



μ MED

INTERNATIONAL CONFERENCE ON MICROPLASTIC POLLUTION IN THE MEDITERRANEAN SEA

HOTEL LA RESIDENZA - CAPRI (NA) - ITALY
Capri September 15th - 18th 2019



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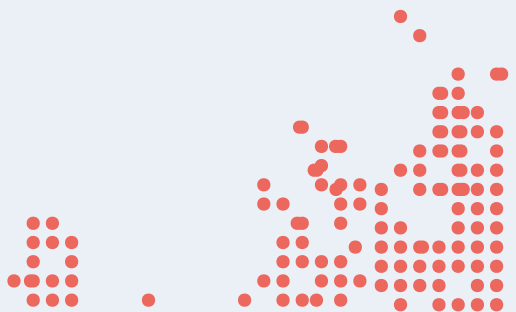
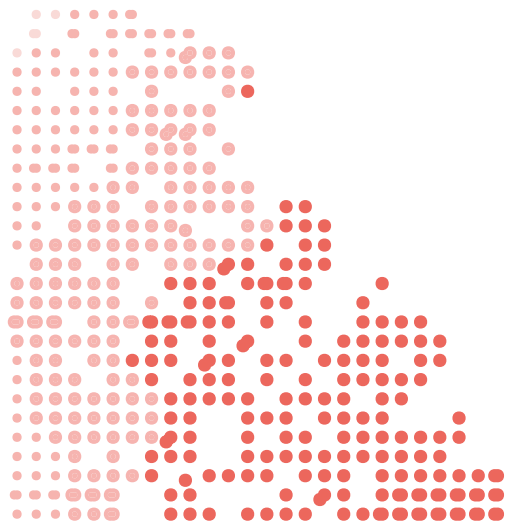
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About

Following the success of the first edition of the “**International Conference on Microplastic Pollution in the Mediterranean Sea**” - μ MED - the Local Scientific Committee and International Advisory Board are pleased to invite the scientific community and stakeholders to the **II edition of the μ MED Conference**, which will be held in **Capri (NA), Italy, from 15th to 18th September**.

The μ MED Conference aims to become a **key event on microplastic pollution**, strengthening the previous experience.

The Conference will gather **experts from scientific and industrial communities, policy makers and environmental organizations**. It will be the occasion to update the state-of-the-art, to highlight the progresses, and to **identify new and effective solutions to mitigate this environmental issue**.

In order to obtain a better knowledge of the microplastic pollution problem, the main topics of the II μ MED Conference have been increased, covering a wide range of multidisciplinary fields: chemistry, polymer science, biology, engineering, medicine, law, physics, ecotoxicology, etc.

Conference Topics

1. Microplastic pollutions in different environments: Freshwater, Marine, Air and Soil
2. **Microplastics in the Mediterranean Sea**
3. Sources, fate and effects of microplastics
4. **Detection systems for microplastic pollution monitoring**
5. Green synthetic approaches for the preparation of environmentally friendly polymers
6. **Recovery and recycling of marine plastics**
7. Wastewater treatment plants as microplastic entrance route
8. **Nanoplastics as emerging pollutants**
9. Degradation of plastics in marine environment
10. **Impact of microplastics on marine life**
11. Microplastics: from marine pollution to human food chain
12. **Mitigation of microplastic impact and innovative solutions**
13. Socio-economic, environmental impacts assessment and risk analysis
14. **Sampling, extraction, purification and identification approaches for microplastics**
15. Adsorption and transport of pollutants on microplastics

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PROGRAMME

15/09/2019 - AFTERNOON

5:00-6:20 PM

Registration

μMED 2019 OPENING

Chairman: Luigi Ambrosio

6:20-7:00 PM

Gaetano Leone UNEP - Mediterranean Action Plan

7:00 PM

WINE & CHEESE

16/09/2019 - μMED INTRODUCTION

8:30-9:00 AM

Registration

Maurizio Avella

Institute for Polymers Composites and Biomaterials - National Research Council, IT

Conference Introduction

Sergio Costa

Environment Minister, IT

Massimo Inguscio

National Research Council, IT

9:00-9:30 AM

Luigi Ambrosio

Institute for Polymers Composites and Biomaterials - National Research Council, IT

Welcome and introduction

Raffaella Giugni

Marevivo, IT

Giorgio Zampetti

Legambiente, IT

16/09/2109 - MORNING SESSIONS

Chairwoman: Maria Westerbos

MICROPLASTIC POLLUTION AND ENVIRONMENTS

9:30-10:00 AM

Susan D. Shaw

Shaw Institute, USA

Confronting the Global Plastics Crisis: Challenge of the 21st Century

10:00-10:10 AM

Steve Allen

University of Strathclyde, UK

Remote Area Atmospheric Microplastic Pollution

10:10-10:20 AM

Friederike Stock

German Federal Institute of Hydrology, DE

Distribution of microplastics in rivers: the example of the German river Elbe

10:20-10:30 AM

Kerry Moss

Nelson Mandela University, ZA

STOP: Survey Techniques for Observing Plastics in riverine systems of Africa

10:30-10:40 AM	Soline Alligant	Laboratoire Eau Environnement et Systèmes Urbains, FR	Microplastic contamination of sediment and water column in the Seine River estuary
10:40-10:50 AM	Stefania Piarulli	University of Bologna, UO CoNISMa, IT	Filter - feeders as aggregator of microplastics in the marine system: A field experimental study

10:50-11:30 AM

COFFEE BREAK

IMPACT OF MICROPLASTICS ON MARINE LIFE

11:30-12:00 AM	Maria Cristina Fossi	University of Siena, IT	The Impact of Microplastics on Filter-feeding Megafauna
12:00-12:10 PM	Dannielle Green	Anglia Ruskin University, UK	Effects of conventional and biodegradable microplastics on the attachment strength and haemolymph proteome of blue mussels (<i>Mytilus edulis</i>)
12:10-12:20 PM	Luca Palazzo	Institute of Anthropic Impacts and Sustainability in marine environment, National Research Council, IT	Fish that live in different zones as possible indicators of microplastic pollution
12:20-12:30 PM	Carl Van Colen	Ghent University, BE	Transfer of microplastics in marine foodwebs: an experimental approach
12:30-12:40 PM	Sarah Meek	University of the West of England, UK	Effects of Seagrass meadows on microplastic deposition.
12:40-12:50 PM	Francesca Garaventa	Institute of Anthropic Impacts and Sustainability in marine environment, National Research Council, IT	Microplastic in water and biota: a Research and Awareness campaign in the Mediterranean Sea
12:50-13:00 PM	Debra Ramon	University of Haifa, IL	Microplastic Ingestion by a Marine Fish Community of Israeli Coastal Waters

1:00-2:30 PM

LUNCH

16/09/2109 - AFTERNOON SESSIONS

Chairwoman: Maria Cristina Fossi

SOURCES, FATE AND EFFECTS OF MICROPLASTICS

2:30-3:00 PM	Johnny Gasperi	University of Paris, FR	Plastic debris in urban water and in freshwater: lessons learned from research projects launched in the Seine Basin catchment
3:00-3:10 PM	Stefania Di Vito	Legambiente, IT	Overview of microplastic presence in Italian freshwater systems: monitoring of principal lakes and rivers
3:10-3:20 PM	Deonie Allen	EcoLab, ENSAT, CNRS, FR	Atmospheric transport and our planetary boundary layer as an environmental compartment of microplastics pollution

3:20-3:30 PM	Claudia Campanale	Water research Institute, National Research Council, IT	Microplastic debris in an Italian river: first monitoring results
3:30-3:40 PM	István Szabó	Szent István University, HU	Characterization of plastic-associated bacterial community in freshwater lake of Vácszentlászló, Hungary
3:40-3:50 PM	Carla Fanizza	INAIL, IT	Microplastic pollution at an urban site in Rome

3:50-4:30 PM COFFEE BREAK

EFFECTS OF MICRO AND NANOPLASTICS ON ACQUATIC ORGANISMS

4:30-5:00 PM	Francesco Regoli	Università Politecnica delle Marche, IT	Insights on ecotoxicological effects of microplastics in marine ecosystems: the EPHEMARE project
5:00-5:10 PM	Auguste Manon	University of Genova, IT	What can model polystyrene nanoparticles teach us on the impact of nanoplastics in bivalves? studies in <i>Mytilus</i> from the molecular to the organism level
5:10-5:20 PM	Carola Murano	Stazione Zoologica Anton Dohrn, IT	Uptake and stress-induced responses of polystyrene microbeads in adults of the sea urchin <i>Paracentrotus lividus</i>
5:20-5:30 PM	Lola Paradinas	Scottish Marine Institute, UK	Seasonal importance on microplastics composition and concentrations in two matrices; coastal waters and wild mussels (<i>Mytilus edulis</i>)
5:30-5:40 PM	C. Martínez-Gómez	Instituto Español de Oceanografía , ES	In vitro effects of mercury (Hg) on immune function of mussel (<i>Mytilus galloprovincialis</i>) are enhanced in presence of microplastics in the extracellular medium
5:40-5:50 PM	Stefano Magni	University of Milan, IT	Microplastics in the river Lambro (Northern Italy): monitoring and toxicity
5:50-6:00 PM	Bárbara Abaroa-Pérez	Plataforma Oceánica de Canarias, ES	Study of chemical pollutants over marine microplastics based on their composition and degradation rate

6:00-7:00 PM POSTER SESSION I

17/09/2109 - MORNING SESSIONS

Chairman: Emanuele Fiore

EVALUATION AND DETECTION OF MICROPLASTICS

9:00-9:30 AM	Richard C. Thompson	University of Plymouth, UK	Marine Litter: are there solutions to this global environmental challenge?
9:30-9:40 AM	Alessio Gomiero	Norwegian Research Centre, NO	Development of a thermo degradation method to assess levels and distribution of microplastics in marine sediments and its application in two case studies: the northern Adriatic Sea (Italy) and the Boknafjord (Norway).

9:40-9:50 AM	Annamaria Cavalleri	PerkinElmer, IT	Optimising the Workflow for Microplastic Analysis by FTIR Microscopy
9:50-10:00 AM	Gabor Bordos	WESSLING Hungary Ltd., HU	Microplastics in Hungarian freshwaters: development of a standard addition sample preparation method
10:00-10:10 AM	Lars Hildebrandt	Helmholtz-Zentrum Geesthacht, DE	First evaluation of continuous flow centrifugation as a novel straightforward and non-size-discriminating sampling technique for microplastic in waters
10:10-10:20 AM	Corinna Földi	Federal Institut of Hydrology, DE	Microplastics – what else? Quantification in environmental samples using pressurized liquid extraction and Pyr-GC-MS
10:20-10:30 AM	Rubén Rodríguez Alegre	LEITAT Technological Center, ES	Microplastics extraction and counting from wastewater and sludge through elutriation and hydrocyclone
10:30-10:40 AM	Julia Reichel	Technical University Munich, DE	Application of thermal extraction/desorption-pyrolyse-GC/MS to investigate sorption kinetics of pollutants and the identity of (sub)microplastic
10:40-10:50 AM	Stefania Federici	University of Brescia, IT	Reference materials and standard needs for environmental micro and nanoplastic pollution
10:50-11:30 AM	COFFEE BREAK		
MICROFIBER POLLUTION: ABUNDANCE, DETECTION AND MITIGATION			
11:30-11:50 AM	Mariacristina Cocca	Institute for Polymers Composites and Biomaterials - National Research Council, IT	Microplastic pollution from textiles: quantitative evaluation and mitigation strategies
11:50-12:00 AM	Paula Félix-De-Castro	LEITAT Technological Center, ES	Mitigation of microfibers release from textiles: chemical finishing and mechanical optimization.
12:00-12:10 AM	Giuseppe Suaria	Institute of Marine Science, National Research Council, IT	Abundance and composition of textile fibres in Mediterranean surface waters
12:10-12:20 AM	Francisco Belzagui	Universitat Politècnica de Catalunya, ES	When Size Matters – Textile Microfibers into the Environment
12:20-12:30 AM	Andrea Stolte	WWF Germany, DE	Derelict Fishing Gear – removing a source of microplastics from the marine environment
12:30-12:40 AM	Liliya Khatmullina	Shirshov Institute of Oceanology, RU	Microplastics settling – flat particles and fibers.
12:40-12:50 AM	Andrej Kržan	PlanetCare Ltd., SL	Filters for fibres from washing
12:50-1:00 PM	Kang Heejun	Korea Institute of Civil Engineering and Building Technology, KR	Reduction of microplastic emission from clothes during laundering with chitosan pre-treatment
1:00-2:30 PM	LUNCH		

17/09/2109 - AFTERNOON SESSIONS

Chairman: Richard C. Thompson

DEGRADATION AND BIODEGRADATION OF PLASTICS IN THE ENVIROMENT

2:30-3:00 PM	Anne-Gaelle Collot	Plastic Europe, BE	The Plastics Manufacturers efforts to tackle plastic waste in the environment
3:00-3:10 PM	Francesco Degli Innocenti	Novamont S.p.A., IT	Biodegradable plastics do not form chemically persistant microplastics
3:10-3:20 PM	Christian Lott	HYDRA Marine Sciences, DE	It depends where it ends – biodegradable plastic degradation differs between habitats, and climate zones
3:20-3:30 PM	Luca Fambri	University of Trento, IT	Controlled aging and degradation of selected plastics in marine environment (12 months of follow-up)
3:30-3:40 PM	Camilla Catarci Carteny	University of Antwerp, BE	Not only diamonds are forever: degradation of plastic films in a simulated marine environment
3:40-3:50 PM	Miriam Weber	HYDRA Marine Sciences, DE	How to proof claims on biodegradability of plastics? overview of available standard tests and their environmental relevance

3:50-4:30 PM **COFFEE BREAK**

MICRO AND NANOPLASTICS AS EMERGING POLLUTANTS

4:30-5:00 PM	Frank J. Kelly	King's College London, UK	Inhalable microplastics: a new cause for concern?
5:00-5:10 PM	Ásta M. Ásmundsdóttir	University of Akureyri, IS	Microplastics and nanoplastics occurrence and composition in drinking water from Akureyri urban area, Iceland
5:10-5:20 PM	Margherita Ferrante	University of Catania, IT	Microplastics in vegetables and fruit
5:20-5:30 PM	Andrew Reynolds	Technological University Dublin, IE	Standardizing in-vivo analysis methods for toxicological effects within freshwater organisms from nano-polystyrene exposure
5:30-5:40 PM	Anna Bellingeri	University of Siena, IT	Impact of nanoplastics on marine diatom <i>Skeletonema marinoi</i> : particles adhesion, ROS production and reduction of colonies length
5:40-5:50 PM	Loriane Murphy	Institute of Technology Carlow, IR	Association of potential human pathogens with microplastics in freshwater systems

5:50-6:50 PM **POSTER SESSION II**

7:00 PM **SOCIAL DINNER at "Il Faro" Anacapri**
Bus Tranfer from Piazzale Europa

18/09/2109 - MORNING SESSIONS

Chairman: Cosimo Carfagna

SOCIO-ECONOMIC AND ENVIRONMENTAL IMPACT OF MICROPLASTICS

9:00-9:30 AM	Julien Boucher	Shaping Environmental Action,CH	The Marine Plastic Footprint & its application to the Mediterranean Basin
9:30-9:40 AM	Ana Rotter	National Institute of Biology, SI	Microplastic pollution: a thriller with many lead roles and unknown ending
9:40-9:50 AM	Mikaël Kedzierski	Université Bretagne Sud, FR	Why and how do we dispose of our plastic waste? An overview of the behavioural bases
9:50-10:00 AM	Esther Kentin	Leiden University, NL	Legislative options for reducing pollution by microplastics in the EU: the meaning of scientific evidence
10:00-10:10 AM	Tosca Ballerini	Expédition MED, FR, Legambiente, IT	The Pelagos Plastic Free Project: plastic marine debris abundance, plastic-associated microbial community composition, and sources of beached waste in the Pelagos Sanctuary
10:10-10:20 AM	Michael T. Sturm	Wasser 3.0 GmbH, DE	Development of a novel technological approach for the reduction of microplastic pollution in various waters
10:20-10:30 AM	Tiziano Battistini	Aquafil Group, IT	Sample preparation and analysis methods of microplastics
10:30-10:40 AM	Svitlana Liubartseva	Fondazione CMCC, IT	Model-based assessment of plastic distribution in the Mediterranean Sea
10:40-10:50 AM	Sören Gutekunst	The Ocean Race, ES	A World Survey on Microplastic Distribution near the Surface from Race Yachts

10:50-11:30 AM

COFFEE BREAK

MICROPLASTICS IN THE MEDITERRANEAN SEA

11:30-12:00 AM	Roberto Danovaro	Stazione Zoologica Anton Dohrn, IT	Impact of microplastics on the deep-sea ecosystems
12:00-12:10 PM	Catharina Pieper	University of the Azores, PT	Into the med: searching for microplastics from space to deep sea
12:10-12:20 PM	Florian Pohl	Utrecht University, NL	Microplastic distribution on the Tyrrhennian seafloor controlled by deep-sea circulation
12:20-12:30PM	Mirta Smodlaka Tankovic	Rudjer Boskovic Institute, HR	Microplastic load in the Northen Adriatic

12:30-12:40 PM	Vincenzo Donnarumma	Stazione Zoologica Anton Dohrn, IT	Microplastic-specific microbial communities in the Gulf of Naples: concentrations, seasonal differences and composition
12:40-12:50 PM	Laura Simon Sánchez	Autonomous University of Barcelona, ES	Seasonal variation of microplastic pollution in Mediterranean islands as an effect of tourism
12:50-1:00 PM	Daura Vega Moreno	Universidad de Las Palmas de Gran Canaria, ES	Analysis of marine microplastics in the water column sampled up to 300 meters depth

1:00-2:30 PM

LUNCH

18/09/2109 - AFTERNOON SESSION

Chairman: Mario Malinconico

CLEANING STRATEGIES

2:30-3:00 PM	Marco Faimali	Institute of Anthropic Impacts and Sustainability in marine environment, National Research Council, IT	The CLAIM project: clean is the aim!
3:00-3:10 PM	Lucia Pittura	Polytechnic University of Marche, IT	Characterization of microplastics in main streams of conventional and innovative urban wastewater treatment plants.
3:10-3:20 PM	Isam Sabbah	Braude College for Engineering, IL	Fate of microplastics in the mainstream of wastewater treatment plant
3:20-3:30 PM	Robin Treilles	Laboratoire Eau Environnement et Systèmes Urbains, FR	Macro and microplastics in stormwater and combined sewer overflows in Paris megacity
3:30-3:40 PM	Gerardo Pulido Reyes	Eawag, SZ	The effect of ozonation used for drinking water treatment on different types of submicron plastic particles
3:40-3:50 PM	Katja Klun	National Institute of Biology, SI	The role of jellyfish mucus stability in capturing microplastic particles
3:50-4:00 PM	Philip Schäfer	neaspec GmbH, DE	nano-FTIR spectroscopy: nanoscale resolved infrared spectroscopy of polymeric nano-particles and self-assembled monolayers

4:00-4:30 PM

Maurizio Avella

Institute for Polymers Composites and Biomaterials, National Research Council, IT

Maurizio Peruzzini

Department of Chemical Sciences and Materials Technologies - National Research Council, IT

Conclusions - Best poster award ceremony







**MICROPLASTIC POLLUTION
AND
ENVIRONMENTS**



Confronting the Global Plastics Crisis: Challenge of the 21st Century

Susan D. Shaw

Shaw Institute, USA

email: sshaw@shawinstitute.org

The plastics crisis is one of the major environmental challenges of the 21st century. Since post-WWII, global production has increased from two million to 380 million metric tons annually, and fueled by the boom in cheap shale gas, production of virgin plastic is expected to double by 2030. Plastic in all its forms has permeated the biosphere, from deep ocean trenches to remote polar regions, and serves as a key geological marker (“techno-fossil”) defining the Anthropocene era. The world’s oceans are choking with plastics, receiving an estimated 8.8 million metric tons annually, an amount expected to increase tenfold by 2020 and outweigh fish by 2050. With an estimated lifetime persistence of hundreds of years, plastics break up into micro- and nano-plastics, facilitating their uptake by marine biota. These petroleum-derived polymers contain chemical additives and contaminants, including known endocrine disruptors and carcinogens that pose ecosystem-wide risks and diminish ocean bacteria’s ability to produce oxygen. There is an urgent need to carry out focused scientific research on the impacts of plastics on human health, but existing scientific evidence is already sufficient to warrant aggressive global actions to mitigate rising plastic production, address wasteful consumption and inadequate infrastructure, and curb the unthinkable flow of more plastics and their toxic additives into the sea.

Remote area atmospheric microplastic pollution

Allen Steve^{1*}, Allen Deonie², Phoenix Vernon¹, Le Roux Gaël², Jiménez Pilar², Simonneau Anaëlle³, Binet Stéphane³, Galop Didier⁴

¹ WESP, University of Strathclyde, Glasgow, UK.

² EcoLab, ENSAT, CNRS, Castanet Tolosan, France.

³ ISTO, CNRS, University of Orleans, BRGM, France.

⁴ GEODE, CNRS, University of Toulouse Jean Jaurès, France.

***Presenter:** Allen Steve, steve.allen@strath.ac.uk

Microplastic studies have focused on the occurrence, abundance and fate of marine plastic pollution over the past decades. Recent terrestrial research has shown microplastic to be found in cities, road dust and agricultural soils as a result of human plastic use. Beyond the direct urban, industrial and agricultural sources of microplastics, microplastic pollution has also been found in remote terrestrial locations. Microplastic has been found on the Tibetan plateau lake shores and sediments, in floodplain sediments in the Swiss mountains' floodplains and in the Arctic ice and snow.

Explanations of how this plastic pollution arrived at these remote locations have been theorised. Recent research on remote atmospheric deposition in the French Pyrenees mountains provides some evidence towards explaining how microplastic pollution is arriving in remote terrestrial places. This research explores: 1) air as an environmental compartment polluted with microplastics that has previously been unaccounted for in mass balance modelling of microplastic; and 2) atmospheric transport of microplastic. The study sampled atmospheric deposition in the Pyrenees and illustrated notable quantities of small microplastic particles (365 MP/m²/day ±69, MP>10µm). The quantity of Pyrenean mountain atmospheric microplastic deposition was comparable to previous city studies (within city studies' quantitative range, e.g. 2-355MP/m²/day, MP>200µm). Due to the location of the sampling station, microplastic particles are suggested to have travelled at least 100 km, but potentially substantially further. The findings from this remote mountain atmospheric deposition study suggest that the atmosphere (air) needs to be considered further as an environment polluted by microplastics – beyond the urban/industry/agricultural source locations. It also suggests that microplastic may be polluting remote locations via atmospheric transport, and that further research is needed to understand the extent and impact of atmospheric pollution and transport of microplastics.





Distribution of microplastics in rivers: the example of the German river Elbe

Stock Friederike^{1*}, Kochleus Christian¹, Dierkes Georg¹, Scherer Christian², Weber Annkatrin², Wagner Martin³, Brennholt Nicole¹, Reifferscheid Georg¹

¹ Bundesanstalt für Gewässerkunde, Koblenz

² Goethe Universität Frankfurt, Department Aquatic Ecotoxicology, Frankfurt am Main

³ Norwegian University of Science and Technology, Department of Biology, Trondheim

***Presenter:** Stock Friederike, stock@bafg.de

Plastic pollution in the aquatic environment has gained worldwide attention in the last years. Meanwhile, intensive research activities have been initiated in these environments; however, the effects and consequences of the plastic pollution are not fully known and have to be better understood. In the frame of the project about macro- and microplastics in German rivers, samples from 11 sites from the German part of the river Elbe were taken in order to study the plastic pollution in water and sediment, detect the sinks of microplastics and better understand the transport mechanisms.

The sediment samples were taken with a Van-Veen-grabber, the water samples from the Elbe with an Apstein plankton net (mesh size 150 μm). The sediment samples were presorted with wet sieving, organic digestion and density separation, filtered on aluminium oxide filters and visually analysed. For the water samples, the organic matter was digested using a reagent composed of equal volumes of 10 M KOH and 30 % H_2O_2 , then, the (micro)plastic particles were isolated from remaining matrix by density floatation using 1.6 g/mL potassium formate solution and pressure filtration. Analysis was done by visual inspection, selected particles measured with pyrolysis GCMS and Fourier-transform infrared spectroscopy.

The results of the sediments of the Elbe point to a microplastic concentration depending on the sampling site (esp. polystyrene and polypropylene particles) and to a decrease in the flow direction whereas the water samples only show a site specific microplastic concentration.

