate (TFR), flow rate ratio (FRR), surfactant concentration, and polymer content to control particle size distribution. Specifically, nanocarriers with an average size of 252 ± 38 nm and surface charge of –6.5 ± 4 mV were fabricated using a TFR of 6 mL/min, an FRR of 1:1, and a 5 mg/mL polymer concentration. Under these conditions, Rhodamine B (Rhod) was successfully loaded with an encapsulation efficiency of 50%. Electron microscopy revealed that the nanocarriers had spherical shapes and smooth surfaces. Nanocarriers smaller than 250 nm showed efficient internalization in various tissue cells, with good biocompatibility and no significant cytotoxicity observed in human peripheral blood mononuclear cells (hPBMCs) at concentrations up to 2 mg/mL after 24 and 48 hours of exposure. Cellular uptake by hPBMCs began at 0.1 mg/mL after 24 hours, with dose-dependent saturation at higher concentrations of 0.25 and 0.5 mg/mL. Moreover, dose-dependent uptake of Rhod-PLA nanocarriers was observed in colon cancer cells (HT29) and C6 glioma cells, indicating promising potential for targeted drug delivery.

FROM WASTE TO HEALTH: NATURALLY DERIVED BIOMATERIALS FOR REGENERATIVE MEDICINE

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Over the past years, the great attention on the circular economy has boosted a significant advancement of naturally derived and biodegradable biomaterials in the biomedical field, owning to their excellent physicochemical and biological properties.
In the field of regenerative medicine and tissue engineering, the synergic contribution of material chemistry and engineering has further allowed the development of different products such as, injectable formulations, films, sponges and innova-

tive structures/scaffolds. Particularly, three-dimensional (3D) bioprinting has enabled the fabrication of 3D customized scaffolds able to mimic the exact extracellular matrix. Furthermore, bioinks, in the form hydrogels, may also embed cells and multiple growth factors to generate tissue analogues with multiple functions (1).

Among naturally derived polymers, gelatin (GEL), chitosan (CTS), gellan gum (GG), collagen (COL), hyaluronic acid (HA) and their derivatives have been widely employed alone or suitably combined. The potential exploitation of wastes as a source of natural compounds and components was analyzed, exploring different biomedical applications.

For example, injectable formulations based on modified HA decorated with plants extracts have demonstrated antinflammatory and antioxidant features which make them potential candidates for neuroprotection. GEL films obtained from marine wastes and agricultural byproducts have been shown to be promising for wound healing meanwhile, marine COL blended with CTS has highlighted interesting properties for liver regeneration. CTS has also been functionalized with extracts from bagasse pulps fibers' valorization to develop biodegradable sponges, as potential pH-responsive wound dressings. Similarly, chemically modified GEL bioactivated with different natural extracts may allow the manufacture of 3D sponges with enhanced regenerative properties and able to control the inflammation. By using modified HA bioinks enriched with cell-derived vescicles, 3D bioprinted patches have been developed for wound healing applications. As further example, bioactivated GG, from bacteria of the *Sphingomonas strain,* or COL have been employed for the design of 3D custom-made scaffolds. Meanwhile, 3D printed nanocomposite scaffolds, based on modified HA, or GG and hydroxyapatite, highlighted their potential use for bone regeneration.

The overall results open an entire new fascinating research field related to the design of high-value products which will provide economic profit and more reliable products for soft and hard tissue regeneration.

1. Petta, D *et al.* (2020). Biofabrication 12, 032001.

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Index of authors