STOP: Survey Techniques for Observing Plastics in riverine systems of Africa

Moss Kerry^{1*}, González-Fernández Daniel², Allen Steve³, Allen Deonie³

¹ Nelson Mandela University, Ocean Sciences Department, Gomery Ave, Summerstrand, Port Elizabeth, South Africa

² University of Cadiz, Biology Department, Puerto Real, Cadiz, Spain

³ WESP University of Strathclyde, Glasgow, UK

***Presenter:** Moss Kerry, kerry.leigh.moss@gmail.com

Only 4% of the world's published plastic research is on Africa, illustrating a large gap in plastic pollution knowledge. It is estimated that 64 to 90% of the oceans plastics originate from fluxes in the terrestrial environment, however determining the exact source is not a simple task. A study along 82 of South African beaches found mesodebris (2-25mm) to be concentrated around urban areas. Many African rivers predominantly run through human settlements that host informal waste dumpsites and lack municipal refuse services. However, little is known about the quantity of plastic pollution entering the river systems and marine environment from land. For the purpose of this pilot study, we incorporated two survey techniques across three rivers discharging into Algoa Bay, South Africa. A visual survey from a bridge using the RIMMEL application and a 2 x 1m STOP frame comprising of a 2000µm mesh net was deployed at Swartkops, Sundays, and Baakens River. The Swartkops River runs through an urbanized and industrial region, Baakens is a canalized urban drainage system, and the Sundays river catchment is sparsely populated, passing through the Addo Elephant National Park before arriving at the sampling site. The pilot study results illustrated; ~8000 macroplastic items and ~250,000 microplastic pieces flowing out of Sundays River, ~520,000 macroplastic items and ~260,000 microplastic pieces flowing out of Baakens, ~29,000 macroplastic items and ~1.2 million microplastic pieces flowing out of Swartkops per year. The STOP project will be deployed across 7 Western Indian Ocean countries with an additional 10 pan Africa countries expressing their interest. As well as a proposal for deploying the STOP protocol in rivers entering the Mediterranean. The collaboration will result in a standardized and comparable plastic waste baseline to which mitigation strategies efficiency can be observed.



Microplastic contamination of sediment and water column in the Seine River estuary

Alligant Soline^{1*}, Gasperi Johnny¹, Gangnery Aline², Maheux Frank², Simon Benjamin², Halm-Lemeille Marie-Pierre², El Rakwe Maria³, Dreanno Catherine³, Cachot Jérôme⁴, Tassin Bruno¹

¹ Laboratoire Eau Environnement et Systèmes Urbains (LEESU), 61 avenue du Général de Gaulle, Créteil, France.

² Ifremer, Laboratoire Environnement Ressources de Normandie, Avenue du Général de Gaulle, Port en Bessin, France.

³ Ifremer, Laboratoire Détection, Capteurs et Mesures, ZI de la Pointe du Diable, Plouzané, France.

⁴ University of Bordeaux, Laboratoire EPOC, UMR CNRS 5805, allée Geoffroy Saint-Hilaire, Pessac Cedex, France.

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Studies on microplastics (<5 mm, MPs) pollution are scarce in estuaries. Recently, scientists pointed out freshwater as an important source of microplastics to the ocean. Estuaries are now investigated as zones of primary interest for the transfer and possible accumulation of MPs between freshwater and ocean. Estuarine hydrodynamics and salinity gradient can affect MP levels, size and spatial distribution compared to lakes and rivers further inland.

The present study aims to quantify the levels of microplastic contamination in intertidal sediment and in the water column of the Seine River estuary.

Four campaigns were conducted between 2017 and 2019. Three sites were selected along the Seine River estuary. At each point, surface water was collected using plankton net, 300 μm mesh, towed for around 5 min. Collected volumes ranged from 5 m^3 to 90 m^3 . Additionally, 1.5 L of wet intertidal sediment was sampled using a Van Veen grab.

In the lab, organic fraction was removed from the water samples using chemical agent (SDS), and hydrogen peroxide. Then, microplastics were recovered in the supernatant arising from a density separation using a sodium iodide solution ($d=1.65 \text{ g}\cdot\text{cm}^{-3}$). Finally, supernatant was filtered, and filters observed under stereomicroscope. Sediment samples are dried at 50°C in a forced-air oven covered with aluminum foil to prevent any airborne contamination. Four replicates of 25 g of dry sediment per sample were subjected to the same steps as water samples were, but in different order.

First results for water samples show concentrations between 1.7 and 7.1 particles $\cdot\text{m}^{-3}$ (for particles >50 μm). Raman characterization of 25% per sample indicates between 20% and 45% of microplastics are PE & PS. In the sediment, first results show presence of fragments and microbeads, few fibers. Some specific fragments and microbeads are found in both sediment and water column. Analysis is still ongoing.

Filter - feeders as aggregator of microplastics in the marine system: A field experimental study

Piarulli Stefania^{1,2*}, Scapinello Sara¹, Primke Sebastian², Gerdts Gunnar², Airoldi Laura¹

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Ingestion of microplastics (MP) has been reported in laboratory settings for a wide range of marine biota but to what extent the uptake by organisms affects the dynamic and fate of MP in the marine system has received less attention. Filter - feeders such as mussels are known for modulating benthic – pelagic coupling and depositional processes of organic material in the marine environment. We evaluated whether *Mytilus galloprovincialis* can act as a vector for MP from the water column to sediments using a field experimental approach through the analysis of biodeposit. We explored whether mussels are able to ingest particles with specific characteristics and concentrate them into their biodeposit. Mussels or empty shells (used as control treatments) were placed in conical traps specifically developed for this study (4 replicates per treatment) for 1 week in every season to allow the filtering and deposition of MP into the biodeposits collectors. Samples (biodeposit and the soft tissue of mussels) were processed and analysed via automated imaging-FTIR to quantify and characterise MP uptaken and egested by mussels in the natural environment.

Results show a size selection operated by mussels that tend to concentrate in their biodeposit small MP (< 50 µm). We found differences in the quantities of MP and polymer typologies between seasons possibly reflecting either variable distribution of MP in the environment due to their physical-chemical properties or due to high/lower uptake rates operated by mussels when exposed to different environmental conditions. Overall, our results demonstrate that aggregation mediated by mussels may represent an environmentally relevant, viable pathway for the transport of MP from the water column to sediments with relative implications at ecosystem and community level.





**IMPACT OF MICROPLASTICS
ON MARINE LIFE**

The Impact of Microplastics on Filter-feeding Megafauna


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In this presentation we reconstruct the scientific story of the invisible war between the charismatic megafauna (baleen whale, filter feeder sharks and manta ray) against the smallest marine debris (microplastics) and their potential toxicological effects. The first warning of this emergent threat was reported by the Fossi and collaborators for Mediterranean baleen whales (*Balaenoptera physalus*) in 2012, and few years later (2014 and 2017) confirmed also, by the same team, for filter feeder shark such as basking shark (*Cetorhinus maximus*) and whale shark (*Rhincodon typus*). The authors report that filter-feeding megafauna are particularly susceptible to high levels of microplastic ingestion and exposure to associated toxins due to their feeding strategies, target prey, and for habitat overlap with micro-plastic pollution hot spots. Given the abundance of microplastics in some hot spot areas, such as the Mediterranean Sea, along with the high concentrations of Persistent Bioaccumulative and Toxic (PBT) chemicals, plastic additives and the detection of specific biomarker responses in the skin biopsies of these endangered species the authors suggest that the exposure to microplastics because of direct ingestion and consumption of contaminated prey poses a major threat to the health of this endangered marine species. For these finding and because many megafauna species investigated by this research team are charismatic and iconic indicators that serve as flagship species for marine conservation, this research field became recently a new “trend topic”. Currently the scientific community and the media are very attracted to this “story” despite this subject at the beginning has been treated with great suspicion. This scientific topic is also developed in the project Plastic Busters MPAs, recently financed by EU (Med-Interreg), focused on the study of the impact of microplastics on cetaceans inhabiting the Mediterranean SPAMI Pelagos Sanctuary. While umbrella species are useful for directing intervention strategies, flagship species could provide a global assessment of microplastics pollution and a mechanism for communicating awareness and stimulating action to tackle marine plastic pollution in all the marine ecosystems (Germanov et al 2018).





Effects of conventional and biodegradable microplastics on the attachment strength and haemolymph proteome of blue mussels (*Mytilus edulis*)

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The contamination of marine ecosystems with microplastics, such as the polymer polyethylene, a commonly used component of single-use packaging, is of global concern. Although it has been suggested that biodegradable polymers, such as polylactic acid, may be used to replace some polyethylene packaging, little is known about their effects on marine organisms. Blue mussels, *Mytilus edulis*, have become a “model organism” for investigating the effects of microplastics in marine ecosystems. We show here that repeated exposure, over a period of 52 days in an outdoor mesocosm setting, of *M. edulis* to polyethylene microplastics reduced the number of byssal threads produced and the attachment strength (tenacity) by ~50%. Exposure to either type of microplastic altered the haemolymph proteome and, although a conserved response to microplastic exposure was observed, overall polyethylene resulted in more changes to protein abundances than polylactic acid. Many of the proteins affected are involved in vital biological processes, such as immune regulation, detoxification, metabolism and structural development. Our study highlights the utility of mass spectrometry-based proteomics to assess the health of key marine organisms and identifies the potential mechanisms by which microplastics, both conventional and biodegradable, could affect their ability to form and maintain reefs.

Fish that live in different zones as possible indicators of microplastic pollution

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Ingestion of microplastics (MPs) by fish has been widely demonstrated, although the capacity of MPs to affect the ecology of fish is still to be understood. The aim of this study is to further investigate the uptake of MPs by species which are environmentally exposed to different habitats and with different feeding behaviours. Therefore, we compare the MPs inherent characteristics that affect their distribution in the water column and the behaviour of fish. The species: *Boops boops* and *Spicara smaris* have been chosen since they favour a midwater layer in the water column; *Oblada melanura* and *Scomber scombrus* are generally found close to the surface; *Mullus barbatus* and *Merluccius merluccius* are deep water species that live close to the seabed and generally found over soft sediments. A minimum number of 30 individuals for each species was collected locally from Sardinia (Italy). For each fish, basic measurements were recorded including length, body weight and gastrointestinal weight. The size classes are taken into consideration in order to note if the life stages are affected in different ways by MPs. The detected MPs are divided into typologies (fragment, film, sphere, rope/filament, sponge/foam, rubber and fiber), size classes (> 50 µm), morphology and colour, while the polymer type is determined by micro-Fourier transform infrared spectroscopy (µFT-IR) analysis. The results from this work can add useful information to show the relationship between the ingestion of MPs and the ecology of fish and eventually take one small step to understand the reasons for the ingestion. Moreover, this research could benefit the identification of appropriate indicators for the presence of MPs in the Mediterranean Sea.





Transfer of microplastics in marine foodwebs: an experimental approach

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Plastic production is rising rapidly and exceeded 380 million tonnes per year in 2016. Because the full degradation of plastic waste takes centuries or even millennia, the millions of tonnes ending up in the oceans every year are an ever-increasing pollutant in the marine environment. Currently, microplastics have become a ubiquitous pollutant in both the pelagic and benthic marine environment. Field studies and lab experiments have reported the voluntary or accidental ingestion of microplastics in at least 220 species, representing a variety of trophic groups. So far, most studies focussed on potential effects of microplastics on the individual level but how ingestion of microplastics alter functioning in marine communities is largely understudied.

In this presentation we report on two experiments that studied two different pathways of microplastic transfer within marine foodwebs. A first study demonstrated that behavioural change in meroplanktonic larvae (*Limecola balthica*) due to the ingestion of 5 μm polystyrene beads decrease their predation by a filter feeding benthic bivalve (*Cerastoderma edule*). In a second study, we demonstrated that the Blue mussel, *Mytilus edulis*, can largely increase the concentration of microplastics at the seafloor through the incorporation of microplastics in biodeposits. Subsequently consumption of microplastic-containing biodeposits by detritivores can keep microplastics in circulation within the food web. Alternatively, bioturbating species may deposit or bury the microplastics deeper into the sediments, therewith partially removing microplastics from marine food webs.

Effects of Seagrass meadows on microplastic deposition

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It is well known that over the last few decades, microplastics have been entering and accumulating in the marine environment. Microplastics have been identified as contaminants of global concern and are known to accumulate in coastal sediments thus are potentially bioavailable and harmful to many marine species and vulnerable ecosystems such as seagrass systems. While there have been a few studies into the occurrence of microplastics in some tropical coastal vegetation systems such as Mangroves, there are no data currently on contamination of seagrass sediments. Seagrass meadows, such as *Posidonia oceanica*, usually feature dense underwater canopies. Despite the fact seagrass meadows of *Posidonia oceanica* are known to influence and reduce the hydrodynamics of water they have been little studied. Seagrass meadows have very high sedimentation rates with minimal resuspension occurring within the canopy, meaning grain size is generally smaller than surrounding un-vegetated sediment. Thus it is possible that under these conditions there is an increase in microplastic deposition and retention within these canopies, potentially making them an important sink for plastic pollution. To test this hypothesis sediments were collected and analysed from a dense seagrass bed in Greece, from the Island of Lipsi. Samples were collected at 5m intervals along a transect which crossed both meadow and bare sand. Microplastics were extracted using Sediment Microplastic Isolation (SMI) Units and identified using a dissecting microscope. Initial data show a positive correlation between total microplastics found and seagrass shoot density, and differences in types of microplastics found in seagrass sediments and bare sand. The number of microplastic fragments found was higher in sediments from seagrass meadows than un-vegetated areas, while microfibrils were slightly more common in un-vegetated areas than seagrass sediments. This research provides much needed information on the deposition of microplastics in coastal waters and potential effects on key coastal ecosystems.



Microplastic in water and biota: a Research and awareness campaign in the Mediterranean Sea

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In 2017 the international tour “LESS PLASTIC MORE MEDITERRANEAN” has been carried out by Greenpeace in collaboration with CNR and UNIVPM to gather data on plastic pollution and to inform people about this environmental emerging issue. During the tour an extended microplastics (MPs) investigation was carried out: water samples were collected using both Manta and Plankton nets (mesh size 330 and 200 μm) from 17 sites along the Italian coasts, characterized by different level of anthropogenic pressure (from ports, river mouths to MPAs). Moreover, in some of these stations, a total of 201 organisms, belonging to 18 species of fish and 3 of invertebrates, were collected in collaboration with local fishermen. Plastic origin of microparticles isolated has been confirmed by FT-IR analysis.

Water analyses confirm MPs presence in all the stations with an average of 0,52 items/ m^3 . The maximum value (3,56 items/ m^3) was observed in Portici (a highly stressed site) but a high number of MPs (2,2 items/ m^3) was even found in the Tremiti Islands MPA suggesting that these isolated systems are subjected to water flow dynamics able to enrich local biodiversity but even to make pristine areas potential hot spot for MPs accumulation.

Organisms investigation pointed out that MPs ingestion is a widespread phenomenon in the Tyrrhenian Sea, since 25-30% of organisms ingested almost one microparticles; slight geographical differences occurred in terms of size and typology of ingested particles (mainly PE, EVA and PP) and a no clear relationships was observed between microplastic ingestion in different species and trophic position, feeding strategy or habitat preference. A special focus is given to the results obtained on the Giglio Island, where a significant recovery of microplastic pollution has been observed in fish (95% vs 35% of fish positive to the MP ingestion), three years after the Costa Concordia wreck removal.

Microplastic ingestion by a marine fish community of Israeli coastal waters

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Ingestion of microplastic by marine fish has been well documented in marine environments throughout the world's oceans. However, sampling efforts often focus either on single or few species, limiting the understanding of the unique species interactions with microplastics as well as its movement within a trophic community. As fish can be characterized by different lifestyles based on habitat and feeding preferences, such interactions can provide insight to the driving factors of ingestion as well as identify more groups more susceptible to this marine contaminant. In Israel, while high concentrations of microplastics have been shown in surface waters, the possible interactions with biota have not been explored. Here, we conduct an initial investigation of microplastic ingestion by fish from coastal waters and assess the interaction dynamics of microplastic via consumption by a local fish community. A total of 771 specimen from 33 different fish species were collected belonging to a range of different trophic levels and categorized by six different habitats; benthopelagic (n=238), coastal-pelagic (n=51), demersal (n=270), pelagic-neritic(n=120), pelagic-oceanic (n=67), and reef-associated (n=25). Pelagic-neritic fish were found to have significantly higher ingestion rates compared to other lifestyles; specifically, two species of planktivorous fish, the Atlantic chub mackerel (*Scomber colias*) and the European anchovy (*Engraulis encrasicolus*), which exhibited a particle contamination rate of 45% and 30% respectively. Additionally, intraspecies differences in contamination was observed between juvenile and adult mackerels (59% and 0% respectively), characterized by a shifting foraging behavior from small zooplankton to larger prey. Here we show that in this specific fish community, fish foraging zooplankton prey in the upper water column (pelagic-neritic zone), where microplastics tend to aggregate, appear to be more susceptible to microplastic ingestion. Importantly, we highlight how considering the lifestyle characteristics when assessing microplastic ingestion contributes to our understanding of its dynamics within a trophic community.





**SOURCES, FATE AND EFFECTS
OF MICROPLASTICS**

Plastic Debris s Urban Water and in Freshwater: Lessons Learned from Research Projects Launched in the Seine Basin Catchment

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Since 2014, several research projects were launched on plastic debris issue at the scale of the Seine Bassin catchment, combining a high population density and a strong anthropogenic pressure. These projects are investigating both macro- and micro-plastics (< 5 μm) in urban water and in freshwater upstream and downstream of Paris Megacity (France). The keynote will provide a global overview on the knowledge gained from these projects and draw the major learned lessons.

A first part of this keynote will be dedicated to macroplastic pollution and will present first levels found in urban water. Based on tagged plastic litters and GPS-trackers, the fluxes of plastic litter in the Seine River were estimated. Our results suggest that for countries having a high GDP per capita as France, the assumption of 2% of mismanaged waste proposed by Jambeck et al. should be revisited.

The second part will focus on the microplastic contamination, by reviewing the levels of microplastics in urban water, in total atmospheric fallout, as well as in freshwater from Paris megacity to the Seine River estuary. Both water column and sediments will be considered. To give perspective, the main scientific barriers and issues related to microplastics in freshwater will be also discussed.



Overview of microplastic presence in Italian freshwater systems: monitoring of principal lakes and rivers

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Recent studies have reported high concentrations of microplastics (1-5 mm) in lakes and rivers, although the understanding of several factors influencing source, transport and fate is still limited. Due to the role of rivers and lakes as suppliers of plastics to the marine environment, it is important to collect more data on microplastics from freshwater environments. There is a knowledge gap related to microplastics abundance and dispersion in rivers and lakes, and, particularly, little is known as regards the situation in Italian freshwater systems. This study compares different lakes and the common factors, which could influence the occurrence and distribution of microplastics.

Starting from summer 2016 Legambiente and ENEA are carrying out a joint research on microplastics presence in the main Italian lakes. Sampling have been performed during the last three editions of the Legambiente's national campaign "Goletta dei Laghi". All in all 10 lakes, many of them in alpine regions in northern Italy (Maggiore, Orta, Como, Iseo, Garda, Cavazzo) and in central Italy (Trasimeno, Bolsena, Bracciano, Albano, Paola), 4 rivers, main tributaries of the River Po (Adda, Oglio, Sarca-Mincio) and important input and output of lake basins (Como, Iseo and Garda, respectively) and 30 beaches of the main lakes (Maggiore, Como, Iseo, Garda, Trasimeno and Bracciano) were monitored. Data collected, analysed by ENEA Casaccia researchers, highlight the influence of the wastewater treatment plant and appears that the difference detected between the samples taken downstream and upstream of the wastewater treatment plant can reach up to 80% of particles/m³. There is an undeniable relationship between municipal discharges, sewage, urban runoff and storm water from rivers-lakes system to sea. The results presented in this work demonstrate significant presence of microplastics in Italian freshwater systems, comparable to other lake findings, and underline different trends during several years.

Atmospheric transport and our planetary boundary layer as an environmental compartment of microplastics pollution

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An increasing number of researchers are starting to ‘look up’ in the search to understand the spatial extend of microplastic pollution. Recent studies illustrate city air pollution by microplastic in Paris, Dongguan and Shanghai. However, microplastics have been found in remote locations as far as the surface lakes of the Arctic, the Swiss mountain floodplains and in the atmospheric deposition of the French Pyrenees. This indicates that this pollutant is not bound to its sources but transported potentially long distances to remote locations. The transport of microplastic from source to sink via rainfall-runoff-rivers to sea is becoming well established, but the counterpart aerosol process has yet to be unravelled.

The planetary boundary layer, the air between earth’s surface and the clouds (free troposphere), is a new environmental compartment of consideration in both microplastic pollution and transport. Very early steps towards understanding the extent of microplastic transport are being undertaken, using known atmospheric transport tools. When Saharan dust is used as an example, studies such as van der Does et al. 2018 on trans-Atlantic transport of ultra-giant particles suggest aerosol microplastic pollution could travel long distances; enabling land-bound cities to pollute marine and remote environments via atmospheric transport.

Current atmospheric transport tools include particle and solute pollution models such as HYSPLIT4 and Flexpart. These models support back-trajectory modelling of air parcels and particles to identify how far microplastic may have travelled (a step towards source identification). However, there are still many unknown variables on microplastic behaviour in the atmosphere: the influence of charge and shape on aerodynamics; their cohesive or non-cohesive behaviour; wet vs dry deposition rates and the influence of precipitation scavenging to name just a few. With these questions and knowledge gaps in mind, early attempts to understand the transport, possible sources and fate of atmospheric microplastics are presented.



Microplastic debris in an Italian river: first monitoring results

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Plastic and microplastic pollution is one such emerging concern. Plastic pollution in the marine environment is well documented, however, there is a lack of knowledge for freshwater and terrestrial environments in terms of extent, occurrence and impacts. Herein we report results from a monitoring study aimed to investigate about abundance, type and composition of microplastic in an Italian river (Ofanto river), evaluating at the same time the presence of temporal trends in response to hydrological parameters (flow velocity, water level of river and precipitation). River surface microplastic samples were collected, during five monitoring campaigns, by three surface plankton nets of 333 μm mesh size fixed in the middle of the river simultaneously for two different time slot for a total of six replicates for each campaign. Chemical digestion was performed in order to remove the labile organic matter and extract all the particles. After processing, a visual and chemical characterization has been carried out. Each particle visually identified, was counted, photographed and categorized according to size, color and morphology. Microplastic content measured in Ofanto river (expressed as mean value \pm dev.st.) ranged from 0.9 ± 0.4 p/m³ to 12 ± 5 p/m³ showing values comparable to or greater than those reported in other studies. A statistically significant difference in the average microplastic concentrations found in different campaigns of this study, has been observed, suggesting thus a temporal variation in plastic abundances. These differences could be explained by the hydrology of the river that influences, with physical forces the concentrations of particles. Microplastics were found at higher concentrations during wet periods (February 2017 and May 2018) indicating a presumable land-based origin probably from surroundings agricultural areas. The work reported here is the first study showing an Italian river context that provides an initial assessment of the extent and nature of this pollution in Ofanto river.

Characterization of plastic-associated bacterial community in freshwater lake of Vácszentlászló, Hungary

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Despite the great benefits of plastics in different aspects of the life, and due to the increase in plastic production and use, plastic wastes are becoming of major environmental concern. It is well known that inappropriate use and disposal also lead to accumulation of plastic litter in different aquatic environments. Microbial biofilm is able to develop on the surface of plastics (plastisphere) in aquatic environments with time. In our study it was aimed to describe the bacterial community associated with plastics in freshwater. Thus, a total of nine self-designed plastic colonizers were submerged under the surface of water in Vácszentlászló lake located in central Hungary, for a period of three months. Three plastic colonizers were cultivated every month and bacterial community associated with plastic samples then were analyzed as follows: a.) culturable bacteria were isolated from plastic surface and identified by 16S rRNA gene sequencing b.) bacterial community was studied using terminal restriction fragment length polymorphism (T-RFLP) and followed by amplicon sequencing c.) besides these molecular analysis of plastic colonizing community, surface water samples from the lake was also taken and analyzed by the same methods. By the results of aerobic isolation, the following notable bacterial groups were identified: bacteria related to fish spoilage, like *Bacillus simplex*, and bacteria with possible biodegradation capability like *Pseudomonas antarctica*, published as hydrocarbon degrader. Some bacterial isolates are also known as responsible for threatening environmental safety like *Aeromonas bestiarum* which is known as a fish pathogen and *Shewanella putrefaciens* which may be an opportunistic human pathogen. By the results of T-RFLPs, the plastic colonizing community shows notable differences comparing to water samples, and it was also verified by the results of amplicon sequencing.

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Microplastic pollution at an urban site in Rome

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The plastic has been widely used in numerous applications gradually replacing materials such as glass and metal. In the last few decades great attention has been paid to the pollution produced by microplastics as it represents an emerging risk.

Air quality data from a one-year study at an urban roadside location in Rome are reported for major pollutants. Continuous concentration data of carbon monoxide, ozone and nitrogen dioxide were measured in Rome in 2017. Moreover, PM_{2.5} samples were collected for one week in winter and summer period. PM_{2.5} mass was measured by gravimetric determination. PM_{2.5} was also collected on polycarbonate membranes for single particle characterization by field emission scanning electron microscope equipped with energy dispersive X-ray spectroscopy (FESEM/EDX). The data of X-ray microanalysis obtained for each particle were analysed by Hierarchical Cluster Analysis (HCA) to classify the particles into groups and identify the most important components of PM_{2.5}. CO and NO_x diurnal variations of all working days followed a similar pattern suggesting a predominant common emission source: the vehicular traffic. Physico-chemical characterization of PM_{2.5} single particles, carried out by FESEM/EDX analysis displayed the particle presence from natural and anthropogenic origin. The HCA analysis identified four principal clusters of particles in winter: aerosol marine, carbonaceous particles, silicates and metal particles. Aerosol marine showed an abundance ranging from 8% to 19% and its contribution to PM_{2.5} was confirmed through the analysis of backward-trajectories calculated by the Hybrid Single-Particle Lagrangian Integrated Trajectory Model (HYSPLIT- NOAA Air Resources Laboratory). During summer, the HCA analysis identified four principal clusters of particles: carbonaceous particles, silicates, carbonates and metal particles. Microplastic particles were detected in all analysed samples in both periods. Preliminary results showed that microplastic particles size were smaller in winter than in summer, besides the most particles detected in summer appeared transparent.



**EFFECTS OF MICRO
AND NANOPLASTICS
ON ACQUATIC ORGANISMS**

Insights on Ecotoxicological Effects of Microplastics in Marine Ecosystems: The Ephemare Project

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The Ephemare project, recently funded by JPI-Oceans, provided novel insights on fate and ecotoxicological effects of microplastics in marine ecosystems. A large selection of biological models was tested, including species of different trophic levels and life stages. Mechanisms underlying uptake, transfer and toxicity of MPs remain largely unknown, but targeted experiments revealed the influence of “size” and “shape” in modulating such processes. Despite the lack of acute toxicological responses, MPs induced slight cellular and physiological effects, particularly on immune responses and reproduction, suggesting potential long-term effects under chronic exposure conditions or multiple-stressors scenarios. Field studies confirmed a high frequency of ingestion, from zooplankton to top predators, in marine food webs from several European areas. Sorption behaviour of pollutants on MPs was shown as a rather dynamic process and desorption was even more difficult to generalize in natural field conditions. Even though MPs can act as vectors of pollutants, they did not increase bioavailability of model chemicals compared to other marine particles. At the same time, MPs altered the timescale of exposure, tissue distribution and the sub-cellular compartmentalization of chemicals, potentially resulting in subtle and long-term detrimental biological effects. The possibility of MPs to modulate responsiveness toward other multiple stressors including climate change deserves attention.

What can model polystyrene nanoparticles teach us on the impact of nanoplastics in bivalves? Studies in *Mytilus* from the molecular to the organism level

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The concept of nanoplastics has recently emerged carrying with it the idea of their possible distinct impact on aquatic organisms. Microplastics may be degraded into nanosized particles under abiotic conditions. However, as for microplastics, the utilization of model nanoplastics may provide first information on their potential toxicity in marine species. Based on our previous experience on the impact of different types of nanoparticles in the Mediterranean mussel *Mytilus galloprovincialis*, thorough studies were carried out utilizing amino modified nanopolystyrene (PS-NH₂), considering several pathway of exposure and effects at different levels of biological organization, in order to provide a full coverage of the impact of this model nanoplastic in mussels. First screening using *in vitro* test showed immunomodulatory effects of PS-NH₂ in mussel immune cells, the hemocytes. Studies on interactions of PS-NH₂ with proteins from biological fluids (e.g. hemolymph) that can adsorb on the surface of NPs, forming a protein corona, contributed to understand the link between their recognition by hemocytes and consequent effects. Comparison with carboxy modified PS-COOH showed distinct effects and interactions. PS-NH₂ also affected early embryo development, with dysregulation of genes involved in shell formation. Further *in vivo* exposure of adult individuals confirmed their immunomodulatory effects from molecular to organism level. In addition, PS-NH₂ induced oxidative stress and inflammation at tissue level (gills, digestive gland). Finally, recent studies showed that PS-NH₂ exposure could induce shifts in hemolymph microbiota composition.

Comparative studies on cold water bivalve species are also in progress utilizing the Antarctic mussel *Laternula elliptica*.

The results obtained so far represent the most extensive information available to date on the responses of marine invertebrates to nanoplastics. These studies may represent the basis for better understanding how nanoplastics can interfere with the health of key species and contribute in to label them as emerging pollutants in marine ecosystems.

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Uptake and stress-induced responses of polystyrene microbeads in adults of the sea urchin *Paracentrotus lividus*

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Plastic pollution represents one of the major threats to the marine environment. A wide range of marine organisms, including fishes, polychaetes, zooplankton, sea cucumbers, mussels, corals have been shown to ingest microplastics for their small dimensions less than 5 mm. The ingested microplastics may translocate to the circulatory system or be transferred via planktonic organisms to a different trophic level. Microplastics ingestion negatively affects many biological processes, such as feeding, energy reserves and reproduction. Very few papers appeared in literature on the effect of microplastics on sea urchin development and virtually none on adult sea urchins. The aim of this work was to evaluate the uptake and the ecotoxicity of virgin polystyrene fluorescent beads (10 and 45 μm in diameter) on adult sea urchin *Paracentrotus lividus*. Uptake was observed both in the digestive and water vascular systems based on microbeads size. They were also found in the coelomic fluid and in the gonads. Treatment of sea urchins with 10 μm particles induced an increase of the total number of immune cells already after 24h, whereas for 45 μm particles the increase became significant only after 48h. To evaluate the oxidative and nitrosative stress status of coelomocytes, a series of assays, including the determination of reactive oxygen species, reactive nitrogen species, in particular nitric oxide, and total antioxidant activity were run. Additionally, general health status was investigated by measuring oxidative stress, enzymatic activities related to biotransformation processes (catalase, glutathione-S-transferase), lipid peroxidation and nitric oxide levels in the gut. Overall data indicate a stress-related reaction to polystyrene microbeads in sea urchins. This represents a first study assessing polystyrene microbeads uptake and ecotoxicity in adult *P. lividus* sea urchin.

Seasonal importance on microplastics composition and concentrations in two matrices; coastal waters and wild mussels (*Mytilus edulis*)

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Microplastics, particles measuring 1µm to 5mm, are ubiquitous in the environment from the deep-sea to mountain tops. It is estimated that between 4.8-12.7 million tons of plastic enters the ocean globally each year with a possible river input of 1.15-2.41 million tons. Coastal waters are the interface between land (representing main source of plastic debris input) and the open ocean where the majority of microplastics are deposited. However, the influence of multiple stressors on the dynamics of microplastics in this environment has not yet been extensively studied. It is hypothesised that the coastline could be a potential sink and source for microplastics that are accumulating on the shore or released into the sea. Microplastics has been studied temporally in coastal waters and fauna, but the results have not proved to be convincing; some studies have highlighted a seasonal change whilst others the opposite.

In this study we have quantified and characterised microplastics observed in coastal waters (from the shore) and fauna (*Mytilus edulis*) at six different sites along the North and West coast of Scotland. Seasonal variation of small plastics was investigated and the effect of hydrodynamic/anthropogenic activity on their distribution along the coast. Microplastics were identified in all water samples for the six locations with concentrations fluctuating between seasons. The predominant plastic shape recorded was fibres. Of the mussels examined (n=240), all the samples contained potential microplastics ranging from 0.14 ± 0.17 to 4.39 ± 3.44 particles per gwwt-1 between the six sites and seasons.

Future work will examine microplastics within intertidal sediments during a lunar cycle to assess the effect of tides on the accumulation on the shore. Additionally, microplastic abundances in coastal water and sediment will be studied during an exceptional event such as a storm, to understand the subsequent effects on microplastics accumulation on sandy beaches.



In vitro effects of mercury (Hg) on immune function of mussel (*Mytilus Galloprovincialis*) are enhanced in presence of microplastics in the extracellular medium

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We investigated the *in vitro* effects caused by mercury (Hg) on phagocytosis capacity and lysosomal membrane stability (LMS) in freshly primary cultures of hemocytes from mussel (*Mytilus galloprovincialis*) and how these effects are modulated by the presence of different polyethylene (PE) microparticles (commercial stocks with mean size range from 15 to < 6 μm) in testing solutions prepared using seawater. Testing solutions of Hg ranged from 10^{-5} to 10^{-9} M and microplastics (MPs) suspensions were prepared at 1 and 100 $\mu\text{g}/\text{mL}$ for each MP type: MPP-635 XF, AQUATEX-325 (oxidized PE) and AQUAMATTE 26HD (oxidized and modified PE). Analysis of the number of particles per mL and the 90th percentile of particle size (D90) of each MP suspension and its corresponding dilutions as well as Hg concentration in testing solutions were measured before and after performing the bioassays. Hemolymph samples from at least 12 individual mussels were used for each experiment/treatment. Concentrations of Hg \geq than 10^{-6} M in the extracellular medium caused acute destabilization of LMS and a significant inhibition of phagocytosis. The different MP types at concentrations tested did not cause acute effects on any of these immune-related functions. However, for some MP types, hemocytes exposed to MP suspensions and Hg concentrations $< 10^{-7}$ M showed acute effects on LMS and phagocytosis. Notably, the results showed quite different number of particles per mL between suspensions of different MP type that had been prepared at the same concentration (up to an order of magnitude). Furthermore, generally microparticle number was directly correlated with dilution factor, indicating particle disaggregation processes in seawater. Total Hg concentration in MP suspensions tested did not exceed 10^{-7} M. Synergetic effects of combined exposure to MPs and Hg are discussed in light of these results.

Microplastics in the river Lambro (Northern Italy): monitoring and toxicity

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Microplastics (MPs) represent an increasing environmental problem in both aquatic and terrestrial environments. Despite the 80% of global plastic pollution has inland sources, the majority of studies were conducted on marine ecosystem. The aim of our study, according to the key principle of Environmental Risk Assessment (ERA), concerned the monitoring of MPs in the River Lambro (RL, Northern Italy) as well as the evaluation of their toxicity (sub-lethal effects) on freshwater species. We proposed a new approach based on the toxicity evaluation of environmental MPs, and not of MP standards, directly collected in the RL with the eventual chemicals adsorbed on their surface. Considering that the RL crosses an industrial and urbanized area and receives treated effluents from about thirty Wastewater Treatment Plants, we sampled MPs in five stations along the river, from the source to its inlet in the River Po. To have comparable samples for both monitoring and toxicity evaluation, we sampled MPs using simultaneously two plankton net with a mesh of 300 μm , equipped with a flowmeter to calculate the filtered water volume in the 30 min of sampling. We characterized MPs using the Fourier Transform Infrared Spectroscopy (FT-IR) equipped with μATR (Attenuated Total Reflectance). To evaluate the toxicity of collected debris, we exposed the freshwater mussel *Dreissena polymorpha* for one week and in static conditions to the environmental mixtures of MPs. Sub-lethal effects were assessed through biomarkers of cellular stress (reactive oxygen species content, activity of antioxidant/detoxifying enzymes superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase), oxidative damage (protein carbonyl and lipid peroxidation content) and cyto-genotoxicity (Tripin blue exclusion method, micronucleus test, apoptotic and necrotic frequencies). The uptake of MPs was certified using the FT-IR on the homogenates of exposed organisms.



Study of chemical pollutants over marine microplastics based on their composition and degradation rate

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Over marine microplastics they are adsorbed large amount of persistent organic pollutants (POPs), mainly polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs). These POPs have much more affinity for the plastics than seawater and therefore they tend to accumulate over microplastic surface. But this affinity and preconcentration is not the same for all kind of plastics. This study compare POPs adsorption rate over the most abundant plastics: Polyethylene Terephthalate (PET), High Density Polyethylene (HDPE), Polyvinyl Chloride (PVC), Low Density Polyethylene (LDPE), Polypropylene (PP) and Polystyrene (PS). Moreover adsorption rate vary on function of physical and chemical degradation state of the plastics. POPs adsorption on microplastic is generated over their surface, mechanical friction of microplastic fragments and pellets (physical degradation) increase their relation surface/volume and therefore their capacity to POPs preconcentration. But also, chemical degradation of the plastic, measure by microplastic yellowness, increases the adsorption rates of these pollutants over microplastic, added to the fact that the most yellowish fragments are also the ones with more microfissures with higher relation surface/volume.



In this study it has been evaluated on one side the adsorption rate of persistent organic pollutants (POPs) over different kind of plastic fragments, with different composition, and on the other, the evaluation over the same kind of plastic (HDPE), with similar size and shape, but at different physical and chemical degradation conditions.



**EVALUATION AND
DETECTION OF
MICROPLASTICS**



Marine Litter: Are There Solutions to this Global Environmental Challenge

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Plastic debris is widely distributed at the sea surface, on the sea bed and on shorelines. Nearly 700 species are known to encounter marine litter, with many reports of physical harm resulting from entanglement in and ingestion of plastic. At the same time it is very clear that plastic items bring many societal benefits. Can these benefits be achieved without emissions of waste to the environment? Progress requires systemic changes in the way we produce, use and dispose of plastic. A key solution to two major environmental problems, our non-sustainable use of fossil carbon (to produce plastics) and the accumulation waste, lie in recycling end-of-life plastics into new products.

Development of a thermo degradation method to assess levels and distribution of microplastics in marine sediments and its application in two case studies: the northern Adriatic Sea (Italy) and the Boknafjord (Norway)

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Plastic waste is of increasing concern in the aquatic environment. A large portion of the plastic waste is produced onshore and reaches the marine environment, which is considered the main sink of plastic debris. Floating plastic particles accumulate in pelagic habitats. However, due to the biofilm formation they eventually sink and accumulate on the seafloor together with non-buoyant by design plastic particles posing risk to the benthic communities. There is, however, a considerable lack of standardized methods to characterize micro plastic particles occurrence and composition. In the present study, a recently validated thermal degradation method to characterize the microplastic occurrence and polymeric composition in marine sediments collected in the northern Adriatic Sea and in an urban fjord located in the south-west coast of Norway are presented. Ten sampling sites were selected from each case study. Small microparticles (< 500 µm) were extracted from 2 kg sediment samples by a MicroPlastic Sediment Separator instrument (MPSS), size fractionated in four discrete classes ranging from 20 to 500 µm and analysed by first visual microscopic inspection and then by pyrolysis gas chromatography/mass spectrometry. Detected plastic particles were identified as polypropylene, polyethylene, polyethylene terephthalate, polyvinylchloride, polystyrene and polyamide. An attempt to identify and quantify the organic plastic additives was performed being 1,2-benzenedicarboxylic acid, dimethyl phthalate, diethylhexyl phthalate, dibutyl phthalate, diethyl phthalate and tert-butylphenol the most recurrent compounds. The preliminary results indicate large differences in total content, grain size and polymeric composition in the two investigated areas. Values ranged from < 1,0 to 170 µg/kg in investigated sediments.





Optimising the workflow for microplastic analysis by FTIR microscopy

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Analysis of environmental samples containing microplastics is essential to determine their prevalence and their impact. A range of analytical techniques have been applied to the analysis of microplastics. Of the techniques adopted, infrared (IR) spectroscopy, and more specifically IR microscopy, has established itself as a primary analytical technique for the detection and identification of microplastics. The microplastics analysis workflow for IR microscopy consists of several steps involved in getting from the raw sample to answers, including the initial sampling through to data analysis. The steps involved may be different depending on the type of initial sample and the amount of sample cleanup required to prepare the sample for infrared (IR) analysis. This paper describes the different types of environmental samples, the sample collection methods, the range of different sample cleanup methods, and then deals more specifically with the best ways to optimise sample filtration for measurement by IR microscopy. The principles of IR microscopy and the different sample measurement modes will be described, comparing and contrasting each type. IR microscopy and imaging experiments can generate significant quantities of data that need to be analysed to get the required information. The different methods for extracting data and information will be explained and suggestions made for best practice.

Microplastics in Hungarian freshwaters: development of a standard addition sample preparation method

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Microplastics have been detected globally in a wide range of environmental elements, but the focus on freshwater areas is moderate compared to oceans and seas. Freshwater studies have mainly been adopting sampling and sample preparation procedures from marine research, but there are no commonly agreed standard methods yet. There is a high uncertainty in sampling which is hard to monitor, and also particle loss can occur during sample preparation that is barely examined. Quality assurance steps are need to be taken to improve the accuracy of microplastic analysis results.

To obtain information on the efficiency of the sample preparation, different salt solutions (NaCl, CaCl₂, ZnCl) and glass equipment (separation funnel and two specially designed glassware) have been tested. For these tests freshwater samples were collected from different rivers and lakes in Hungary using a jet pump and stainless-steel filters. At every location ca. 2,000 L water was sampled. As surrogate standard, fluorescent polystyrene particles (0.97-1.2 g/cm³; 100 and 300 μm in diameter) were added to the samples. The main benefits of this standard are that it is easy to track with a UV light microscope and these particles are not present in natural environmental samples. Experiments were run in triplicate. Results show that ZnCl with one of the specially designed jars shows the highest recovery level between 90-100%, while separation funnel with NaCl the lowest values (37%).

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First evaluation of continuous flow centrifugation as a novel straightforward and non-size-discriminating sampling technique for microplastic in waters

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Microplastic has been detected in a plethora of environmental systems and organisms. Different kinds of sampling, sample processing, e.g. density separation, chemical and enzymatic sample purification, and detection approaches have been applied. Despite some recommendations for these steps from organizations such as the NOAA or the MSFD Technical Subgroup on Marine Litter (EU), there is no standard operation procedure for microplastic sampling. Many studies on microplastic abundances in environmental samples do not indicate a sound methodological validation of the applied methods and procedures. Recoveries for the overall applied procedures, especially for the sampling process, are mostly not provided or, if yes, only the particle-sizes given by the producers are stated. From author's point of view sampling still poses the weakest link in the analytical process related with environmental microplastic analysis, since most studies apply size-discriminating sampling techniques. As small microplastic bears the highest potential of biological uptake, translocation and adverse effects, it is highly important to quantitatively sample these particles. The present study provides an approach to eliminate the trade-off between clogging-problems when using small meshes (10 - 50 μm) for small microplastic sampling and the size-discrimination of high volume microplastic sampling employing larger mesh sizes (100 - 333 μm). For the validation of the instrumental setup, five polymer types (referring to 73% market share) were employed namely polyethylene, polyethylene terephthalate, polypropylene, polystyrene, expanded polystyrene and polyvinylidene chloride microplastic powders. These respective particles covered a broad size range from 1 μm to 1 mm and a density range from 0.94 g mL^{-1} to 1.63 g mL^{-1} . Recoveries ranged between 95.0% \pm 2.3% and 99.1% \pm 0.3% for virgin powders. For powders deployed in river water for 40 days, recoveries even ranged between 96.1% \pm 0.6% and 99.4% \pm 0.2% (1 SD ($n = 3$)).

Microplastics – what else? Quantification in environmental samples using pressurized liquid extraction and Pyr-GC-MS

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Fast and reliable quantification of microplastics in environmental samples is currently a challenging task. To enable monitoring of MP, a fast and robust method in sample preparation and subsequently analysis is of extraordinary need and urgency. Therefore the combination of pressurized fluid extraction and pyrolysis GC-MS has been developed. The full automated extraction includes a pre-extraction via methanol for matrix elimination and a subsequent main extraction for MP using tetrahydrofuran in pressurized liquid extraction to relatively enrich microplastics on silica which is hence analyzed by means of pyrolysis GC-MS.

Several commonly occurring organic matrices known to result in GC interferences were tested to be eliminated by pressurized fluid extraction. For the most frequently used synthetic polymers PE, PP, and PS extraction efficiencies of 113-131, 80-98, and 70-118 %, respectively, and limits of quantification down to 0.005 mg/g were achieved.

The new developed method was validated and applied to environmental samples with complex matrices such as roadside soils, potting soils and sewage sludge. In all these matrices PE, PP, and PS were detected with contents ranging from 0.8 to 3.3, 0.01 to 0.36, and 0.06 to 0.61mg/g.



Microplastics extraction and counting from wastewater and sludge through elutriation and hydrocyclone

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Microplastics are directly released from the wastewater treatment plants (WWTP) to the oceans and rivers because of their resistance to biodegradability, being consumed by marine species changing the food chain. This affects the human health due its toxicity and carcinogenic properties. Some studies focus in the microplastics extraction through elutriation and hydrocyclone separation.

This project studies the extraction and the microfibers recount on samples of different WWTPs located near textile industries.

A pre-treatment based on Fenton process was performed to remove the organic matter.

The elutriation process was done using a methacrylate column. 5 L samples were introduced and filled with tap water at $0.05 \text{ m}^3 \text{ h}^{-1}$, extracting by flotation the less dense portion. Then, the sample is pumped into a multi-hydrocyclone (12 units) at $3 \text{ m}^3 \text{ h}^{-1}$ flow rate.

The microfibers were analysed by FTIR and raman microscopy. The presence of microplastics was observed in all the samples. Different forms were identified as microspheres presumably coming from personal care products.

Application of thermal extraction/desorption-pyrolyse-GC/MS to investigate sorption kinetics of pollutants and the identity of (sub)microplastic

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The methods available for the analysis of microplastic in environmental matrices are currently mainly designed for particles in the size range of 1 μm to 5 mm. Particles below 1 μm have hardly been investigated so far. However, due to their small size, these particles may act as a potential vector, sorbent or source of metabolic inhibitors and toxins. Furthermore, as a result of the higher surface to volume ratio, there is probably a higher adsorption capacity for chemical substances, e.g. pollutants like pesticides and thus a higher ecotoxicological relevance. For this size range in particular, standardized and established detection methods and toxicological evaluations are still lacking.

The objective of this study is to investigate sorption processes of various pollutants, e.g. pesticides, on (sub)microparticles. Here, thermal extraction/desorption-pyrolyse-GC/MS (TED-Pyr-GC/MS) is employed. Ad- and Desorption experiments are carried out with selected contaminants and (sub)microparticles of various types and sizes of plastic. An innovative development of the TED-Pyr-GC/MS enables identification of the adsorbed pollutants and the type of plastic in one single analysis. Initially, the pollutants are desorbed from the particles by thermodesorption and analyzed using GC/MS. Subsequently, the polymers are decomposed by pyrolysis and the decomposition products can be identified via GC/MS analysis. The results should enable the assessment of different sorption behaviors of micro- and submicroparticles regarding the respective contaminants. In first experiments, the sorption properties of the pesticides phenathrene and benzophenone on the polymers polystyrene (PS), polymethyl methacrylate (PMMA) and polyethylene (PE) were investigated. For a mass balance of the pollutant, both particles and the aqueous phase are investigated. The content in the aqueous phase is quantified using a stir bar sorptive extraction (SBSE). Additionally, the application of TED-Pyr-GC/MS to analyze contaminants in samples from toxicological assays with and without particles will be shown.



Reference materials and standard needs for environmental micro and nanoplastic pollution

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Methods for the analysis of microplastics and sub-micrometer particles pose a serious challenge in the environmental pollution assessment. In this framework, there is a strong need to create good reference materials at the laboratory scale in order to perform a more representative characterization of the interaction between plastic particles and the environment. Minute fragments resulting from the breakdown process in the marine environment are far from being modelled by ideal particles commercially available for laboratory studies. Also, sub-micrometer particles cannot be considered as nanomaterials synthesized with the desired size, shape, surface, and composition. For these particles there are no reasons for a selection in size, they could have asymmetrical shape and heterogeneous surfaces. Moreover, small microplastics and nanoplastics have the limit to be small in size and this fact strongly hampers their characterization. Techniques as Raman Spectroscopy, Infrared Spectroscopy, pyrolysis-gas chromatography-mass spectroscopy (pyr-GC-MS), thermogravimetric analysis coupled to solid-phase extraction (TGA-SPE) and thermal desorption gas chromatography mass spectroscopy (TDS-GC/MS) are already established for the study of macro and meso pieces, but critical limitations occur at the micro and sub-micrometer scale.

In this contribution, we will present a methodological pathway to study micro and nanoplastics toward the preparation of more realistic reference materials. Starting from commonly used disposable plastic items, we use different techniques to fragment or degrade macro-pieces in a controlled way, such as mechanical disruption or photodegradation. We include methods for preconcentration and separation of the plastic particles into specific size fractions. Finally, our approach combines consolidated techniques in the field of nanomaterials analysis, such as imaging techniques, size distribution and physical-chemical characterization techniques, as vibrational spectroscopy, posing particular attention to the innovative portable instrument solutions.



**MICROFIBER POLLUTION:
ABUNDANCE, DETECTION
AND MITIGATION**

Microplastic Pollution from Textiles: Quantitative Evaluation and Mitigation Strategies

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The washing processes of synthetic fabrics have been identified as one of the main source of microplastic pollution in marine environment. The mechanical and chemical stresses produced on the fabrics during a wash, cause the release of microfibrils to wastewater. Due to their size, some of them are not blocked by the sewage treatment plants, reaching seas and oceans and becoming a threat for marine species.

In the last years, an analytical protocol was developed and proved to be a useful tool for the evaluation of the extent of the release from textiles during washings of synthetic clothes at lab scale, allowing the identification of specific trends in the microplastic release, as a function of textile nature and geometry, used detergent and washing conditions. In addition, a procedure to evaluate the microfibre release during laundering performed in real scale washing tests was developed and applied to identify the effect of different textile structures and parameters on the release of microfibrils. This method allows to determine the contribution of the washing process of synthetic clothes to microplastic pollution.

With the aim to mitigate the environmental problem caused by the microfiber pollution several mitigation strategies to reduce the impacts of microplastic pollution have been set up and tested. In details, new functional finishing treatments were developed with the aim to create a protective coating on the surface of synthetic fabrics, which reduces the amount of microfibrils shed during a washing process and thus mitigating the environmental impact of microplastics. One of the treatments is based on the use of pectin, a natural polysaccharide, the others were performed by using an electrofluidodynamic method to apply a homogeneous thin coating of biodegradable polymers on fabric surface.

Mitigation of microfibers release from textiles: chemical finishing and mechanical optimization

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Concern about the presence of microplastic particles (MPs) and their effect on the environment and human health is increasing among scientists, policy makers and the society. This is so due to their persistence in the environment, bio-accumulative characteristics, the fact that they are a vector of contaminants and microorganism (i.e. biofouling) and they are potentially trophic transferable. The European Strategy for Plastics as a part of the transition towards more circular economy, the EC together with the ECHA are looking at scientific evidence for all intentionally-added microplastics. Microplastics release from textiles (microplastics shedding) are considered unintentionally added microplastics. Therefore, they are not being considered in the scope of the next legislation in Europe. However, synthetic textiles and clothing account for a large source of microplastic pollution. They are released due to textile abrasion during laundry and to their exposure to chemicals and detergents, causing the breakdown of synthetic fibers into smaller microfibers. In this scenario, LEITAT driven by its commitment to circular economy has been participating in different projects and initiatives focused on microplastics and microfibers. In the MERMEIDS project, a protocol was developed for identifying and quantifying microfibers shedding during washing processes with the aim to establish and understanding the main parameters influencing microfiber shedding. LEITAT is now participating together with other experts and sharing its experience in developing a standardized protocol at laboratory scale and domestic scale to be used across Europe as well as dealing and collaborating in industrial, national and European projects for mitigating microfibers shedding. This article presents the potential processing steps from the textile value chain (fibre, yarn, fabric, preparation, dyeing and finishing processing) affecting microfiber shedding as well as technological solutions based on textile structures, mechanical and chemical finishing for mitigating microfibers release from textiles.



Abundance and composition of textile fibres in Mediterranean surface waters

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We present the results of a microfiber pollution survey performed during two sampling expeditions in the central-western Mediterranean Sea between October and November 2017. A total of 108 surface water samples were collected at 36 sampling stations. At each station, three-replicates samples were collected using a 10-liter stainless steel bucket, triple rinsed in seawater prior to use. Samples were gravity-filtered through 20 μm nylon mesh filters and stored frozen for subsequent analysis. In the laboratory, all fibers were counted by the same individual using standardized criteria. A subsample of 336 fibres were randomly selected for polymer identification using a FT-IR microscope (Bruker LUMOS). Only matches $\geq 70\%$ with reference spectra were accepted as verified polymers. Prior to each scan, fiber length and diameter were also measured to the nearest micrometer. Microfibers were found in all samples with a mean concentration of 5.1 ± 2.3 fibers/liter across the study area. Fibers concentrations showed a relatively high spatial variability, but no clear trend in relation to distance with land. Maximum concentrations (12.2 fibres/liter) were observed in the Sardinian Channel, while the lowest concentration (2.1 fibres/liter) was found in a sample collected in the Western Tyrrhenian Sea. Half of the collected fibers (50.1%) were clear in color (white, grey, transparent), followed by dark/black (22.1%), blue (21.75%), red (3.8%), orange/yellow (2%) and green (0.2%). Mean fiber length was 1.79 ± 1.82 mm (Max: 14.62 mm; Min: 0.14 mm) while mean fiber diameter was 22.6 ± 18.4 μm (Max: 200 μm ; Min: 5 μm). FTIR analysis revealed that most of the fibres were made of non-synthetic materials, with the majority being made of cotton (47.3%), wool (5.4%) or other cellulosic materials (40.5%). Only 6.85% of the characterized fibers were actually synthetic, with polyester being the most abundant polymer (4.2%), followed by nylon, polypropylene and acrylic.

When size matters – textile microfibers into the Environment

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Within the microplastic pollution, textile microfibers have been predominantly found across the environment and in products for human consumption. Hence, microfibers are one of the most important primary microplastic sources (emitted to the environment in a microplastic size).

In order to understand this source, estimations of its mass flow to the environment have been conducted. The typical calculation approach applies a textile property that relates the mass and the length of the fibers: the “*linear weight*”. A common linear weight unit is the decitex (dtex), which expresses the mass in grams per 10000 meters of fiber. Explained this, published estimations have applied 300 dtex in their calculations, which is an average value for yarns. Therefore, according to those publications, the global mass flow of microfibers to the oceans is between 0.2-0.5 million tons/year. However, *microfibers* must be considered as *individual filaments*. In this way, from the literature, it was found that an appropriate linear weight for the microfibers is between 1 to 5 dtex.

Therefore, in this work, the global mass flow to the oceans was re-estimated, resulting in 17,830 tons/year, which is approximately 3% to 9% of previously published calculations. Nonetheless, in our experiments, measured microfibers are about 25 times smaller than the considered in those estimations. Then, the number of microfibers particles is increased from 3.6E+15 to 1.4E+17. Finally, the microfibers limit-size should be re-evaluated, as they have a special morphology. Hence, we suggest to adopt the diameter limit of 5mm with a length:diameter ratio > 3:1 proposed by the ECHA.

Summarizing, despite the decrease in the microfibers mass flow, a higher number of smaller and easily ingested microfibers is heading towards the oceans. Thus, as these microplastics are very light weighted, it is important to put special attention onto the *number* and the *size* of detached microfibers.



Derelict fishing gear – removing a source of microplastics from the marine environment

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Lost fishing gear is omnipresent in the marine environment. The Mediterranean acts as a hotspot of microplastics, with a dominant fraction being fibres. The origin of these fibres – fishing nets, ropes, or land-based waste water, is unknown. Fishing nets take decades to centuries to degrade in the marine environment, remaining a source of microplastic fibres with a potential of entering the marine food web and returning to our plates. In the North Sea and Atlantic, mussels and pelagic fish for human consumption were shown to contain 1-2 microplastics on average, most of which were fibres. In the framework of the MARELITT Baltic project, WWF Germany together with colleagues from Estonia, Poland, and Sweden, has developed best-practice methodologies for the search, retrieval and treatment of lost fishing gear. A special focus was placed on the possibility to manage and potentially recycle retrieved fishing nets from the sea. Because passive nets are weighed down with sink lines, degradation of the plastic sheathing causes toxic lead to be released into seafloor sediments. Retrieving and processing this type of hazardous waste fishing gears is challenging. In this presentation, I will report on the results of our retrieval campaigns and the efficient search with sonar techniques in hot spot areas for fishing gear loss. The requirements needed for the treatment of retrieved fishing gears and the missing infrastructure for a proper waste management of mixed marine litter will be discussed. As fishing gear is one of the major sea-based sources of marine microplastic fibres, the best-practice approach developed during the MARELITT Baltic project will help to mitigate the impact of lost fishing gear microfibres on the marine environment.

Microplastics settling – flat particles and fibers

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Terminal settling velocity of microplastics (< 5 mm, MPs) of previously undeexplored shapes – flat particles and fibers – was measured in "ideal" conditions (steady homogeneous water) in order to expand current knowledge on MPs dynamics and to develop valid parameterizations of MPs settling. Generated by fragmentation of packaging material (bottles, containers, films, bags), which include most of the objects produced from plastic for mass consumption, solid flat fragments and fragments of flexible thin films could presumably account for the majority of MPs in the marine environment. According to different field observations fibers also represent the most common type of MPs. In total, 9 sets of flat particles of different density, thickness, and rigidness in the size range of 0.5 – 5 mm, and 11 sets of fine fibers of different lengths were prepared; 190 measurements of terminal settling velocity were acquired. Flat particles fell with their maximum area perpendicular to the vertical axes and demonstrated various secondary motions during the fall. On the basis of previously published and experimental data, a semi-empirical formulation of the settling rate of flat MP particles was obtained; the average approximation error compared with experimental values varied from 9% to 35% for different sets of particles. The calculated terminal settling velocities for the flat plastic particles in the range of MPs' sizes and densities varied from 0.04 to 226 mm/s. Fibers settled smoothly without any preferred orientation. Settling rates of fibers with lengths < 5 mm varied between 0.5 and 2.9 mm/s; fibers of equal length showed scattered values of terminal settling velocity which may be explained by the presence of light turbulent or convective pulses in the experimental tank.

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Filters for fibres from washing

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Fibres from washing are estimated to be the largest single source of microplastics in oceans and have been found in marine environments around the globe. Washing of clothes, which are today approximately 60 % made of artificial materials (plastics), is the largest contributor to this class of pollutant. The washing machine is a convenient choking-point for fibre removal.

Filtering devices that allow efficient retention of fibres before they are released into the wastewater system have been developed for domestic and commercial washing machines. Both existing and new washing machines can be equipped. The filters combine efficiency of fibre capture with practical use and affordability. Currently available filters from a leading producer and their characteristics will be presented.

Reduction of microplastic emission from clothes during laundering with chitosan pre-treatment

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A Sewage treatment plant is a pathway of microplastics from domestic and industrial activities as many researches reported. Out of all shapes of plastics, fiber type is the most abundant microplastics in sewage. The main emission source of fiber type microplastics is the washing process of synthetic clothes. More than 90% of microplastics are reduced by sewage treatment process, but untreated fibers are eventually discharged into the ocean. Since the microplastics removal rate of the sewage treatment plant is limited, it is necessary to reduce microplastics before entering the sewage treatment plant. In this work, we tried to find a way to reduce the amount of fiber released from clothes by pretreatment clothes before the washing process. The pretreatment solution is based on the chitosan, which is an eco-friendly and natural polymer material that can avoid any additional contamination. In this work, we tried to reduce the amount of fiber released from clothes during the washing process by pretreating them with a chitosan solution which is an eco-friendly and natural polymer material that can avoid any additional contamination. The number of released fibers were counted from two groups, one with the chitosan pretreatment and the other with no pretreatment. When using the chitosan solution for pretreatment, more than 90% of fiber was reduced than untreated clothes. This study results indicate that the chitosan pretreatment is a promising solution for reducing fibers from laundry wastewater.





**DEGRADATION AND
BIODEGRADATION OF
PLASTICS IN THE
ENVIRONMENT**

The Plastics Manufacturers Efforts to Tackle Plastic Waste in the Environment

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Plastics contribute many solutions to hygiene, health & safety and resource efficiency. Leakage of plastics (macro and micro) causes serious environmental issues and their prevention is amongst the plastics industry top priorities. We are working towards developing solutions as well as scientific facts on both plastics litter and microplastics. This includes for example the implementation of the Operation Clean Sweep Programme to prevent leakage of our plastics material and Life Cycle Assessment (LCA) tools that can help stakeholders make knowledgeable and informed decisions. Multidisciplinary approaches are needed to advance science and we are currently working jointly with the industry, academia and regulators worldwide to both identify the current gaps in science as well as develop new environmental risk assessment tools for microplastics. These developments will be the object of our presentation at the conference.



Biodegradable plastics do not form chemically persistent microplastics

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Biodegradation of plastics is a heterogeneous reaction in which the reactant (the solid item) and the catalysts (the enzymes in the liquid phase) are in two phases. Microorganisms colonise the surface of solid items and form a biofilm. Extracellular enzymes are secreted by the biofilm and reach the surface of the item starting depolymerisation. Monomers and oligomers are released and assimilated by the surrounding microbes thus becoming part of the microbial metabolism. Respiration is the ultimate biodegradation process leading to CO₂ and H₂O. Biodegradation rate and level are determined by exposing plastic samples to environmental matrices (e.g. compost, soil, marine sediment/water) and measuring the evolved CO₂. Samples of progressively smaller size show proportionally higher biodegradation rates, proving that the available surface area limits the process. Best regression models were the double reciprocal plot (the Lineweaver-Burk approach used in enzymatic kinetics) and the squared-Y model. Both models imply a progressive decrease in dependent variable Y (rate) with increasing values of independent variable X (surface area) suggesting a maximum rate reached at a given surface area. The results show that the biodegradation process that occurs at the interface is very fast. If it were possible to mill the test material, to obtain nanoplastics, the biodegradation would be extremely fast, lasting a dozen days, with a time frame similar to that required by the OECD for the classification of "readily biodegradable" chemicals. Therefore, the chemical permanence time of biodegradable plastics is very short. On the other hand, the physical permanence time depends on the surface area, i.e. on the thickness of the plastic items. The results suggest that biodegradable plastics do not generate persistent microplastics, because as erosion increases the surface area, this in turn increases the biodegradation rate to levels similar to those required, by the OECD, for chemicals to be defined as readily biodegradable.

It depends where it ends – Biodegradable plastic degradation differs between habitats, and climate zones

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The fate of plastic items, claimed as biodegradable, in the natural environment has been rarely studied systematically. However, several observational studies show that claims by manufacturers or distributors, sometimes printed on the item itself, did not match the expectations raised, and items did not degrade under natural conditions as claimed. We studied the performance of intrinsically biodegradable polymers and blends under marine conditions in a series of experiments in mesocosm and in field tests from 2013 to 2019. Our data show that all materials that were proven to biodegrade in optimized laboratory tests also showed disintegration in open-system tests. However, huge differences in disintegration rates were observed between different habitats and different climate zones. The variations in biogeochemical conditions such as temperature, nutrient-related parameters, and sediment characteristics (e.g. grain size) were reflected by variations in the degradation performance of a specific material. Thus, we conclude that whether or not, or how fast an intrinsically biodegradable material is actually degrading once lost to the natural environment strongly depends on the habitat where it is ending up. These findings have to be considered when biodegradable plastic is discussed as a substitute for conventional plastic as a measure against plastic pollution of the environment.

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Controlled aging and degradation of selected plastics in marine environment (12 months of follow-up)

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The presence of polymeric debris in marine environment is one of the greatest menaces not only for the oceanic ecosystems, but also for local seas such as Mediterranean. The formation of secondary microplastics, caused by the fragmentation of larger items, strongly depends on the structural degradation of the polymeric materials and on the related decrease of their mechanical properties.

The aim of this study is the evaluation of the behaviour of some common polymers (PP, PS, PET, PLA, PE) after the exposition of selected items to natural aging conditions in marine environment at Villasimius (CA), Italy. Plastic cups, bottles, foams and films were maintained in containers positioned i) in the beach sandy shoreline, and ii) in seawater at various depth, i.e. on the surface at about 0.5 mt, and underwater at about 1.5 mt. The progressive decay of physico-chemical properties was evaluated following the evolution of properties after 3, 6, 9 and 12 months of exposition to UV-visible radiation and/or sea water.

Infrared (FTIR) spectroscopy was carried out to assess the extent of the oxidation phenomena, associated to the formation of a carbonyl peak, and water absorption. Tensile tests were performed on specimens taken from longitudinal and transversal direction. The variation of oxidation onset temperature (OOT) was determined by DSC and it was related to the thermal stability of the material.

Moreover, different rheological tests were used to evaluate the molecular weight of the polymers, polylactic acid and polystyrene were tested with capillary viscometry, whereas polyolefins were studied with rotational melt viscometry. Colorimetry tests were also performed to evaluate the yellowing of the items, attributable to the oxidation of macromolecules.

The three environments showed different features, such as different extent of solar radiation, heat, humidity, biological interactions, and consequently the specific behaviour of the various plastics item has been compared.

Not only diamonds are forever: degradation of plastic films in a simulated marine environment

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In recent years, biodegradable polymers have been hailed as one of the potential solutions to the microplastic pollution problem, due to their ability to fully degrade rather than break down in smaller pieces over time. However, complete degradation of biodegradable polymers is often achievable only under strictly controlled conditions (i.e. increased temperature and pressure), which are not found in the natural environment – particularly in aquatic and marine habitats. This study aims to compare the degradation performance of plastic films made of two different biodegradable polymers – polylactic acid (PLA) and polyhydroxyalkanoates (PHA) – to that of polyethylene (PE) films, in a simulated marine environment. Plastic films of the three chosen polymers, of equal dimensions, were exposed to natural sunlight within a novel setup - which simulated the sea surface - for six months. Films were chosen as they are among the most frequently reported type of plastic litter in coastal environments worldwide, and because of the increasing adoption on the market of biodegradable films for packaging. Results showed that, after six months, no consistent degradation could be observed on any of the films – not even the biodegradable ones. Between PLA and PHA films, the latter weathered slightly more than the former, but not at a significant level. Interestingly, differences were reported among the different polymer films in terms of type and extent of fouling, brittleness, surface charge and surface microstructural changes, and microplastic formation. Overall, this work suggests that biodegradable plastic behaves rather similarly to traditional plastic in the marine environment over a half-year span. Albeit further experiments on even longer timescales are needed, this study provides evidence that, unless properly disposed of in an industrial composter facility, biodegradable plastic may only contribute to the very problem it was intended to solve.





How to proof claims on biodegradability of plastics? Overview of available standard tests and their environmental relevance

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The emergence of so-called bio-plastics on the market in recent years, and the regulatory shift towards biodegradable plastic items such as bags for fruits and vegetables in some European countries have led to the introduction of several labels and certificates indicating a certain performance of these plastic items with regard to biodegradability. Apart from the confusion amongst the general public about the definition of “bioplastic” and “bio-degradable plastic” several claims communicated to the consumer are misleading or at least difficult to understand. In order to prove claims such as “biodegradable”, or “compostable” national and international standard tests exist. We present an overview of the standard tests available internationally and how they reflect the conditions in the natural environment where plastic might end up if littered or lost. We also discuss possible gaps in the testing schemes applied today in order to comprehensively assess the risks and chances these new materials might bring along.

A world map composed of red dots on a dark blue background. The dots are arranged in a grid pattern, with the density of dots varying to create the shape of the continents. The map is centered on the Atlantic Ocean.

**MICRO AND NANOPLASTICS
AS EMERGING POLLUTANTS**

Inhalable Microplastics: a New Cause for Concern?

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Microscopic plastic particles – microplastics – are a global issue for aquatic habitats. Recently, they have been reported in atmospheric deposition, and indoor and outdoor air, raising concern for public health due to the potential for exposure via inhalation. However, very little is known about airborne microplastics, including their spatial and temporal concentrations; chemical composition; and, importantly, whether they occur in the inhalable size range. Here we present data on the presence of microplastics ($>25\ \mu\text{m}$) in total atmospheric deposition sampled over one month at an urban background site in London, UK. Using Nile Red staining, bright field and fluorescence microscopy and Fourier-transform Infrared spectroscopy, ten times more fibrous microplastics were found than non-fibrous. This equated to an average deposition rate of 706 fibrous microplastics/ m^2/d , with polyacrylonitrile being the predominant polymer type. We also present developments for an analytical protocol compatible with Raman microscopy and an active pollen sampler, which has been validated to detect microplastics down to $2\ \mu\text{m}$ in mock samples, and $2.5\ \mu\text{m}$ in environmental particulate matter (PM). As global pressure to reduce road transport and fuel burning emissions increases, PM composition is likely to shift. In combination with a predicted increase in plastic use, especially in the textile sector (4%/year), the proportional concentration of airborne microplastics will become increasingly important. It is therefore timely to establish baseline knowledge of global airborne microplastic burdens and begin to understand what their potential role in PM-associated health effects might be.

Microplastics and nanoplastics occurrence and composition in drinking water from Akureyri urban area, Iceland.

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Microplastics as a potential health and environmental problem has gained an increasing attention recently. Microplastic is defined as plastic pieces smaller than 5 mm in diameter, and the sources of microplastic are many. State of the art literature reports that microplastics are ubiquitous worldwide. While several authors report fragments of different polymers being observed practically in all environmental compartments of marine, freshwater and terrestrial ecosystems; others point out the accumulation of micro- and nanometric sized plastic particles thorough the marine and terrestrial food webs is posing a risk to marine and terrestrial life and ultimately to the human health. Despite of studies pointing out the occurrence of micro plastics in freshwater systems including surface and groundwater basins, very little is known about the occurrence of microplastics in the drinking water and their implications on human health. According to the WHO men should consume 3 L and women should consume 2.2 L of beverage per day. Most of these beverages consist of tap water, or drinks derived from tap water. The risk of plastic uptake from drinking water is currently unpredictable and furthermore, these plastic particles add to the plastic potentially consumed in other sources, such as sea salt, beer, food and seafood. The research tasks of the present work were: map published and available literature, develop and optimize a standardized fast, sensitive protocol for sampling and quantification of nano/microplastics particles in drinking water and finally analyze and detect microplastic particles in drinking water supply systems with special focus on different polymeric composition and size fractions. Study area is located in Akureyri (Iceland) populated by approximately eighteen thousand inhabitants. A total of about 25 liters of drinking water were analyzed from each of the eight different sampling sites in the drinking water suppling grid of the urban area. A fast and sensitive method based on a GCMS-pyrolysis was developed and adopted for the study. Polyethylene and Polyvinyl chloride were the only polymers above the detection limits. Levels ranged from non-detectable to few µg/L. Space related trend are presented.



Microplastics in vegetables and fruit

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In recent years, microplastics (MPs) represent a threat both the environment and human health. Vegetables and fruits have never been investigated for MPs since they come indirectly into contact with plastic debris due to soil and water contaminations.

Aim of this study was to investigate the presence of MPs into lettuces and apples bought from three sites: Large Scale Retail Channel (GDO), zero Km market (OKm) and local market (LM).

The method applied in the study has been nationally and internationally protected. The code of the submitted request of international patent's extension in several country of world is PCT/IB2019/051838 of 7 March 2019, coupled with Italian patent number 102018000003337 of 07 March 2018. In brief, it consists in mineralization of 100 mg dried of each sample (3 apples and 3 broccoli), particle sedimentation (having density>1), dispersion on an aluminum and copper alloy stub, reading by SEM-EDX for particle identification, counting and measurement of diameters.

MPs were detected in all samples: from GDO, MPs in apples and lettuces were 1.20E+06 and 1.17E+06 with main diameters of 2.14 μm and 2.2 μm , respectively; from OKm, MPs in apples and lettuces were 1.97E+05 and 5.02E+05 with main diameters of 3.62 μm and 2.6 μm , respectively; from LM, MPs in apples and lettuces were 4.09E+05 and 1.62E+06 with main diameters of 3.04 μm and 2.1 μm , respectively.

MPs concentrations (p/g) were very high. Apples and lettuce from OKm showed the less number of microplastic for gram with the biggest particles sizes. Moreover, lettuces were stronger contaminated respect to apples. It can be explained by the difficult of translocation of MPs in the aerial parts of the plants. Accumulation of MPs into vegetables could be become useful to carry out a phytoremediation method able to reduce environmental contamination of MPs.

Standardizing in-vivo analysis methods for toxicological effects within freshwater organisms from nano-polystyrene exposure

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New technological and chemical techniques have produced a growing interest in developing the application of “nanoplastics”. Nanoplastics can store certain chemicals in a manner that reduces the overall concentration needed to be effective, along with forming structures to control chemical release and ensure optimal application of the ingredients. Despite rising interest in these products, there is a noted lack of research in the potential toxicological effects related to the nanoplastics. Our current testing focuses on nano-polystyrene, a substance already found in residual effluence & waste water. The purpose of this research is to determine if existing non-invasive testing method can be produced for determining morphologic and toxicity effects of specific nano-polystyrene spheres. The tests are based on freshwater organisms to act as a realistic but controlled comparative assessment to the potential results from nano-carriers present in effluent wastewater products. Fluorescent nano-polystyrene (NPS) would be used in combination with fluorescence and light microscopy to analyse uptake locations within organisms. Later detection analysis would be conducted by a combination of compound-detecting spectral techniques such as Raman and UV-Vis fluorescence spectroscopy.

Four test organisms were chosen to represent the most vulnerable levels on the freshwater food chain. Micro-algae would represent the aquatic algae that freshwater species are dependent on and risk detrimental change to growth rates from NPS exposure. *Daphnia magna* represented primary mobile filter-feeders, dependant on the algae and can demonstrate the toxicological impact of NPS exposure. Zebra-mussels represented static filter-feeders to provide bioassays on specific organs toxicity of NPS aggregating into sediment. Zebrafish embryos represented secondary consumers at their most vulnerable stage and were used to present indications of morphological malformations from NPS exposure. The combined information should determine whether these nano-plastics are inherently toxic or incur toxicological effects following alterations from the surrounding freshwater environment.



Impact of nanoplastics on marine diatom *Skeletonema marinoi*: particles adhesion, ROS production and reduction of colonies length

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Marine diatoms are among the most abundant taxa of microorganisms associated to plastic fragments found in the marine environment. However, few information is current available concerning the impact of smallest plastic fragments, named nanoplastics, at both single cell and population level. The aim of the present study was to investigate the impact of 70 nm polystyrene nanoparticles (PS NPs) functionalized with carboxylated groups (–COOH) to the marine diatom *Skeletonema marinoi*. PS NPs behaviour and surface charge in exposure conditions were characterized by dynamic light scattering (DLS), while impact on growth was investigated through a 14-days toxicity test as well as the induction of reactive oxygen species (ROS). The interaction between algal cells and PS NPs was assessed via environmental scanning electron microscopy (ESEM), transmission electron microscopy (TEM), and differential interference contrast (DIC) microscopy, using different sample preparation procedures. Furthermore, an analysis of *S. marinoi* colonies length upon PS NPs exposure was performed. Results showed no inhibition of growth under all tested PS NP concentrations (1, 10, 50 µg/mL) but a significant increase of both extracellular and intracellular ROS was observed. TEM images revealed PS NPs adhesion to diatoms external surface, mainly localized at fulcortopula process (FPP), the structure responsible for linking cells to one another to form colonies. *S. marinoi* colonies length resulted significantly reduced upon PS NPs exposure probably as a consequence of PS NPs adhesion. The comparison of different techniques to observe diatoms-NPs interaction, highlighted how current methods might alter the interaction occurring in natural conditions between PS NPs and biota. PS NPs-diatoms adhesion could have serious implication at population level by impairing diatom's buoyancy as well as at community level due to their trophic transfer up to the food chain.

Association of potential human pathogens with microplastics in freshwater systems

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Microplastics (MPs) have gradually become a global environmental pollution concern and are readily colonised by environmental microorganisms. MPs persist in the environment and serve as potential vectors for spreading bacterial pathogens of humans, due to their low biodegradability, high surface area to volume ratio and hydrophobicity. In the pelagic and benthic environments, surfaces are always colonised by microorganisms, forming biofilms. As a consequence, microorganisms absorbed onto MPs can be transferred towards other marine species along the trophic web. Despite recent findings on microorganisms associated with MPs in marine environments, there is a lack of information on microbial colonisation of microplastics in freshwater ecosystems. Due to their small size, MPs in rivers provide a solid surface on which dense biofilms can form, just like on natural particles. Therefore, we employed a 14-day experiment to investigate bacterial colonisation on polyethylene (PE) MPs within the river Barrow Carlow, Ireland. MP beads were placed *in-situ* in the river as well as in river water samples in the laboratory and left for 14 days. High throughput 16S ribosomal DNA (rDNA) gene sequencing was used to profile bacterial communities on the surfaces of microplastic particles. Different bacterial communities and lower microbial diversity were observed in the laboratory samples compared to the *-in-situ* river water samples. Results revealed the presence of potentially pathogenic species, such as *Enterobacter* spp, *Helicobacter* spp, *Arcobacter* spp, *Clostridium perfringens* and *Escherichia coli*. This suggests that MP do have the capacity to support and transport potential harmful bacteria.





**SOCIO-ECONOMIC AND
ENVIRONMENTAL IMPACT
OF MICROPLASTICS**

The Marine Plastic Footprint & its Application to the Mediterranean Basin

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Forecasting plastic leakage, i.e. “plastic footprinting”, is a necessary first step towards the identification of mitigation and remediation solutions to ultimately close the plastic tap.

The presentation will offer a state of the art of plastic footprint methodologies and describe key stages of the calculations and data requirement.

Furthermore, two examples of applications will be presented.

(1) The first example will illustrate the use of the plastic footprint methodology to guide eco-design in the industry (from the Plastic Leak Project).

(2) The second example will present the result of a recent IUCN project where the Marine Plastic Footprint has been applied to the Mediterranean basin, including also an assessment of the quantities of plastic accumulated in the Mediterranean Sea.

To conclude the limitations of these approaches will be discussed as well as key perspective to render footprinting approaches more actionable in the future.



Microplastic pollution: a thriller with many lead roles and unknown ending

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(Applied) science conduct is in the process of explosive revolution. This is obvious from a couple of major points: scientific communication, quadruple helix approach and the concept of the responsible research and innovation. There is a visible gap between the academic training and exercising science in practice, where a lot of knowledge in tackling scientific hypotheses, entering new research areas, obtaining scientific funding and collaborating are self-trained.

The issue of microplastic pollution has been in the recent years recognized as a top priority area by the general public, the policy makers, the media as well as the scientific world. However, while scientists are still trying to evaluate the adverse effects of microplastics pollution on the environment, food and humans, the public and policy makers are eager to finally use the potential solutions.

In this perspective, the issue of microplastic pollution is an excellent example of transdisciplinary scientific collaboration, scientific communication, involvement of public and NGOs, efforts for legislation change, including the close collaboration with policy makers and finally offering approaches that can be used in practice and in future commercialized by small & medium enterprises as well as the industry.

The talk will present some introduction to the terminology, facts and figures regarding microplastics research in the EU, what scientific communication has done to catalyse this area of research and (inter)national changes of legislation. Finally, we will present ongoing research activities that have yielded / promised solutions for microplastic pollution.

Why and how do we dispose of our plastic waste? An overview of the behavioural bases

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The presence and consequences of plastic waste in the environment have been the subject of intensive researches in recent years. While the state of contamination and the impact of this plastic waste on the environment and ecosystems is beginning to be better understood, the fundamental reasons why we throw away our plastic items, and the reasons that guide the way we throw them away, remain poorly understood. The objective of this review is to identify, through the scientific literature, the main elements that make it possible to try to explain the choices of a person getting rid of a plastic waste. This work proposes to address these topics through our knowledge of waste management in historical times and our waste management behaviours. These decision factors will be illustrated through examples related to expanded polystyrene. Expanded polystyrene (EPS) is a plastic foam usually used in packaging and insulation including as food trays, drink/food containers and fish boxes. Good insulating and mechanical properties of EPS explain its very wide use in the industrial world. Although EPS is recyclable, it seldom is, because of its contamination by food or because of the low cost-effectiveness of transporting and recycling expanded material. As a result, it is often used to produce energy or landfilled. Its lightness means EPS is easily blown from garbage bins and landfills. It is not biodegradable and tends to fragment, with small pieces travelling long distances. In a second part, through the review of studies on climate change, we will explain why it is so difficult to change these behaviours. This project is part of the OceanWise European project. This project aims to jointly develop a set of long-term measures to reduce the impact of expanded polystyrene products (EPS) in the North-East Atlantic Ocean.



Legislative options for reducing pollution by microplastics in the EU: the meaning of scientific evidence

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In January 2019 the European Chemical Agency published the restriction dossier on intentionally added microplastics, which proposes the phasing out of certain microplastics in several products, such as cosmetic products, detergents and fertilisers. The restriction dossier had been prepared on request of the European Commission in line with the EU Plastics Strategy, aiming at a reduction of plastic pollution. The Commission has also commissioned a preparatory study on options for reducing the releases of microplastics from wear and tear, identifying source prevention measures and downstream measures. The study might be the first step in instituting legislation on microplastics from wear and tear. These developments are promising, though no regulation is in force yet and it remains to be seen whether and if so which measures will be put in practice.

This presentation addresses the policy options for reducing pollution by microplastics and identifies the legal framework for regulation in the European Union. An overview of the restriction procedure regarding intentionally added microplastics will be given, as well as possible regulatory frameworks for microplastics from wear and tear. Special attention will be given to the role of science in these processes. EU regulation usually requires scientific foundation, including risk or hazard assessments. Recently, it has been suggested that significant uncertainties on the impact of microplastics exist, and that the current scientific evidence might not satisfy the hazard risk criteria set in certain procedures. Further discussion of the criteria and of the present level of evidence on microplastics is therefore particular urgent for the success of legislative measures.

The Pelagos Plastic Free Project: plastic marine debris abundance, plastic-associated microbial community composition, and sources of beached waste in the Pelagos Sanctuary

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The Pelagos Plastic Free project was developed by Legambiente and Expédition Med and was partially funded by the Pelagos Secretariat to tackle the problem of marine plastic pollution in the Pelagos Sanctuary through integrated approaches that consider governance, science, and scientific literacy. High accumulation of Plastic Marine Debris (PMD) occurs in the Pelagos Sanctuary, overlapping with the foraging habitat for fin whales. PMD is covered by a biofilm of microbial communities (the Plastisphere) distinct from the open water and acting as a potential vector for pathogenic species. Scientific research was carried out through Citizen Science Laboratories in collaboration with several European research institutes. 60 manta net tows were carried out aboard the R/V Ainez in July-August 2018 with the help of 29 volunteers. The total number of plastic fragments varied between 4 - 1895 items per tow (mean = 166 ± 287 SD), of which 46-100% were microplastic fragments (<5 mm). Microplastic abundance varied between 0.012 - 5.329 items/m³ (mean = 0.445 ± 0.767 SD). Total genomic DNA was extracted from PMD biofilms, followed by next-generation sequencing of the V6 hypervariable region of 16S rRNA gene. Plastisphere communities varied regionally, and between plastic samples and seawater. SEM images of plastic fragments showed bacteria, diatoms, other protists, and small invertebrates. Beach litter monitoring has been carried out so far in 8 beaches with the European Environment Agency protocol and 50 volunteers were trained. Total number of waste items varied between 173- 1512 items/100 m (mean = 685 ± 573 SD). Non-identifiable plastic objects, including polystyrene fragments, of size 2.5-50 cm accounted for 49% of total waste items. Overall plastic contribution was 86%. Poor management of urban waste is the main source of PMD. It is urgent to develop multiple stakeholders' collaborations to promote circular economy and stop production of marine litter.





Development of a novel technological approach for the reduction of microplastic pollution in various waters

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An increasingly serious and widespread problem is the introduction of plastics into the water cycle. The poor degradability leads to the plastic waste remaining in the water for a long time and over time it fragments into smaller and smaller plastic particles, so called microplastics. New technologies are needed to prevent the microplastic spread into the ecosystem. Wasser 3.0 PE-X offers a new material and technology based solution for various water. The innovative approach for the removal of microplastics from various waters combines a chemically induced agglomeration and a new technological implementation step. The particular challenge in removing the microplastics is their small size and their inert properties against most of the physical and chemical additives for flocculation. The new concept is based on the development and use of special organosilanes, which can attach to microplastic particles and link them subsequently by forming an inclusion compound. This leads to the localized agglomeration fixation of microplastic particles in large agglomerates, which can be easily removed from water. With an easy implementation to existing systems, an economic aspect and a strong impact not only in the field of wastewater treatment, but also in seawater or industrial application (e.g. seawater desalination).

Sample preparation and analysis methods of microplastics

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One of the main topic related to the study of the environmental problem constituted by microplastics (MP's) is the creation of international standard methods (CEN- ISO) for the identification and quantification of these materials in different shape and matrices. In this perspective Aquafil S.p.A and CNR STIIMA recently started a partnership collaboration to develop a new test method for extraction, purification and identification of microplastics. Aquafil is one of the leading players in the production of Polyamide 6 and a leader in the research of new production models for sustainable development. Aquafil is also the creator of ECONYL® Regeneration System, a process starting from Nylon waste (Fishing nets, Carpets, fabric, various pre consumer waste) collected around the world and chemically regenerated into Nylon monomer. CNR STIIMA of Biella is part of Institute of Industrial Technologies and Automation of Italian National Research Council and its studies are particularly dedicated to research and innovation on textile materials and processes, standardization and technology transfer to the textile sector. Aim of the co-operative work is to obtain a quali-quantitative method for MP's and for the identification of morphological and dimensional distributions including the evaluation of the characteristic surface and weight of each polymer and for each particle dimensional class. Chemical pre-treatments of the sample (textile processes, rivers or sea water), based on oxidizing agents and/or acid/basic digestion have been studied to remove "non plastic particles" and preserve microplastics integrity. The main analytical techniques investigated are molecular spectroscopy coupled with optical microscopy and image analysis, that allow the simultaneous chemical identification and counting of multiple particles having micronic dimensions. The Optical Microscopic inspection of the sample suggests the right kind of analysis (e.g. μ ATR, Reflection, Transmission) to perform. The automated analysis led to a higher accuracy and precision of measurements, reducing also significantly the time needed. The results obtained will be useful for the preparation of a standard method for MP's to be applied to samples of different origin.



Model-based assessment of plastic distribution in the Mediterranean Sea

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Mapping of floating plastic concentration and their fluxes is necessary to understand the generation, distribution and fate of microplastics in the Mediterranean. Field observations, laboratory experiments and numerical models combined together can significantly contribute to advance the understanding of the sources, pathways and fate of microplastics in all the environmental matrices: water, sediment, and biota.

Drift of floating debris was studied with a 2D Lagrangian model with stochastic beaching and sedimentation of plastics. An ensemble of $> 10^{10}$ virtual particles was tracked from anthropogenic sources (coastal human populations, rivers, shipping lanes) to environmental destinations (sea surface, coastlines, seabed) using high-resolution currents and waves provided by the Copernicus Marine Environment Monitoring Service (CMEMS).

Extremely high spatio-temporal variability in sea-surface plastic concentrations without any stable long-term accumulations was revealed. A substantial accumulation of plastics was detected on the coastlines and the sea bottom, where the former sink greatly dominated the latter one.

According to the model, the most contaminated areas were located in the Cilician subbasin, Catalan Sea, and near the Po River Delta and Venice Lagoon. Also, highly polluted local patches in the vicinity of sources with limited circulation were identified (e.g., the Gulf of Izmir (Turkey), the Saronic Gulf (Greece), the mouth of the Buna-Bojana River (Montenegro), the Gulf of Naples (Italy), the Gulf of Marseille (France), and the Valencia Gulf (Spain)).

Solution to the inverse problem, quantifying the national source-receptor relationships among the 19 Mediterranean countries, revealed that the plastic pollution of almost every country's coastline was mainly caused by its own terrestrial sources of plastics.

A world survey on microplastic distribution near the surface from race yachts

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Gaps in scientific knowledge and the challenges of measuring the distribution of microplastic pollution in our vast ocean are compounded by the absence of a standardised methodology for accurate estimation of marine microplastic concentrations and a single platform for data sharing. For other oceanographic parameters, such as dissolved carbon dioxide and sea surface temperature, the challenges of acquiring in-situ measurements from remote areas mean that extensive areas are essentially unsampled and our understanding of critical variables remains difficult to validate.

A unique campaign to combine scientific sampling with elite sailing was undertaken during the 2017-18 Volvo Ocean Race. The initiative capitalised on the often remote route of the extreme round-the-world race to generate an internally consistent picture of microplastic distribution using a pioneering combination of sampling and analysis techniques. Direct measurements of oceanographic and environmental variables were recorded and samples were captured for analysis of microplastic concentration.

Using a flow-through system onboard two of the racing boats seawater was filtered through a stainless steel filter set-up of three to isolate microplastics in the size range of 30 - 100, 100 to 500 and 500 µm to 2 mm. Laboratory analysis was conducted with a method combining Raman spectroscopy for identification, holographic imaging for size determination and microfluidics for fast analysis. Microplastic particles were detected in 93% of samples collected along the route, including some of the most remote locations sampled. The highest concentrations recorded were in the South China Sea, near the south European coast and in the Mediterranean.

Here we will report on the techniques used to examine the distribution of microplastic concentration along the race route and how the very successful collaboration demonstrated the efficacy of racing yachts as vessels of opportunity to capture high-quality oceanographic data and the value of using the sport's platform to promote awareness of ocean science and in particular microplastic pollution.





**MICROPLASTICS IN THE
MEDITERRANEAN SEA**

Impact of Microplastics on Deep-Sea Ecosystems

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Microplastics contamination of marine ecosystems is an issue of global concern, but the mechanisms determining impact on marine life are still largely unknown. We investigated the abundance of microplastics in the deep sea and conducted studies on the effects of microplastics on the model organism *Coralium rubrum*. We demonstrate here that the exposure to microplastics, at concentrations present in some marine ecosystems, determines macroscopic and molecular effects, ultimately leading to death. Immediately after exposure to microplastics, corals increase enormously the release of mucus and reduce their feeding rate. The unprecedented evidence of the multiple biological effects of microplastics on marine organisms and call for urgent action to control microplastics contamination of marine ecosystems and adopt targeted conservation measures.



Into the Med: searching for microplastics from space to deep sea

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Microplastics (MP) behaviour and fate within the marine realm is poorly understood. In fact, most studies focus on surface and/or near-surface water column estimations, which can underestimate the global plastic marine debris budget and hinder realistic model outputs. At the sea surface, the vertical distribution of MP is affected by wind-driven vertical mixing, sources and surface currents, among others. This forcing enables particles to be either mixed down with others (e.g. biological) or fragmenting and thereby, altering their size and/or floating/sinking behaviour. In this sense, a cruise was made (PE442 aboard *RV Pelagia*) from Terceira Island, Azores (open ocean) into Sicily, Italy (Mediterranean Sea) from 26th July to 9th August 2018. The main aim of the present study is to characterize the vertical distribution and composition of MP in these waters, and to infer if their main patterns are shaped by specific physical/biological mechanisms. For this, a total of 146 water samples (2 L each) were collected at eight chosen depths (from surface up to 1500m) using real-time profiles obtained by an ultra-clean multivalve CTD system. Samples were filtered on board and MP were subsequently identified mainly in terms of particle colour, type, and size. Concurrently, satellite-derived data was processed for the time and cruise area to infer near-surface spatial gradients and patterns of chlorophyll a and sea surface temperature. Preliminary results show that MP presence varies with depth with MPs present at the sea surface, just below the DCM layer, and at times in deep waters, with important differences between the open ocean *versus* MED areas. This study shows promise in shedding new light over water column MP behaviour. It also provides evidence of plastics transfer from sea surface to deep sea, their accumulation and fate with depth, and their possible association with biotic/abiotic processes.

Microplastic distribution on the Tyrrhennian seafloor controlled by deep-sea circulation

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Microplastics occur on all of the world's ocean floors. The deep seafloor is presumed to be their ultimate sink. These highly bioavailable tiny particles are foci for accumulation of pollutants; hence trophic transfer of microplastics is of both ecological and societal concern. Despite their ubiquity and importance, the processes that control the seafloor distribution of microplastic have not yet been demonstrated. We report seafloor microplastic distribution patterns in the Tyrrhenian Sea and relate these to the strength and orientation of ocean currents at the seafloor. Sediment cores were selected across a range of depositional environments identified with multibeam bathymetry, shallow seismic, and near-bed current modelling. Microplastics were found in all cores; dominated by fibres with lesser fragments - up to 181 and 9 pieces per 50 ml of sediment, respectively. These are the highest levels recorded in deep-sea sediments to date. The highest concentration is associated with an inter-canyon area close to the base of the continental shelf. The canyons link major rivers passing through urban and industrial catchments with offshore particulate transport. Sediment and microplastics are inferred to be transported by gravity currents within the canyons and subsequently reworked by ocean bottom currents. Microplastics are found in lower densities in drift moats, and higher densities in areas of drift accumulation. Using modelled bottom current shear stresses we demonstrate, for the first time, that microplastics dispersal and distribution on the seafloor is strongly controlled by bottom currents and basin physiography, and not solely by vertical settling as many models currently assume. We conclude that integration of existing knowledge of particulate transport processes in modern sedimentary systems, and the biological activity within them, can provide much-needed constraints on the transfer of microplastics to deep-marine environments, their distribution, ultimate fate, and implications for benthic ecosystems.



Microplastic load In the Northern Adriatic

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Plastic is the most common artificial material used today. As plastics degrades only at very slow rates it inevitable ends up in marine ecosystems, often ground down to microplastics. The microplastic concentration on the sea surface ranges from one thousand to hundreds of thousands of particles per km². The amount and distribution of microplastic in the sea depends on several factors, from the proximity of urban centers and the coast to the wind and sea streams. The data on microplastic waste in the Mediterranean Sea are generally scarce and show great variations between the investigated areas. The North Adriatic (NA) is a dynamic ecosystem with numerous and steep ecological gradients but also a relatively long seawater retention time, which puts it in a particularly vulnerable area for negative impact of microplastics. The aim of this study was to assess the spatial and temporal distribution of microplastic in the water column of the NA during one year. Microplastic was found in all samples, both from the surface and from the water column. High concentration variability was recorded at all stations during the monthly sampling regime. The average concentration was 179×10^3 MP particles/km² for the surface samples and 992 MP particles/dm³ was the average concentration for the water column samples.

Microplastic-specific microbial communities in the Gulf of Naples: concentrations, seasonal differences and composition

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Plastic pollution is a global issue, but still little is known about its effect on the marine biome. Both onshore and offshore, plastics undergo transformations by physical, chemical and biological factors, fragmenting into pieces millimeter and even micrometers in size. These little particles host microbial communities, which may take part in these transformations and represent the so-called "Plastisphere". We present here an investigation of the microbial plastispheres associated with microplastics collected at 3 sites in the Gulf of Naples (Italy) in two different times of the year (January and July 2018). Particles were counted and characterized for their chemical composition by Fourier-transform infrared spectroscopy (FT-IR) in ATR mode. The microbial community attached was analyzed by DNA amplicon sequencing of 16S rRNA gene hypervariable regions V4 and V5 (by Illumina) and Scanning Electron Microscopy (SEM).

Average microplastic concentrations in terms of particles per m³ were higher in July (3.13 ± SE 1.08) than in January (1.39 ± SE 0.12), although not significantly different. July samples presented significantly (p<0.001) more diatoms per mm² (386 ± SD 389) than January samples (227 ± SD 507). Conversely, January samples showed more bacteria (8905 ± SD 14392) per mm², than July ones (average 7264 ± SD 12776) per mm², although not significant (p>0.1)

From sequencing it resulted that the bacterial community attached to microplastics was different from the free-living one, with Bacteroidetes dominating the free-living and Proteobacteria the attached. In addition, different polymers were hosting their own specific bacterial communities (e.g. polystyrene vs. polyethylene), with Bacteroidetes phylum being more abundant on polyethylene and Firmicutes on polystyrene, suggesting that the substrate has a role in selecting species.

In conclusion, these data highlight a high diversity harbored by microplastic in seawater and suggest that plastic pieces represent a selective habitat for microbes, whose composition is affected by polymer structure, environmental factors, circulation and time of residency in the water.



Seasonal variation of microplastic pollution in Mediterranean islands as an effect of tourism

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The ubiquitous presence of larger-size plastics and microplastics (<5mm) is one of the key challenges threatening our aquatic ecosystems. The Mediterranean Sea has been recognised as one of the largest accumulation area of plastic debris. In addition, the coasts of the Mediterranean are the leading tourist destination for seaside vacation, strengthen the anthropogenic pressure in a very concentrated time frame. In this study, we investigated the seasonal variation of microplastic occurrence in sandy beaches of 8 Mediterranean islands during both the low and high touristic season. For each island, three different sites were selected for comparison: one very popular and touristic beach, one popular beach but mainly used by locals and one remote beach. Sand samples, five per survey, were periodically collected during 2017. Microplastic extraction was adapted from previous methods, and particles were characterized by size, color, shape and polymer type.

The preliminary results show that the 24 monitored beaches are contaminated with microplastics. The concentrations found in touristic sites are higher than in remote sites. Different season trends were observed: while in most beaches the concentrations are higher during the high touristic season, in few cases we observed an increase of microplastics through the year. Our results, at large geographical and temporal scales in the Mediterranean, provide new insights on the dynamics of these pollutants in coastal ecosystems linked to seasonal tourism.

Analysis of marine microplastics in the water column sampled up to 300 meters depth

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The presence of microplastic at open ocean is a well documented fact. These microplastics can proceed from macroplastic fragmentation (secondary microplastic) or from virgin pellets (primary microplastic). But they have been determined basically in the first 5 meters of the water column or near the seabed, not at different depths on the water column.

Microplastics found below surface can have similar density to seawater, as nylon of fishing nets or fibers. But there are other kind of samples with different composition, as polyethylene (PE) and polypropylene (PP), with a density significantly lower than seawater density, but due to their size and shape can appear hundreds of meters below seawater surface.

Based on this idea, specific sampling of microplastics at the water column were done. Stations were located near Canary Islands, but at oceanic areas, far enough for avoiding island influence. 72 liters of seawater were filtered per sample with a mesh size of 100 μm . Sampling was done between 0 and 300 meters at four different depths. Sampling period vary between April 2017 and March 2019. At samplings carried out, the presence of microplastics at each depth was observed, with some variability related with oceanographic conditions and seawater density profile.

Microplastics found were small fragments of fishing nets, lines, paint shavings, fibers and small fragments. This preliminary study assumes that there is an indeterminate microplastic size with neutral buoyancy according to oceanography dynamic that is not taken into account at prediction models, which can underestimate the tons of plastic existing at marine environment.

The identification of plastic composition was made by analytical pyrolysis with gas chromatography-mass spectrometry (Py-GC-MS) for several samples collected between 50 and 300 meters depth.





CLEANING STRATEGIES

The CLAIM Project: Clean is the Aim!

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Global production of plastic materials have steadily increased in the past fifty years leading to accumulation of Plastic Marine Litter (PML), recognized today as a major environmental problem, adversely affecting not only nature and biodiversity, but also society and human well-being.

In response, a four-year-long Horizon 2020 CLAIM project “Cleaning marine Litter by developing and Applying Innovative Methods in European Seas”, with the involvement of 19 institutions from 13 EU- and 2 non-EU countries is working for the prevention and in-situ management of both visible and invisible marine litter, through development of new technologies and redefining methodological approaches to innovate new ways to protect our seas from further pollution (www.claim-h2020project.eu).

CLAIM aspires to provide practical tools for a step change towards the mitigation and efficient ecosystem management of marine litter pollution in the Mediterranean and Baltic seas. 5 cost-efficient and innovative marine cleaning technologies and monitoring and modelling/mapping tools are being developed for tackling the menace of visible and invisible plastics. The cleaning technologies are being tested close to major input sources of marine litter (i.e. rivers and WWT plants). An ecosystems approach will guide the project through the evaluation of the potential benefit from proposed litter cleaning methods to ecosystem services and human well-being. This presentation will summarise the preliminary results of the first two years of activities.





Characterization of microplastics in main streams of conventional and innovative urban wastewater treatment plants

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In this study, the effectiveness in removing microplastics (MPs), including both fiber- and particle-shaped, was evaluated in a wastewater treatment plant (WWTP) of the Central Italy: MPs were extracted from different steps of water and sludge line of a full-scale plant based on activated sludge (CAS) processes and of a pilot-scale AnMBR system, consisting of an upflow anaerobic sludge blanket (UASB) reactor coupled with an anaerobic side-stream ultrafiltration unit, that is fed with the same wastewater treated by CAS.

The CAS process revealed an abatement efficiency of 83%: the influent contained 3.1 MPs/L, which were reduced to 1.9 MPs/L after primary settling, to 0.8 MPs/L after biological treatment and to 0.5 MPs/L in the effluent. Settling processes and entrapment in suspended solids favoured the abatement of microplastics from the liquid fraction and their accumulation in sewage sludge, that was confirmed by a concentration of 5.3 MPs/g measured in secondary sludge. μ FT-IR analysis highlighted a certain heterogeneity of polymers in the inflow and in the primary effluent, in respect to a dominance of polyesters after biological treatment, of ethylene/propylene copolymers in the final effluent and of polyethylene and polypropylene in secondary sludge.

In comparison, the innovative AnMBR system removed the 94% of microplastics, providing 1.7 MPs/L dominated by synthetic fibers and polyethylene particles after UASB reactor, further reduced to 0.2 MPs/L after the ultrafiltration unit, where only microfibers were found.

The study suggested as the innovative anaerobic configuration coupled with the AnMBR membrane minimizes the input of MPs in the environment, in respect to traditional wastewater treatment schemes. Moreover, results on MPs characterization extracted from primary, dewatered and UASB sludge will provide a complete assessment of the destiny of microplastics in conventional WWTPs and will highlight a possible effect of anaerobic processes on MPs degradation.

Fate of microplastics in the mainstream of wastewater treatment plant

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Wastewater treatment plants (WWTPs) are acting as an important route of microplastics (MPs) to the environment. It is therefore pertinent to examine MPs in wastewaters through sampling programs covering extended periods. In this study, the efficiency of a municipal WWTP to remove MPs from wastewater was studied by collecting raw, primary secondary and tertiary treated wastewater samples during a 12-month sampling campaign. The WWTP is a typical conventional activated sludge (AS)-based process with tertiary treatment using rapid sand filtration and disinfection step before releasing the effluent for unrestricted agriculture use. The microplastic particles and fibers of the different unites of mainstream were identified by using an optical microscope, FTIR and Raman microscopy. Overall, the retention capacity of microplastics in the WWTP was found to be 97.5%. Most of the MP fraction was removed at the secondary stage (before the sand filter). The efficiency of the sand filtration step was also examined. The main related finding is that sand filtration permeate contained 1.97 MP/L in comparison with the final effluent of the AS process (2.72 MP/L). According to this study, the relative abundance of particles was lower than fibers in treated effluent compared with the raw wastewater. The secondary and tertiary treatment processes removed particles more efficiently than the plastic fibers. The fibers made up around 74 % of the total microplastics in the raw wastewater, and in treated effluent water 91 %. Total MPs detected in the effluent were significantly higher in winter and spring samples compared with summer and autumn. Most of the MP particles detected were in the 200 μm < d_p < 450 μm size range. The MP fibers ranged between 1000 μm and 2000 μm in length and 10 to 20 μm in width. While most of the fibers were of PET origin, the MP particles consisted mainly of PE, PVC, PC and PP.



Macro and microplastics in stormwater and combined sewer overflows in Paris megacity

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Stormwater and combined sewer overflows (CSO) might represent an important source of macro and microplastics in the environment, but such fluxes are poorly investigated. In order to fill this knowledge gap, macro and microplastic concentrations were investigated (i) in stormwater and grid refusals at the outlet of a runoff water pipe located in Sucy-en-Brie (Paris suburb, France) and (ii) in CSO discharges at the Clichy outfall (Paris suburb, France). Macroplastics were sorted, weighted and classified. To study microplastics during rain events, 500 litres of water were filtered using plankton net with 80 μm mesh size. Microplastics were then extracted using H_2O_2 30 % digestion followed by NaI density separation ($d=1.6 \text{ g.cm}^{-3}$). A microscope and Fourier Transformed Infrared spectroscopy were applied to identify microplastics. Preliminary results from stormwater outlet of Sucy-en-Brie showed that macroplastic mass percentages were in the range of 1.5 to 37.5 %. Moreover, 8 main plastic categories were found to contribute to 95% of total macroplastics and were identified as sanitary textiles, plastic bags or films, food packaging, bottles, cigarette buds, garbage bags, and polystyrene fragments. Macroplastic concentrations in stormwater from March to June 2018 ranged from $3 \cdot 10^{-5} \text{ kg/m}^3$ to $1 \cdot 10^{-4} \text{ kg/m}^3$. Three rain events were sampled at Sucy-en-Brie to study the concentration of microplastics. Microplastics with size of 1 to 5 mm during first rain event were analysed. Results indicated that microplastic concentrations were in the range of $4.6 \cdot 10^{-2}$ and $9.3 \cdot 10^{-3}$ fragments/L, in which polyamide and polyethylene predominated.

The effect of ozonation used for drinking water treatment on different types of submicron plastic particles

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Microplastics have been recently detected in bottled drinking water as well as in drinking water sources. Studies addressing the fate and removal of submicron sized plastic particles during drinking water treatment are currently lacking. Ozonation is a key treatment step in modern drinking water treatment plants due to its excellent disinfection and oxidation properties. Therefore, we evaluated the impact of ozone treatment on the physicochemical properties of three types of submicron plastic particles in terms of morphology, surface charge and particle aggregation state. Two plastic particles, a rough polyacrylonitrile/polystyrene co-polymer (PAN/PS) and a smooth polyacrylonitrile particles (PAN), were synthesized and showed a hydrodynamic diameter of 215nm (PDI:0.077) and 150nm (PDI:0.043), respectively, in agreement with the diameter derived from transmission electron microscopy (TEM) images. Commercially available 100nm polystyrene spheres represented the third plastic particle (PS). In individual batch experiments, the plastic particles (10^7 - 10^8 particles/mL) were reacted with ozone (0.5, 1 and 5 mg/L) in either buffered-DI H₂O or in a lake water (used as a source of drinking water for the city of Zurich, Switzerland). After the ozone treatment in the experimental media, the hydrodynamic diameter of the particles suspended in buffered-DI H₂O and lake water increased in the range from 253-409nm, 550-620nm and 116-150nm for PAN/PS, PAN and PS, respectively. All the plastic particles kept a negative surface charge once suspended in the experimental media, but, after ozonation, the Z-Potential decreased from -20 to -30 mV for particles suspended in the buffered-DI water and from -14 to -17 mV for particles suspended in the lake water. Initial results from TEM investigations of particles which underwent ozone exposure revealed a very similar size and surface structure as observed for the pristine particles. Thus, ozonation does not appear to either fragment plastic particles nor dramatically change their surface charges. Whether more subtle changes on the surface properties are induced by the ozone treatment is currently under investigation.



The role of jellyfish mucus stability in capturing microplastic particles

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If stressed, jellyfish can release mucus in larger quantities from gland cells present in their epidermis and gastrodermis. Mucus production is a form of chemical defence of jellyfish, which is indicated by the presence of discharged and undischarged nematocysts and toxins in the released mucus. Recently, the biotechnological potential of jellyfish mucus was recognized, in particular as a starting biomaterial for development and production of biofilter to capture nanoparticles (NP) and potentially microplastic (MP) present in the outflows of the wastewater treatment plants. This task is one of the main goals of the EU project “GoJelly – a gelatinous solution to plastic pollution” (<https://gojelly.eu/>). The aim of this study was to characterize jellyfish mucus’s biochemical composition and its stability to efficiently exploit its properties for further biotechnological applications: (i) stability: the time frame from harvesting jellyfish on a vessel and laboratory mucus extraction step and preservation can be long; (ii) bacterial degradation of jellyfish mucus that should be minimized. To address these properties we performed experiments for mucus stability at different temperatures, following the changes biochemical composition and bacterial community growth rates in time. We collected mucus from two different jellyfish species (*Aurelia aurita* s.l. and *Rhizostoma pulmo*) seasonally re-occurring in the Northern Adriatic Sea. Daily subsamples for bacterial community abundance as well as protein, carbohydrate and amino acid composition of mucus were collected and analysed. The storage temperature effect on mucus composition and structure stability were tested by analysing its ability to capture/adsorb different fluorescent MP particles. We will present our preliminary results and guidelines for mucus storage to ensure its optimal MP adsorption characteristics. Our results indicate that this material is one of the possible new solutions for microplastic pollution mitigation.

nano-FTIR spectroscopy: nanoscale resolved infrared spectroscopy of polymeric nano-particles and self-assembled monolayers

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nano-FTIR is an emerging new optical super resolution microscopy technique that enables IR spectroscopy and chemical mapping with down to 10nm spatial resolution. Utilizing broadband laser sources like a mid-IR supercontinuum laser for tip illumination and interferometric light detection analogous to classical FTIR spectroscopy enables near-field spectroscopic measurements at nanometer scale spatial resolution with unprecedented signal quality.

Owing to its orders of magnitude higher detection sensitivity compared to standard FTIR spectroscopy and extremely high spatial resolution nano-FTIR has been successfully employed for determining the chemical composition of multi-component thin film polymer blends, probing the local secondary structure in individual insulin and collagen fibers and detecting individual single protein complexes.


We demonstrate for the first time nano-FTIR spectroscopy and infrared mapping of a thin self-assembled polymer film with a thickness of only 7nm. nano-FTIR spectra are reproducible and are in good agreement with standard FTIR spectra from similar, yet bulk materials. These first studies open a whole new perspective and opportunities towards nano-chemistry and nano-biology as well diverse studies of nanoplastics.







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Yellowness index determination using a mobile app

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POSTER SESSION I

P1

In recent years the number of publications related to the study of marine microplastics has increased significantly. Many of these scientific articles improve methodologies for sampling, collection and identification of plastic type. The identification of the plastic composition is done mainly by infrared spectroscopy (FTIR) y by analytical pyrolysis with gas chromatography and mass spectrometry (Py-GC-MS).

These microplastics can be present in marine environment for long periods of time, being transported thousands of kilometers before reaching the coasts. The aging and degradation of the plastic produce a yellowing of it. The main index that defines the degradation state of the plastic is the Yellowness index (YI) (%), however, standardized methods for YI determination are complex and require specialized equipment. This produce that many authors determine YI only from a qualitative or comparative point of view, without giving quantitative values to the samples they process.

In this study we studied marine pellets found on the coasts of Canary Islands (Spain). These samples were analyzed by FTIR to determine their composition. Samples of High Density Polyethylene (HDPE) composition was selected. These pellets were analyzed with a colorimeter, determining the YI for each sample following international standardized method E0313-15E01. Results for each sample were compared with the obtained using the color measurer *Pantone® Studio*, an app easy to use which allows the determination of sample's color instantly and easily. The comparison between these two results has allowed obtaining a quantitative scale to measure the Yellowness Index (YI) using this app for mobile devices.

Risk assessment analysis on microplastics

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The accumulation of microplastics (MPs), plastic particles with a diameter smaller than 5 mm, in marine environments has raised health and safety concerns. Because of their small size, MPs are potentially bioavailable to a wide range of marine organisms. There is increasing evidence that MPs may be transferred through the food chain from prey to predator and may eventually lead to bioaccumulation of MPs or associated toxic substances. However, there is currently only limited evidence of transfer of chemicals from ingested plastics into tissues. Effects on the marine food-chain can by extension pose potential risks to human health through the consumption of seafood, and may lead to socio-economic costs.

Careful risk assessment consists of several steps:

- Modelling MP particles distribution;
- Sediment analysis;
- Mapping aquaculture;
- Studying effects of microplastics on marine organisms;
- Studying presence of chemicals and bacteria on microplastics;
- Potential economic impacts.

Environmental concentrations of MPs are presented, followed by results from literature and experiments on the potential effect of MPs on the area of socio-economic interest. Based on the data collection on web there is a socio-economic hazard of MPs on the sea coasts that indicate a potential hazard for human health. Models and field studies have indicated the coincidence of microplastics and aquaculture areas, and seafood sampled from the field and supermarkets contain microplastics. In general, the risk analysis of microplastics on the coasts is currently incomplete and uncertain due to limited information on exposure levels and established effect levels.

Rationale policy measures are therefore difficult to develop. It seems however, that cleaning up of the microplastics already present in the marine environment is an impossible feat due to the wide spread of these particles in riverine, estuarine and marine systems as well as the technical limitations to filtering out only plastics



Cigarette Butts as a Source of Microfibers to the Environment

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POSTER SESSION I

P3

Microplastics ubiquity is a currently well-established and rising contamination concern. Nevertheless, some investigations have reported *non-synthetic polymers* as the predominant type of microparticles polluting the environment. For instance, microparticles contamination in deep sea sediments is four order of magnitude more abundant than in most contaminated superficial areas. Most of these microparticles were identified as *rayon* microfibers.

In this way, two main observations arise from those findings: 1) rayon is a textile polymer with a relatively low global production rate; and 2), depending on the polymeric identification method, rayon could be easily confused with other *cellulosic polymers*. Henceforth, these cellulosic microparticles on the sea bed could have other unidentified sources that needs further investigation.

In the present study, one possible source of non-synthetic microparticles was investigated: the *microfibers released from cigarette butts*. These are mainly *artificial fibers* made up of cellulose acetate, a *chemically modified cellulose*. In our studies, it was found that one cigarette butt can release *more than 1000 small microfibers per day* (<0.2 mm), contributing as much as textile microfibers to the microparticle pollution. Also, this material was found to be hardly oxidized when applying UV light or the Fenton reaction, which is consistent with the slow degradation rate (up to 30 years) mentioned by the literature. In addition, the effects of cigarette butts on algae were measured and it was concluded that they pose an additional and inherent risk due to the hazardous substances adsorbed during the puff of the cigarettes.

Lastly, cigarette butts have been widely identified in the environment as persistent and hazardous pollutants but still no attention has been put onto them as a microparticle source. Thus, we consider that *the microplastic terminology should be re-evaluated*, as other types of artificial microparticles could have similar or even worse impacts throughout the ecosystems.

Amino-modified hyper-crosslinked resins for textile wastewater treatment

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Hyper-crosslinked resins (HCLR) are high surface area materials extensively studied for their adsorption properties towards gases and organic pollutants from water. HCLR are particularly interesting for the wide possibility of tailoring their porosity and adsorption properties through different synthetic procedures, by using specific precursor monomers or by including particular functional fillers into the polymer matrix to obtain hyper-crosslinked nanocomposites. Recently, we evaluated the effect of a polar functionalization of divinylbenzene (DVB) – vinylbenzyl chloride (VBC) based hyper-crosslinked resins on the HCLR porosity distribution and adsorption properties, obtaining interesting insights into the mechanism of pore size modification and on the effect on the adsorption properties of the resin towards different gases and organic pollutants. In this work, a new route is exploited to functionalize DVB – VBC based HCLR by introducing amino groups into the hyper-crosslinked aromatic structure. The grafting of –NH₂ functional groups on the HCLR has a significant effect on the surface area, the pore size distribution and the adsorption properties of the resin. In particular, the adsorption isotherm of the HCLR changed from the typical BET multilayer adsorption isotherm to the monolayer Langmuir type. The neat and functionalized hyper-crosslinked resins porosity and adsorption properties were investigated through the evaluation of the specific surface area and the pore size distribution. Finally, the functionalized resin was tested for the decontamination of wastewater from textile dyes.



Assessment of the impact of the washing conditions on the microplastic release of PET fabrics

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POSTER SESSION I

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Over 240 million tons of plastic are used every year and the microplastics (plastic particles with dimensions of less than 5 mm, including very small and nano particles), are a subject of intense research for high pollution especially found in the sea.

A large proportion of microplastic fibers found in the marine environment may be derived from effluents of washing of synthetic clothes. A publication indicated that the number of fibers released from washing 6 kg of laundry could reach more than 700,000 fibers. Synthetic fibers account for approximately 60% of the total global fiber production, and polyester (polyethylene terephthalate (PET)) and polyamide (nylon) prevail.

In this study the amount of microfibers release from two different 100 % polyester fabrics, in different washing condition (different programs and temperatures) was investigated comparing the use of detergent alone vs detergent with stain remover. The data were taken after 1, 2, 3, 4 and 5 cycles and four replicates were carried out for each test to verify the reproducibility of data and to decrease the statistical error. The material collected from filtration was characterized with qualitative and quantitative methods. Morphological and structural changes were analysed by Light Microscopy and Scanning Electron Microscope. Infrared spectra of unwashed polyester fabric, residues after washing were acquired. The thermal properties were measured by Differential Scanning Calorimetry.

The structure of sample and washing conditions significantly influence microplastic release.

First investigation of microfibre release from the washing of laminated fabrics for outdoor apparel

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In the last decades in EU, the total consumption of clothes per person has increased by 40 %, as a consequence of low prices and of easy access to fashion. In parallel, the request for high-tech functional fabrics has been increasing for the application in outdoor apparel. The main properties of these functional fabrics are waterproofness, breathability and climate-regulating performances. These properties are obtained through the combination of high performance materials in a laminate structure.

Since the washing of synthetic clothes has been identified as one of the major source of microplastic pollution in marine environments, several studies have investigated which textile characteristics may influence the release. Such investigation is of critical importance to provide indications to the textile industry on the ways to mitigate the environmental impact of their products. In this respect, the peculiar structure of laminated fabrics is of particular interest for the evaluation of their release of microfibrils during washing.

For this purpose, washing test at lab scale were performed on several type of laminated fabrics, which differ for raw materials used, textile construction and characteristics. For each type of laminated fabrics, samples obtained from different steps of the laminate production were tested, from raw materials to laminates with or without durable water repellent (DWR) treatments. The number of microfibrils released was evaluated by filtering the water coming from each washing test and analysing the related filters by scanning electron microscopy (SEM). The outcomes of this investigation allowed to identify trends in the microplastic release from laminated fabrics, pointing out which factors in the choice of the materials and production may have an influence on the release. In particular, comparing the quantities of microplastics released from the raw material and from its laminate, the results indicate that lamination seems to reduce the amount of microfibrils released.



Understanding nanoplastics through the nanomaterials analysis

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POSTER SESSION I

P7

Plastic pollution is a great issue affecting our Planet, especially marine ecosystems. While macroplastic and microplastic pollutants are already established, nanoplastics are now emerging as important actors of this contamination.

One of the main drawbacks with the study of nanoplastics is their isolation from environment, due principally to their small size, and this fact also strongly hampers their characterization. Among the analytical methods available, some authors start using Nanoparticle Tracking Analysis (NTA) or Dynamic Light Scattering (DLS) to determine nanoplastic particles size, coupled with spectroscopic techniques to define physical-chemical characteristics.

Nanoplastics are not nanomaterials synthesized with the desired size, shape, surface, and composition. For nanoplastics there are no reason for a selection in size, they could have asymmetrical shape and heterogeneous surfaces. As a consequence, another issue is how to produce nanoplastics at the laboratory scale, in a way that mimics what happens in nature. Create a good reference material may allow scientists to better understand the characteristics of nanoplastics and how nanoplastics interact with marine environment and living ecosystem. In fact, nanoplastics could penetrate into the living organisms in a different way than macro- and microplastics, potentially showing unexpected effects in terms of ecotoxicity.

In this work, we present a preliminary study on the production, isolation and characterization of nanoplastics. Starting from commonly used disposable plastic items, we use different techniques to fragment or degrade macro-pieces into nanoplastics in a controlled way, such as mechanical disruption or photodegradation. We include methods for preconcentration and separation of the nanoplastic into specific size fractions. Finally, our approach combines consolidated techniques in the field of nanomaterials, such as imaging techniques, size distribution and physical-chemical characterization techniques, to study nanoplastics.

Half-life as a specific material property-Modeling the performance of biodegradable plastics in different environments

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The replacement of conventional plastics with biodegradable plastics is discussed as one possible contribution to mitigate plastic pollution in the environment. Several standard tests exist to assess the degradation under various conditions such as in industrial compost, soil or the marine environment. However, so far, a single parameter to compare test results of different plastic materials, i.e. pure polymers and blends, in different conditions is lacking. Here, we show the mathematical modeling of results from tests under marine conditions in the laboratory, in mesocosms and in the field. We describe the calculations applied and discuss the constraints of our method. As an outcome, we propose to use the disintegration or degradation half-life of a material under specific test conditions as a specific material property. This value can serve to compare the degradation of different materials in the same environment and to compare the performance of the same material exposed to different conditions. The principle of biodegradation half-life will help plastic producers, manufacturers and distributors with material development and choice of application. It will also enable decision makers to easily compare different scenarios and to evaluate whether or not a certain material application will perform in the environment as technically or politically desired, claimed or expected.



Analysis of plastic litter detection using remote sensing systems

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POSTER SESSION I

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Nowadays, Unmanned Aerial vehicles show very interesting performances for marine plastic litter detection. However, these systems suffer from some important limitations, mainly due to the coverage, cost and distance issues. For these reasons, space-borne based plastic litter detection from satellites is gaining interest. Due to the wider swath coverage, free or lower cost of data preparation, multiple passes, remote sensing satellite systems seem a promising instrument for the plastic litter detection problem. The main limitation of space-borne data is related to resolution issues. Up to now, a few studies have explored the potential applications of satellite remote sensing for detection of plastic waste. Generally, optical remote sensing sensors have been used for the direct detection of high densities of plastic. Typically, plastic litter shows significant reflections in the 0.4–2.8 μm region of the spectrum, where most multi and hyperspectral systems operate, such as Sentinel-2A & B of European Space Agency (ESA). The spatial resolution of Sentinel-2 seems to be sufficient to detect aggregated objects, plastic litter and other natural sources of debris, as well as anthropogenic sources.

Moving to radar frequencies, space-borne Synthetic Aperture Radar (SAR) has indisputable benefits of weather independent and day-and-night wide-swath earth observation. Besides that, the very high resolution SAR data with polarimetric information allows the application of SAR systems in specific targets identification. It is understandable that plastic litters can have effect on the SAR backscattering and on the polarimetry.

In this paper an analysis of the previously reported space-borne systems will be conducted. The aim is to evaluate the possibility of developing a hybrid target indicator with multi band optical and high resolution SAR data. Concerning the optical systems, modelling studies will be conducted in laboratory in order to generate and possibly create a dictionary of spectra characteristic of plastic marine litter. Model samples and mixtures simulating the composition of plastics containing also the most common additives, antioxidant and dyes will be created in the laboratory.

Is plastic a risk to human health?

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Overall plastic is a risk for the biota and the health of ecosystems, Homo sapiens is also included in the biota of ecosystems. Starting from this assumption, much work has yet to be done to evaluate the effects of plastics on human health and the risks to ecosystems health. Humans are at the top of the food chain of ecosystems and can come into contact with plastics under different exposure routes: from food, including water, to inhalation, to those linked to lifestyles (clothing, cosmetics, etc.). Negative effects of plastic on biota are well known and described in many scientific papers. First of all, the death of sea turtles, marine mammals and sea birds can be caused by ingestion of plastics, starvation, suffocation, infection, drowning and entanglement. Furthermore mechanical disorders on marine vertebrates and effects on the physiological system of fishes and other aquatic organisms or the passage through the lymphatic system or brain cells have been recorded. Some scientific evidences highlight possible interactions with human health but are not exhaustive and suitable for a risk assessment as a whole. The direct exposure routes are known: for example the air that we breathe contain microplastics, or the exposure via chemical by-products , they have been found in the lungs, detected in the faeces or can accumulate in food. Some estimates show the amount of plastic that arrives in our body through various routes daily/weekly or annually. The major route of exposure appears to be food from aquatic ecosystems. Moreover, pilotes studies among nylon fiber workers suggest a link with several pathologies such as respiratory irritation The plastic represent also a newly route of the spread and transmission of bacteria, viruses and or protozoan, due to biofilm forming on its surface These so called "new vector" represent a new issue in terms of prevention strategy planning for human health. Should we enhance scientific studies in order to collect evidence or invoking the precautionary principle? Both of them. Now or in the near future. Chronic exposure to plastic in humans is an issue of great concern to our health. Adopt the precautionary principle now allows to react quickly to a possible risk for human, animal, plant health and is necessary for the protection of the ecosystems as a whole.



BLUEMED Pilot: Towards Plastic-free, Healthy Mediterranean Sea

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The health of Mediterranean sea is crucial for the future in order to guarantee our social wellbeing and prosperity. Plastic pollution represents today a serious risk for the environment and human health, and also indirectly affecting our lives by damaging those key economic sectors that rely on sea resources as for Fisheries and Tourism. To close the gap between international governance structures, technical innovation and the management of plastic litter across Mediterranean basin, the Euro-Mediterranean Group of Senior Officials BLUEMED Working Group steering the BLUEMED Initiative (www.bluemed-initiative.eu) agreed to launch a BLUEMED Pilot Action “Plastic-free, Healthy Mediterranean sea”. In line with its objectives, the BLUEMED Pilot action consists in creating a shared strategic environment to promote the implementation of best practices and actions to specifically address the Mediterranean challenge as a whole. With the support of the partners of the BLUEMED Coordination and Support Action, a project funded by the EC H2020 R&I Programme, national delegates from Mediterranean EU and non-EU countries, in consultation with their communities of stakeholders undertake key activities to showcase how, working together, R&I can tackle the litter and the plastics challenge in the Mediterranean, in the framework of the Blue Economy. In line with the priority goals of the BLUEMED Initiative, the Pilot will channel the potential of the bio-economy to contribute to addressing plastic pollution at sea and deliver on the targets of the relevant strategies, with measurable goals within a set timeframe.

Implementation of a sensitive thermal degradation method based on GCMS-pyrosis to estimate fluxes of microplastics in sewage treatment plants

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Substantial amounts of microplastic particles are likely to be collected in the sewage system, mainly deriving from cleaning of synthetic clothing, and waste water disposal facilities have been identified as point sources of microplastic in the aquatic environment. Today's waste water treatment plants (STP) are not designed to manage microplastics. The main objective of this study was to develop a standardized method to collect, quantify and characterize microplastic particles. This study aims at contributing to the knowledge about the possible significance of waste water treatment facilities as point sources of micro-plastic particles into food chains both in water and land. Special attention is addressed to the sewage sludge, a valuable by-product of the sewage treatment process being adopted in agriculture production. According to Scandinavian practice stabilized waste water sludge shall preferably be utilized on agricultural soil. However, more research is needed to understand the incidence, potential accumulation and biological adverse effects of micro-plastic in aquatic and terrestrial ecosystems. We have implemented a pyrolysis-gaschromatography-mass spectrometry (Pyr-GC-MS) methodology to waste water filtrates, sludge and plant material. Applied to waste water deriving from the Stavanger area, a typical Scandinavian urban settlement of about 250.000 inhabitants, the data obtained so far suggest a microplastic content (100-500 um) in the influx water of approximately 0.5 % of the water dry matter content, divided fairly evenly between the five plastic types tested. Application of the methodology to a wider range of WWTPs will bear out whether similar figures generally holds in a Scandinavian context.



Resuspension of microplastics particles of different shapes by unidirectional constant flow: laboratory tests

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POSTER SESSION I

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Critical shear stress (critical flow velocity) at which microplastics particles (MPs) settle to the bottom or are re-suspended back into the flow is one of the key factors to learn when trying to describe their transport and accumulation. In contrast to natural sediment grains (sand, granules, and pebbles) the MPs have much lower density and the variety of shapes. Thus, MPs motion in the near bottom layer substantially differs from that of bad-load material. In order to observe this specific behavior, test runs were performed (supported by the Russian Science Foundation grant No 19-17-00041) in a 10-m long laboratory channel. To represent the variety of 'native' bottom MPs, the 3-D plastic and amber fragments, flat PS flakes, rigid fishing line cuts, and flexible threads/fibres were used (two sizes for each particle type). Experimental runs were performed under the step-wise change of the unidirectional flow velocity over the different bottom sediments (natural coarse sand, gravel, pebbles).

Laboratory tests show that settling/re-suspension behavior of bottom MPs particles under the influence of unidirectional flow is highly dependent on the particle shape. While flat fragments may stay motionless under the high-velocity flow (being captured within viscous boundary layer), flexible threads and fibres are easily captured by the flow. Thus general mobility of the particle on the bottom increases (i.e., the critical shear stress should decrease) from 3D-particles – via flat fragments and elongated rigid lines – to flexible threads and fibres. The differences in the behavior become more pronounced with the increase of the bottom roughness. Over the pebble bottom, MPs particles tend to be clogged between sediment grains and are kept there even under strong flow. Thus, sorting of MPs particles by shape might be expected in natural environments.

Microcapsules contribute to microplastics pollution

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Fabric softeners use microencapsulation technology to enhance the durability of fragrance delivery in washed clothes. Microcapsules are designed to protect the fragrances during storage as well as during stages of the washing process. Generally they are made of cross-linked, non-degradable polymers. A significant part of microcapsules is not retained by clothes and is released into the wastewater system and into the environment. Results of microcapsule analysis in commercial products will be presented.



POSTER SESSION I

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Oil extraction as separation method for microplastic in sediment samples

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POSTER SESSION I

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Microplastic (MP) are widely distributed in the environment and have nearly detected in every area. To analyse environmental samples, like from the Mediterranean Sea, it is necessary to separate MP and other matrices. Right now, there is no standardized method to separate MP from different matrices in environmental samples. Especially for sediments it is an important preparation step as compared to water samples the sample volume cannot easily be concentrated on a filter. An easy and effective method is the separation with oil due to the lipophilic characteristics of plastic. Oil does not have toxic properties, like other substances used for separation, and does not depend on a specific density, what is fundamental for commonly used density separation methods. Since there are only a few publications about this method, it is extensively validated in this study by using spiked MP. Therefore, 8 different polymers with densities lower and higher than water and 9 different particle shapes were analysed. Together with the extraction method a plastic free separation unit was used to separate MP. In total, more than 200 experiments will be carried out and evaluated by identifying the recovery rates of the different polymers and different shapes. The experiments were conducted with oil to define the theoretical load capacity of particle sizes < 5 mm and additionally with sediment samples by adding oil.

At the conference the experimental setup will be presented and the results will be shown.

CHEMOPLASTICS: the multivariate side of microplastic pollution

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The staggering plastic consumption in the world imposes, as soon as possible, a very reliable solution to monitor the pollution. The contamination processing requires analytical techniques capable of very quickly recognizing the type of plastic to act promptly. To achieve this purpose vibrational spectroscopy is one of the most useful technique. Within this latter, to make the process more efficient, the use of portable instruments is a very smart approach as this kind of instruments significantly reduce the costs and time of analysis and allow you to collect a lot of data in a very short period. The large amount of data collected requires a very robust and reliable method to analyse them, and to clear up this issue, chemometrics is a very powerful approach. Chemometrics applies statistical tool to the analysis of chemical data, the design of chemical and physico-chemical experiments, and simulations. It obtains valuable information from broad and complex data sets and facilitates the revealing of hidden relationships between variables. Chemometric methods can be divided into two types: unsupervised and supervised approaches. The most commonly used unsupervised techniques are Principal component analysis (PCA) and Cluster analysis (CA). Supervised methods employ different data information to develop classification or regression models of some sort that can then be applied to predict new samples. In classification techniques the number of classes is known, as the membership of samples to the classes. Classification methods belong to supervised pattern recognition techniques and include: Linear discriminant analysis (LDA), K-nearest neighbour (KNN), Support vector machine (SVM) and Partial least squares discriminant analysis (PLS-DA). The aim of the work is to study the potential of chemometrics combined with portable spectroscopy techniques for a reliable detection of plastics in the environment.



Sorption kinetic and reversibility of current-use pesticides and personal-care products to polyethylene microplastic in seawater

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POSTER SESSION I

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Plastic debris access to the marine environment through direct discharges and transport from coastal areas and are present in all marine compartments. The potential impact of plastics in the marine environment is mainly fourfold: (i) inducing direct physical effects; (ii) releasing part of their constituents (monomers, base polymers and additives); (iii) being a pollution vector and iv) contributing to the rafting and transport of organisms providing new habitats. The third issue is relevant because plastics act as passive samplers from the surrounding environmental compartments (air, water, soil, etc. and particulate matter), either in continental or/and marine areas, accumulating hydrophobic organic contaminants. The majority of environmental and sorption studies have considered persistent organic contaminants and PAHs, but many more substances can also be sorbed as it has been confirmed in coastal areas. The role of plastics as pollution vector is relevant because they can favour the transfer of these contaminants of emerging concern through different environmental compartments and their bioavailability will depend on their sorption interactions and reversibility.

In this study the sorption of several current use pesticides (CUPs) and personal care products (PCPs) on polyethylene has been characterized in seawater. Specifically, the sorption kinetic and reversibility of triclosan, galaxolide, tonalide, pendimethalin, propyzamide and methyl-chlorpyrifos have been determined at dark conditions and different temperatures. The partition coefficients varied from 300 to 10000 mL/g depending on the hydrophobicity of the contaminants and the experimental conditions (temperature, contaminant and microplastic concentrations). The sorption process was reversible for all cases and the extension was mainly dependent on the corresponding hydrophobicity, as it was found in real plastic debris.

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Study on the occurrence of microplastics from marine pollution to human food chain (In SiRiMaP PON Project)

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RASFF notifications and EFSA website report the occurrence of high levels of microplastics in seafood. Ingestion of water contaminated with microplastics is the main exposure route for commercially important fish species (Atlantic cod, Atlantic horse mackerel), and crustaceans.

Plastics enter the marine food chain when they degrade producing microplastics. The food web is extremely complex. Plastic debris has an adverse impact on Zooplankton, organisms at the bottom of the food chain, that is eaten by different fish. Fish ingest microplastics due to their continuous uptake of water. Microplastics get into the next level of the chain when other animals eat fish contaminated with microplastics. Eventually, microplastics move all the way up to the top of the food chain, and being highly persistent in the environment, pose a serious issue to humans at the end of the food chain.

The potential accumulation of microplastics in food chain could have adverse effects on human health. Risk associated with ingestion of microplastics into the human body is a function of hazard and exposure. In addition, chemicals may be absorbed on the surface of microplastics, transferred from ingested microplastics to animal tissues, and may cause impairment of key body functions.

Currently, there is still a knowledge gap regarding the threats, toxicities, and adverse health effects in humans posed by the ingestion of microplastic-contaminated food, and no regulatory requirement to increase human food safety. To assess the risk of transfer of microplastics along the marine food chain, microplastics both in benthonic and pelagic Tyrrhenian and Adriatic seafood will be researched. Based on the physiological and nutritional characteristics, the fish species will be studied as markers to evaluate the potential impact of microplastics on the marine ecosystem and human food safety (SiRiMaP-PON project, 2019-2021-4.6 action.)



Pump-underway ship intake: an opportunity for marine strategy framework directive (MSFD) monitoring needs. First observations of microplastics on oceanic and coastal waters off the Canary Islands (subtropical NE Atlantic)

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POSTER SESSION I

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Broad scale sampling methods for microplastic monitoring in open ocean waters is a current challenge in oceanography. Large amount of samples is required to understand distribution, abundance and fate of these particles in the environment. The underway water system of research vessels (RVs) has been approached for microplastic sampling in some studies up to date. We present a sampling methodology founded on the design of a microplastic sampling device connected to the pump-underway ship intake system as an opportunity for oceanic monitoring needs concerning microplastics.

This methodology provides four main advantages: (1) the device is fully made with standard materials, cost-effective and affordable, and it can be self-mounted by the researchers without additional skilled personnel or equipment on-board; (2) it can be employed to report data taking advantage of oceanographic campaigns without interfering their regular vessel activities; (3) it is highly versatile and reduces the time needed to recover each sample; (4) it is suitable to sample microplastic particles down to 50 μm .

As preliminary field application results and first reported data from the Canary Islands oceanic and coastal waters, we investigated microplastics (0,05 – 1 mm) sampled from subsurface waters (- 4 m) using the pump-underway system of a RV in the Subtropical NE Atlantic. Sampling was performed in three consecutive oceanographic campaigns over a year, repeating the same procedure, retrieving water while on navigation and on coastal and oceanic stations. Microplastic particles (> 50 μm) were found in the total stations and transects sampled. Fibres (64.42%) were predominant over fragments (35.58%), being the concentration values over the data reported in other areas in the Atlantic.

This system was proved to be an efficient sampling method to report data on microplastic abundance and distribution, addressing the threat that microplastics pose to the marine environment and ecosystems.

Degradation of expanded polystyrene (EPS) beads in sea environment

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Nowadays microplastics are ubiquitous in the environment. They can be found in indoor and outdoor, in water and sediments and in terrestrial and aquatic organism. Their main sources vary but come largely from tyres, cloth washing, urban dust, cosmetics and fishing gear.

The most abundant plastic polymers that constitute microplastic particles and fibres are: polyester, polystyrene, polyamide, polyethylene, etc. These polymer types show different colours, shapes, morphologies. However, surface degradation due to weathering, bacteria and UV aging must be investigated to better assess and classify them. The aim of this work is the study of morphological and structural change of expanded polystyrene beads (EPS) before and after 70 days of incubation in the sea through Scanning Electron Microscopy (SEM) and Infrared spectroscopy (FTIR) characterization. In addition, the analysis of adsorbed organochlorine compounds, heavy metals and polycyclic aromatic hydrocarbons have been carried out.

To expose the EPS beads to waterborne contaminants in an efficient way custom mesh bags filled with 7g of EPS beads were deployed underwater, anchored to the sea bottom with a rope.

The marine site where the plastic beads were incubated was close to the harbour of Leghorn, one of the main commercial port in the Mediterranean area. For these reasons the site where the samples were deployed was expected to present a rather low water quality with an high level of contamination. During the incubation period, the bags were periodically checked to monitor that proper incubation condition were being maintained.

The morphological analysis highlights moderate levels of degradation and colonization of the incubated beads. The contaminant analysis shows a strong increase of the compounds investigated after marine incubation.



Modelling the global distribution of beaching of marine plastic

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POSTER SESSION I

P21

While floating plastic can be carried into the open ocean by the surface currents, an unknown fraction ends up beaching on coastlines. Plastic beaching negatively affects coastal ecosystems and tourism, and likely explains part of the discrepancy between plastic inputs and measured plastic concentrations in the open ocean. However, the global distribution of beaching and the origin of beached plastic is barely understood.

We run Lagrangian simulations where particles, representing floating plastic, are inserted at the ocean coasts scaled according to coastal population densities. They are then advected by surface currents from the 1/12° HYCOM/NCO-DA global reanalysis and surface Stokes drift from the 1/4° WaveWatch III global reanalysis for 10 years. We implement an approximation for beaching of particles, based on surface currents, distance to land and time spent in the vicinity of the coast. Beached particles cannot be resuspended, so the model yields a high-end estimate of beached plastic.

Our analysis reveals a global pattern of beached plastic, with the highest beaching concentrations being in areas with high population densities, such as South East Asia, the Mediterranean and the Indian subcontinent. The lowest beaching concentrations are found at remote coastlines of Australia and in polar regions.

We also calculate the places of origin of beached plastic, indicating whether coastlines receive plastic from local or remote sources. First results indicate that beached plastic in Southern Australia originates from remote sources, while in Brazil, Western Africa and the Western United States plastic originates mostly from local sources.

Our work identifies coastlines that are particularly threatened by plastic pollution, serves as a first step towards constraining the amount of plastic that is removed from the ocean via beaching and can help to direct beach clean-up efforts.

Identification of microplastics by holographic microscopy enabled support vector machine

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Micro-plastics derive from industrial activities and progressive fragmentation of larger plastic items. Plastics represent a major global threat because marine plastic litter can persist for decades, and has the potential to affect the food chain. However, due to the small size, it is very difficult to map the distribution of micro-plastics in water and the existing sampling methods have not reached yet a satisfactory level of accuracy. High-throughput microscopy systems able to reliably identify plastic-like particles inside heterogeneous mixtures are highly demanded. Here we tackle the problem using Digital Holography (DH) microscopy combined to machine learning. We analyze micro-plastics of various compositions and spanning over a wide range of characteristic scales (from micron size to millimeters). DH is label-free, high-throughput, and gives access to the sample phase-contrast. We exploit the rich content of information of the holographic signature to design new distinctive features that specifically characterize micro-plastics and allow discerning them from marine plankton of comparable size. Then, we use these features to train a plain support vector machine, remarkably boosting its performance. In this sense, holographic microscopy brings added-value to machine learning, which goes against the current trend of works relying on deep learning networks requiring a huge amount of data to be trained. Nevertheless, we reach a classification accuracy higher than 98%, and remarkable sensitivity and precision. To the best of our knowledge, our results outperform any other high-throughput imaging approach proposed so far.



Miniaturized Handheld MicroNIR: A tool for microplastic analysis

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POSTER SESSION I

P23

Plastic Pollution is a global concern now that has been affecting the environment since the times mass production of plastic began. They can take up to 500 years or more to degrade but before they do so, they break down into micro particles called microplastics (1mm-5mm) that persists in highest peaks to the deepest points in oceans explored, majority being reported along the coastlines. Analysis of microplastics has become increasingly common. The techniques for the polymer identification are Raman Spectroscopy, Infrared Spectroscopy, pyrolysis-gas chromatography-mass spectroscopy (pyr-GC-MS), thermogravimetric analysis coupled to solid-phase extraction (TGA-SPE) followed by thermal desorption gas chromatography mass spectroscopy (TDS-GC/MS). The vibrational techniques complement each other. However, there remains a conflict of interest when low cost miniaturized instruments come into action. Polymers in plastics have unique Near-Infrared (NIR) spectral fingerprints which can be utilized for sorting and quality assurance throughout their shelf life. The critical understanding of the polymer class is necessary for recycling purpose. In this work, the use of miniaturized handheld MicroNIR for distinguishing colored plastic resin is described. The handheld micro NIR is a miniaturized palm sized-portable spectrometer ($\approx 250\text{g}$ in weight and $< 200\text{mm}$ in length and 50mm in diameter) that employs a Linear Variable Filter (LVF) to collect the spectra in 0.25-0.5seconds. In total, 250 plastic samples belonging to various classes of polymer were used. The NIR signal arises from the combination and overtone bands. The intricate understanding of the spectra thus requires allocating resources to the idea of chemometrics models. The knowledge obtained from this classification model and use of the miniaturized hand held MicroNIR would be helpful in analyzing the plastics on-site, with great flexibility and rapidity.

Plastics and microplastics: the OECD's approach

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In 2018 the OECD organized the "Global Forum on Environment" on the theme: "Plastics in a Circular Economy - Design of Sustainable Plastics from a Chemical Perspective" with the direction of Working Party on Resource Productivity and Waste.

The contents of the Forum, the possible hints and contacts with the research as well as the possible implications with the economy of the participating Countries are still today objects of analysis and debate within the OECD.

It is in fact since 2015 that the Working Party on Resource Productivity and Waste works on the theme of sustainable plastic materials, after facing a profound examination of plastic pollution starting from the pressing theme of the marine litter and addressing all aspects of sustainability: waste management and circular economy up to the governance of economic systems.

The Working Party has recognized the need to approach the issue of environmental pollution of plastics, from plastic materials and from plastic items, through an optimal management of plastic waste, in a perspective of circular economy and sustainability of the raw materials used, as well as the safety of plastics from a chemical perspective of the additives and reagents used in the plastic cycle.

The effects on ecosystems, with an eco-toxicological approach, have been investigated and scientific evidences confirm the high risk with decreasing ecosystem energy and services and consequently the risk for human health which, in reality, must still to be confirmed, but for example must be preserved with a socio-economic and cultural perspective of sustainability and primary prevention.

The Global Forum was used to put under the magnifying glass, the various management examples operating on a local scale in the Forum global context - as is the issue of marine litter - to be able to implement these processes all around the world.



Solid-liquid-liquid microextraction (μ SLLE) for determining persistent pollutants at marine microplastics

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POSTER SESSION I

P25

Persistent organic pollutants (POPs) enter the ocean through the air-ocean interface, or from coastal inputs, especially in the mouths of rivers and gorges. Their stability and persistence, on top of their pervasiveness from man-made pollution, means that POPs can be found in a range of different matrices in the environment, especially in hydrophobic matrices as microplastic. Microplastics with POPs over their surface, can be transported over large distances. They are to be found in all oceanic areas all over the world, including remote polar regions such as the Arctic Ocean. Microplastics enter by ingestion to the food chain with the bio-accumulation and bio-magnification of POPs associated.

The current pollution of the marine environment requires fast and reasonably-priced analytical techniques that allow us to routinely check the concentration of persistent organic pollutants (POPs) to be found in different samples, especially coastal samples. Traditional methods used to extract and determine POPs in solid environmental samples often require a significant number of steps between sampling, extraction and analysis, taking a lot of time and effort. Miniaturised extraction systems enable us to analyse samples quicker and cheaper in both financial and environmental terms. These techniques are highly referenced for analysing liquid samples, but not for solid samples, and even less so for analysing pollutants from microplastics. A miniaturised solid-liquid-liquid extraction technique (μ SLLE) using micellar solution as extractant, it has been developed to extract, pre-concentrate and analyse up to 27 POPs from samples of marine sediments and microplastics.

The pollutants determined include organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs).

This technique is quick and it does not require to dry the extract. The pollutants are analysed using single quadrupole gas chromatography with mass spectrometry (GC-MS).

Erosion behaviour of different microplastic particles

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Until now, we hardly know anything about the behaviour of microplastics in rivers. Questions like 'How is microplastic transported?', 'Where does it deposit?' and 'When is it eroded again?' need to be answered before we can accurately simulate microplastic transport in rivers using hydro-numerical simulations. For this reason, sedimentation and rising experiments with different microplastic particles were conducted and the results published last year. In the next step, physical experiments are being carried out to investigate the erosion behaviour of microplastic particles with different particle shapes, densities and sizes on different soles. Polymer types heavier than water, namely PET, PVC, PA and PS, in the particle shapes pellets, spheres, fragments and fibres are studied. In the experiments, the critical shear stress of the individual particles is determined in circular channel, which has the advantage that an endless flow can be generated in which the shear stress at the bottom of the channel is precisely defined and can be increased slowly. Subsequently, the determined critical shear stresses are set in relation to the particle shapes, densities and sizes and it is tested whether the methods for describing the erosion behaviour of sediments, such as the Shields diagram, can also be applied to microplastics. If this is not the case, the sediment transport equations are modified until they can also represent the behaviour of microplastics.

At the conference the experimental setup will be presented and first results will be shown.



POSTER SESSION I

P26



Microplastic analysis in bottled water with Raman and Infrared microscopy

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POSTER SESSION I

P27

Microplastics, i.e. plastic particles less than 5 mm in diameter and down to few hundred of nanometers, are spread across the world, appearing everywhere, from the deepest sea trenches to Antarctica. In fact, even bottled water is not immune to microplastics and they can carry a variety of hazardous chemicals, such as bisphenol A (BPA), phthalates and persistent organic pollutants (POPs) which can affect human health. Here we will show how Infrared and Raman microscopies are techniques capable of analyzing not just the bulk but specific individual particles, providing information on each of them about size, shape and chemical composition.







POSTER SESSION II

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- P1** First record of the occurrence and composition of microplastics in sediments in Eyjafjordur, Iceland.
HALLDÓRSDÓTTIR Valdís, ÁSMUNSDÓTTIR Ásta Margrét, GOMIERO Alessio, ÖYSÆD Kjell Birger
- P2** Interconnection of mytilus innate immune response and hemolymph microbiota after nanoplastics exposure
AUGUSTE Manon, LASA Aide, BALBI Teresa, PALLAVICINI Alberto, VEZZULLI Luigi, CANESI Laura
- P3** Plastic accumulation in the jellyfish *Pelagia noctiluca* from the Tyrrhenian Sea
BERGAMI Elisa, MATANI Loris, VACCARI Lisa, CORSI Ilaria, SEMENOV Alexander, MACALI Armando
- P4** Microplastic in Coastal Areas - Impact of Waves, Sediments and Saltwater on the Degradation Behaviour
BORN Maximilian; SCHÜTTRUMPF Holger
- P5** The role of humic acids on the effects of nanoplastics in fish
BRANDTS Irene, BALASCH Joan Carles, TVARIJONAVICIUTE Asta, BARRETO Angela, MARTINS Manuel A., TORT Lluís, OLIVEIRA Miguel, TELES Mariana
- P6** Preliminary data on the polymer type identification from estuarine environmental samples
BRÁS GOMES Gonçalo, MORGADO Vanessa, PALMA Carla
- P7** Qualitative and quantitative screening of organic pollutants associated on microplastics from Ofanto River (South Italy)
CAMPANALE Claudia, BAGNUOLO Giuseppe, DIERKES Georg, MASSARELLI Carmine, URICCHIO Vito Felice
- P8** Assessment of microplastic pollution in Sarno River
COCCA Mariacristina, DE FALCO Francesca, DI PACE Emilia, AVOLIO Roberto, ERRICO Maria Emanuela, GENTILE Gennaro, AVELLA Maurizio
- P9** Microplastic ingestion in the *Ephyrae* stage of *Aurelia* sp. affects acute and behavioural responses
COSTA Elisa, GAMBARDELLA Chiara, PIAZZA Veronica, LAVORANO Silvia, VASSALLI Massimo, SBRANA Francesca, GARAVENTA Francesca, FAIMALI Marco
- P10** Holistic approach to the marine microplastic: sampling, characterization, consequences
DĄBROWSKA Agnieszka

- P11** Marine microplastic at Santuario Pelagos
DĄBROWSKA Agnieszka
- P12** Microplastics uptake and egestion dynamics in Pacific Oysters, *Magallana gigas* (Thunberg, 1793), under controlled conditions.
GRAHAM Philip , PALAZZO Luca, CARBONIA Stefano, TELFER Trevor, **DE LUCIA Giuseppe Andrea**
- P13** Extraction protocol optimization for detection of microplastics in digestive system contents of loggerhead turtle (*Caretta Caretta*).
DI RENZO Ludovica, MASCILONGO Giuseppina, DI GIACINTO Federica, ZEZZA Daniela, DI FRANCESCO Gabriella, OLIVIERI Vincenzo, BERTI Miriam, PETRINI Antonio
- P14** Study of plastics debris collected on north beaches of the Garda Lake after the severe storms of autumn 2018.
FAMBRI Luca, BOMBARDELLI Giada, GAVAZZA Claudia, Battocchi Paola, Tomasi Renzo
- P15** Microplastics and polycyclic aromatic hydrocarbons occurrence in *Solea solea* from the Adriatic Sea
FRAPICCINI Emanuela, PELLINI Giulio, GOMIERO ALESSIO, GUICCIARDI Stefano, Betti Mattia, SCARCELLA Giuseppe, ANNIBALDI Anna
- P16** Cyanobacterial exopolysaccharides are key drivers of polystyrene nanoparticles fate and toxicity in the marine environment
GRASSI Giacomo, CORSI Ilaria, CASSIER-CHAUVAT Corinne, CHAUVAT Franck
- P17** All that glitter is litter – the effect of conventional and biodegradable glitter on aquatic plants and invertebrates
GREEN Dannielle
- P18** Ecotoxicological effects of microplastics in marine zooplankton
MORGANA Silvia, GAMBARDELLA Chiara, COSTA Elisa, PIAZZA Veronica, GARAVENTA Francesca, FAIMALI Marco
- P19** Impact of microplastic beads and their associated microbial communities on the sea urchin *Paracentrotus lividus*
MURANO Carola, DONNARUMMA Vincenzo, ADAME Alvaro, CASTELLANO Immacolata, AGNISOLA Claudio, ZETTLER Erik, AMARAL ZETTLER Linda, CORSI Ilaria, CASOTTI Raffaella, PALUMBO Anna

P20 Occurrence of microplastics in gastrointestinal tracts (GITs) of *Coryphaena hippurus* from Mediterranean Sea

SCHIRINZI Gabriella, PEDÀ Cristina, ANDALORO Franco, BAINI Matteo, BATTAGLIA Pietro, D'ALESSANDRO Michela, GENOVESE Martina, FARRÉ Marinella, PANTI Cristina, FOSSI M. Cristina, **ROMEO Teresa**

P21 Microplastics in the River Thames water column: levels comparable to some of the highest recorded in the world.

ROWLEY Katharine, CUCKNELL Anna , SMITH Brian , CLARK Paul, MORRITT David

P22 Do microplastics affect the freshwater blackworm *Lumbriculus variegatus*?

STOCK Friederike, KOCHLEUS Christian, STIEDL Joscha, BREUNINGER Esther, BRENNHOLT Nicole, REIFFERSCHIED Georg

P23 Effects of polymethylmethacrylate nanoplastics on lipid metabolism in *sparus aurata*

BARRÍA Camila, BRANDTS Irene, BALASCH Joan Carles, TVARIJONAVICIUTE Asta, BARRETO Ángela, MARTINS Manuel A., TORT Lluís, OLIVEIRA Miguel, **TELES Mariana**

P24 Measuring the size and the charge of microplastics in aqueous suspensions with and without microorganisms using a zeta-sizer meter

TZIOURROU Pavlos, BOURIKAS Kyriakos, KARAPANAGIOTI Hrisi K.

P25 Microplastic release from plastic bottles - Comparison of two analytical methodologies (SEM-EDX and μ -FTIR)

WINKLER Anna, SANTO Nadia, TERMOLADA Paolo, PAROLINI Marco, PASINI Valerio, ORTENZI Marco Aldo, BACCHETTA Renato

P26 Following the fate of microplastic along the Ticino River, Italy

WINKLER Anna, DE FELICE Beatrice, BALESTRIERI Alessandro, PAROLINI Marco, ANTONIOLI Diego, GIANOTTI Valentina, LAUS Michele, ORTENZI Marco Aldo, TREMOLADA Paolo



First record of the occurrence and composition of microplastics in sediments in Eyjafjordur, Iceland.

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POSTER SESSION II

P1

There is a growing concern regarding plastic pollution, its distribution and effects on the ecosystem, both within the scientific community and among the public. Scientists have measured microplastic (plastic fragments less than 5 mm in size) in almost every corner of the world, including in the marine environment, both in the sediment and the water column. The accumulation of the microplastic in the food web is threatening the ecosystems health. Microplastic has been reported in fish and other marine organisms as well as in birds, mammals and lately in human stool in number of publications. The sewage is an important source of microplastic pollution in the costal sea, depending on the sewage treatment, more or less of the microplastic ends up in the sea. This study is the one of first records of the occurrence and composition of microplastics in Iceland. The sampling site Eyjafjörður, is the longest fjord in Iceland, located at the north coast. Several small towns are situated by the fjord, the far biggest being Akureyri with 18.000 inhabitants. None of the towns have any sewage treatment installed. The samples were prepared for analysis using a density separation with zinc chloride and filtered on 1 µm glass fiber filter. A fast and sensitive method based on a GCMS-pyrolysis was developed and adopted for the study. The results show considerable amount of microplastics in sediments in vicinity of the sewage outlet of Akureyri.

Interconnection of *Mytilus* innate immune response and hemolymph microbiota after nanoplastics exposure

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Marine invertebrates, including mussels can host complex and specific microbiota, which role remain unclear but which abundance and composition is known to be influenced by several abiotic or biological parameters. Previously, exposure to nanotitania-nTiO₂ have shown to change the hemolymph microbiota composition of the marine mussel *Mytilus galloprovincialis*. Results attested for complex interactions between immune cells, the hemocytes, activated in presence of nTiO₂, creating unfriendly medium in hemolymph, that likely lead to affect the most sensible commensal bacterial communities present. In the new era of plastics pollution, growing interest is now turned to the nano-size, too often underestimated but highly reactive at the cellular level. In this line, *M. galloprovincialis* were exposed 96h to the amino modified nanopolystyrene PS-NH₂ (10 µg/L). The microbial composition was evaluated by 16S rRNA gene based profiling, in hemolymph before and after the exposure, together with the surrounding seawater and a bench of functional hemocytes and hemolymph parameters. The results showed that PS-NH₂ at this concentration did not cause strong adverse effect in mussels while was enough to activate defence functions of hemocytes. Hemolymph at the beginning of the experiment displayed a lower microbial diversity as mainly dominated by the genus *Vibrio*. After 96h, as attested by their presence in surrounding seawater, *Vibrio* was depurated from the mussels likely leaving the opportunity to other genera to spread in hemolymph. The results underlined the diverse effects of the combined factors triggered by PS-NH₂ exposure on the bacterial communities, with some genera favoured (e.g. *Psychrobium*) while other were more sensitive to the stressing conditions (e.g. *Shewanella*). Even though the specific role of microbial communities remains largely unknown in *Mytilus*, the present data attested for a perturbation in the natural microbial composition caused by the presence of nanoplastics, which could further lead to affect mussel fitness.

Work supported by the EU Commission H2020 ITN project PANDORA Probing safety of nano-objects by defining immune responses of environmental organisms (GA 671881).



Plastic accumulation in the jellyfish *Pelagia noctiluca* from the Tyrrhenian Sea

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P3

The biomonitoring of marine debris is a growing research field, which can provide useful information about sources and distribution of plastics, but also about the overall health of marine environments. Common sentinel species for plastic pollution mostly cover benthic filter-feeding organisms or endangered marine vertebrates, but monitoring programs involving a broad range of synergetic bioindicators are missing. Jellyfish have recently been reported as target organisms to floating plastics, being able to internalize a number of anthropogenic debris. Being a widespread energy source in pelagic food webs and occurring at high densities, jellyfish may represent a suitable invertebrate bioindicator to monitor plastic pollution in pelagic waters, along with their common predators, such as sea turtles and many fish. An extensive sampling of the mauve stinger *Pelagia noctiluca* ($n > 200$) was conducted near Ponza island (Tyrrhenian Sea) in April 2018 to understand the extent of plastic accumulation in these planktonic organisms. Extraction of macro-, meso- and microplastics was conducted following the method from Li et al. (2016) adapted for gelatinous organisms. Our preliminary results showed that individuals of *P. noctiluca* were able to internalise a variety of anthropogenic debris, ranging from 34 cm to 1 mm in size and including diverse polymers, such as low-density polyethylene (LD-PE), polystyrene (PS), polyamide (PA), polypropylene (PP), nylon and varnish. Here, the data acquired are discussed according to the hydrographical conditions and considering the key trophic role of jellyfish, which should be included in future monitoring surveys as a novel bioindicator for plastic pollution.

Microplastic in coastal areas - impact of waves, sediments and saltwater on the degradation behaviour

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Mechanical degradation behaviour of microplastics is scarcely researched, and only very few studies evaluate the combined impact of waves and sediment on the abrasion or degradation of microplastics. To assess this impact of different grain sized sediments and wave action on microplastics, experiments in a so called 'slosh box' are planned.

The slosh box has an oscillating movement which causes an agitation of the water and sediment resulting in wave action as well.

Already carried out preliminary studies using sediments in different size classes and water in the slosh box have shown to simulate the shore run-up of waves quite accurately, even resulting in a sawtooth-movement of the sediments. Through changeable water heights, different parts of coastal zones can be simulated while using varying sediment and water types (fresh/salt) to evaluate the possible impact of saltwater and grain sizes on the degradation process as well.

The tests are planned to run long term (>30 days) to ensure a continuous interaction of the microplastics with the sediments and the milling like processes they induce through wave action.

Through the adjustable oscillation frequency of the slosh box, increased accelerations of the water body and thus increased wave heights are possible, which should theoretically result in an amplified abrasion of the microplastics.

To evaluate the impact of these experiments on the microplastic particles a microscopic analysis will be carried out.

The aim for a participation at the conference is to present the experimental set-up and first results



The role of humic acids on the effects of nanoplastics in fish

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POSTER SESSION II

P5

Plastic pollution is a worldwide problem, highlighted by the fact that bulk plastics undergo different degradation processes, fragmenting into micro- and nanoplastics (NPs). These plastic particles, have been reported as ubiquitous pollutants in marine environments worldwide, being potentially more bioavailable to marine biota, as well as a source of entry of other contaminants into organisms. Dissolved organic carbon (DOC) is abundant in marine and freshwater environments, constituting one of the greatest cycled reservoirs of organic matter in our planet. Humic acids (HA) are a general category of natural and heterogeneous organic substances, making up between 60 and 80% of the DOC previously reported play a relevant role in the fate of organic chemicals, influencing their bioavailability and toxicity to aquatic organisms.

The present study aimed to assess stress-related effects of polystyrene (PS) NPs, individually or combined with HA, on *Dicentrarchus labrax*, at different levels of biological organization. For this purpose, fish were exposed, for 96 h, to two concentrations of PS NPs (0.02; 20 mg L⁻¹), HA (1 mg L⁻¹) and to the mixture of each NPs concentration with HA. Molecular and biochemical biomarkers were assessed in head kidney, plasma and skin mucus. Expression of target genes involved in several stress-related key functions, such as immune response, cellular stress and stress-related hormone secretion, was evaluated in head kidney and biochemical parameters were assessed in plasma and skin mucus.

Preliminary data on the polymer type identification from estuarine environmental samples

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Microplastics (MPs) are widely recognized as a contaminant of emerging concern in the marine environment. This is a work in progress as part of a project aiming for the quality assessment of the aquatic environment for aquaculture activity in Portugal, and, in this context, we report the first results in surface waters in one of the estuarine study areas (Mondego, Portugal). This work aims for the investigation of MPs presence in 4 estuarine environments in Portugal. Water samples are taken with a manta tow net with 200 cm length and 300 μm mesh size and sediments with a Van Veen Grab. Polymer identification of MPs can help identify its sources, degradation, and fate. We used attenuated total reflectance Fourier transform infrared spectroscopy with microscope (micro ATR-FTIR) technology to identify MPs' polymer type. Although it gives rise to low intensity spectra, they are very well defined with well resolved bands. Estuarine environments are rich in organic matter, which is mostly composed of natural cellulosic fibers (cellulose, hemicellulose and lignin). Distinguish microplastics from organic matter proved to be a challenge in some cases. This is a question that needs some attention when it comes to polymers with a simple infrared spectrum: like polyethylene. Some spectra can be misunderstood because polyethylene characteristic peaks can be overlapped. Particles will be counted, sorted by size and type and characterized by polymer type. Microplastics data from surface water and sediments will be crossed to study the distribution of MPs. Preliminary results from water samples revealed a density of 0.8 particle per m^3 and that low-density polyethylene is the most dominant plastic polymer type (about 90%).



Qualitative and quantitative screening of organic pollutants associated on microplastics from Ofanto River (South Italy)

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There is considerable complexity involved to understand the impact of microplastics on the natural world due to their heterogeneous physico-chemical properties that make these synthetic polymers a multifaceted stressor. Although data on microplastics exposure levels in marine environments and organisms have rapidly increased in recent decades, limited information is available on chemicals associated with microplastics.

Based on the first findings and the concerns of the scientific community herein we report results related to the identification and quantification of persistent organic pollutants adsorbed on microplastics collected from an Italian river (Ofanto) during different campaigns. Some of these compounds are added during plastics manufacture, while others adsorbed from the surrounding ambient.

Polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and polycyclic aromatic hydrocarbons (PAHs) have been selected as target compounds to be quantified while a qualitative general non target-screening of plastic additives has been carried out by high resolution gas chromatography mass spectroscopy.

Matrix blanks, (virgin polymers), were analyzed together with field samples in order to compare the concentration of adsorbed environmental contaminants to the amount of natural chemicals originating from plastics.

The quantitative analysis of pollutants, underlined the presence of these contaminants on almost all samples. PAHs were found in all samples. The major pesticide quantified, associated with the plastic debris, was been DDE that showed values above the Italian regulatory limit set for soils. PCB 52 and five of the 16 EPA-PAHs were observed in virgin pre-production pellets suggesting a probable contamination or a use of these pollutants as plastic additives.

Non-target screening of compounds associated to microplastics provided an overview of more than 90 organic chemicals identified, including hydrocarbons, ultraviolet stabilizers, antioxidants, plasticizers', lubricants and intermediates. This work is the first study that shows a detailed overview of the variety of chemicals associated on microplastics collected from an Italian river context.

Assessment of microplastic pollution in Sarno River

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In the last years, a growing concern has been arising about the contamination of marine ecosystems by microplastics, defined as plastic fragments smaller than 5 mm. Their impact on the environment is unpredictable and quite dangerous since they can adsorb organic pollutants and be ingested by marine organisms, potentially reaching the human food chain.

The occurrence of plastics in marine environments has been widely reported in several papers. However, few data concerning freshwater systems have been reported, even though a great impact of rivers on microplastic pollution of marine habitats is expected. In fact, rivers can act as vectors for the transport of litter into oceans. A new study has recently started, founded by “Fondazione per il Sud”, to gather information about the occurrence of microplastics, the abundance of different particle shapes, and polymer types in the Sarno river. Sarno river, who was defined as “the most polluted river in Europe”, is located in the South - West of Italy and flows from Sarno reaching the Tyrrhenian Sea in the Gulf of Naples, and collects water from two important effluents, the Cavaioia and Solofrana torrents.

The main actions planned are related to the development of an analytical procedure to evaluate microplastics in the Sarno water, to quantify and identify their chemical composition as well as their shapes and morphologies, and finally to develop guidelines to prevent/mitigate the microplastic pollution in Sarno river. The obtained results will be correlated with the sampling sites, population density, proximity of nature reserves, direct sources (e.g., sewage treatment plants and waste disposal) and diffuse sources (transport by river).



Microplastic ingestion in the Ephyra stage of *Aurelia* SP. affects acute and behavioural responses

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We report for the first time a correlation between microplastic (MP) ingestion and ecotoxicological effects in gelatinous zooplankton (cnidarian jellyfish). The ephyra stage of the jellyfish *Aurelia* sp. was exposed to environmental and high concentrations (0.01-10 mg/L) of fluorescent 1-4 μm polyethylene MPs for 24 and 48 hours and acute (percentage of immobility) and behavioural (percentage of frequency of pulsation) endpoints were investigated. Confocal and holo-tomographic investigations allowed localizing MPs on gelatinous body and mouth, either attached on the surface and ingested. It is possible to ascribe to these interactions (adhesion and ingestion) ephyrae survival and behaviour impairing at all concentrations already after 24 h of exposure. Adhesion may contribute to acute and behavioural effects with a mechanical disturbance bringing about the loss of radial symmetry, due to MPs. In fact, contaminated ephyrae moved to clean seawater showed a full recovery after 72 h. In conclusion, short-term exposure to MPs impairs the health of ephyrae jellyfish, inducing toxic effects on both survival and behaviour. Polyethylene MPs temporarily affect both immobility and frequency of pulsations of *Aurelia* sp. jellyfish. This study provides a first step towards understanding and clarifying the potential impacts of MP contamination in gelatinous zooplankton.

Holistic approach to the marine microplastic: sampling, characterization, consequences

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The aim of this paper is to present the holistic strategy to tackle the increasing problem of ubiquitous marine microplastics. The entirely new, simple and efficient device for pelagial water sampling will be shown together with the characterization of the collected debris. Secondly, the comprehensive discussion of Raman spectroscopy and SEM-EDS possibilities and drawbacks is included. Finally, the speech will address the strategies to evaluate the ecotoxicology of plastic materials in macro, micro and nanoscale.



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Marine microplastic at Santuario Pelagos

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The aim of this paper is to present the results of research on microplastics and nanoplastics collected in the pelagial waters of Santuario Pelagos and at the Italian and French coastline. The sampling method, characterization techniques and impact assessment will be discussed in detail. As the field studies were done on STS Pogoria, the sail training activity and science engagement within European Maritime Day will be mentioned.

Microplastics uptake and egestion dynamics in Pacific Oysters, *Magallana gigas* (Thunberg, 1793), under controlled conditions.

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Microplastics (<5 mm) are becoming ever more present in the marine environments due to human population growth. Therefore, an increase in this type of pollution is expected over the coming years and decades, becoming a growing threat for different marine organisms. Through aquatic animals, microplastics can enter in the human food chain, and can be perceived as a risk for consumers' health. Indeed many studies report the presence of particles in marketable shellfish including the Pacific oyster *Magallana gigas* (Thunberg, 1793). The aim of this study is to examine the potential risk of microplastics entering in the human food chain through this shellfish species, investigating the dynamics of the uptake and egestion of microplastics in Pacific oysters under controlled conditions.

M. gigas collected from a farm in the San Teodoro lagoon (Italy), were exposed to 60 fluorescent orange polystyrene particles L⁻¹ of known sizes (100, 250 and 500 µm). The uptake of each particle size was 19.4 ± 1.1%, 19.4 ± 2 % and 12.9 ± 2 % respectively. After exposure *M. gigas* were left to depurate for 72 hrs, during which 84.6 ± 2% of the particles taken up were released whilst 15.4 ± 2% were retained inside the shell cavity. No microplastic particles were found in the animals' soft tissues. The results of this study, suggest that depuration is an effective method to reduce presence of large microplastic particles in *M. gigas* and that the burden that could theoretically be up taken by consumers from these shellfish is negligible when compared to other routes. Being the depuration an effective method to reduce the presence of larger microplastic may be advised to use it as a standard procedure even when no depuration would be compulsory due to sanitary reasons such in the case of class A waters.



Extraction protocol optimization for detection of microplastics in digestive system contents of loggerhead turtle (*Caretta Caretta*)

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Loggerhead sea turtle (*C. caretta*) has been identified by the European Commission as Mediterranean indicator for the marine litter in biota. Within the European INDICIT project, 62 loggerhead turtles stranded along Adriatic coasts of Abruzzi and Molise regions (Italy) have been examined. In addition to routine diagnostic activities, contents of digestive system were analysed applying INDICIT protocol. The categorization of items found in esophagus, stomach and intestine were based on the general morphology of plastics (sheet-like, filament, foamed, fragment, other), on other general rubbish or litter characteristics. Data on microplastics (≥ 1 mm), as weight and number of items, were recorded within Descriptor 10 of Good Environmental Status (GES) analysis foreseen by Marine Strategy Framework Directive (MSFD). Further investigations on 16 gastrointestinal contents were made through an experimental protocol for the search of microplastics in the range between 0.45 μm – 0.99 mm. Proportional quantities of samples (grams) were obtained from the first washing of the contents for each digestive tract. Two different phases of filtration on sub rates were developed to obtain a significant specimen to be analyzed by microscope. The items founded were classified in plastics or fibres, according to the shape and colour. Results were compared with INDICIT ones by evaluating a potential correlation between the presence of microplastics and mucosa lesions of the intestinal system. Applying in different species, the optimized protocol could be useful to better understand the impact of microplastics in biota.

Study of plastics debris collected on North beaches of the Garda Lake after the severe storms of Autumn 2018

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This presentation is a recent contribute related to the study of plastic contamination as consequence the severe storms that hit the north side of Italy in Autumn 2018. The average rainfall in Trentino reached 275 mm during the three days 27-29 October, with max peak of 600 mm according to Meteotrentino; and gales of up to 120 km/h brought down thousands of trees not only in the Fiemme valley. In following days all the rivers increased their flow and enlarged their width determining a kind of intense cleaning of their side banks, as in the case of the Sarca river that has a water basin about one forth of Trentino, and it is the main tributary of the Garda lake. Moreover, to avoid the danger of flooding, the waters of the Adige river were redirected to Lake Garda through the Torbole tunnel (Trento) from 29 October until 1 November with a flow of 350 m³/s.

Various organic/vegetable and inorganic materials were first dispersed into the lake, and then cumulated on the shoreline, due to the local south wind "Ora". In November 2018 a proper campaign of plastics retrieval and collection started in the lake-shore of Riva (with the contribute of the students of the local Liceo Maffei), Arco and Nago-Torbole. Many plastics wastes were found, not only microplastics (pieces up to 5-10 mm), but also macroplastics (even objects with large size up to about 1 mt length, or of about 25 kg weight).

Lab characterization and testing was performed i) to identify the polymer (by using FTIR, density analysis, DSC) and ii) to determine the grade of physico-chemical degradation after long term in environmental expositions. The most common categories of products (films, bottles, foam, fragments, cups, ...) and polymers (PE, PS, PU, PP, PET, PA6, PVC, ...) were identified and classified.

Moreover many plastic item exhibited a severe level of aging, similar to that of marine plastics waste.

The retrieval campaign has been repeated after four months in the same positions (identified by gps) and many other microplastics were again collected, expecially near the shore.



Microplastics and polycyclic aromatic hydrocarbons occurrence in *Solea solea* from the Adriatic Sea

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In aquatic environment, microplastics (MPs) are emerging contaminants ubiquitously present in all compartments from surface water to benthic sediment, including aquatic biota (via ingestion).

MPs and persistent organic pollutants (POPs), such as polycyclic aromatic hydrocarbons, PAHs, are strongly related because the latter can easily be adsorbed by MPs. According to our knowledge, many studies were conducted to access the interaction between MPs and PAHs, most of these are experimental studies and few field studies have been carried out on organisms. Therefore, more supportive knowledge towards how POPs sorb on MPs, which factors regulate their interaction and the role of MPs as vectors of POPs to marine organisms are needed.

Moving from these considerations, this is a preliminary work that gathers two researches previously published by authors, in which MPs in the gastrointestinal tract and PAH levels were measured in *S. solea* tissues and in marine sediments collected from the northern Adriatic Sea during the Solemon survey 2014. In the present study, the most commonly found polymers in the gastrointestinal tract of *S. solea* (polyvinyl chloride, polypropylene, polyethylene, polyester and polyamide) and three PAH congeners (phenanthrene, fluoranthene and pyrene) were investigated to find their possible relationship on the sediment and *S. solea* of the North Adriatic Sea, where intensive processes of industrialization and urbanization occur. These three PAHs were chosen as they are more present in the most tested plastic polymers.

Preliminary results showed a positive relationship between three investigated PAH congeners and the polypropylene presents in the gastrointestinal tract of *S. solea*, suggesting that this polymer is the one that sorb and concentrate the highest amount of PAHs. However, the relationship was significant ($p < 0.05$) only in sediment samples and not in *S. solea* tissues.

Cyanobacterial exopolysaccharides are key drivers of polystyrene nanoparticles fate and toxicity in the marine environment

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Polymeric nanoparticles are increasingly used in many everyday products and there is evidence of their formation and accumulation in the marine environment, due to biotic and abiotic degradation of floating plastic debris. Marine phytoplanktonic organisms represent important primary producers that provide essential ecosystem services to coastal and oceanic environments. In particular, cyanobacteria represent a large part of phytoplankton communities in marine waters worldwide. Their environmental relevance makes them important model organisms for the study of the ecotoxicological impact of plastic nanoparticles on the marine environment. Moreover, cyanobacteria produce and extrude exopolysaccharides (EPS), which largely contribute to the marine natural organic matter (NOM) pool, capable of interacting with NPs influencing both their behaviour in the water column and their biological outcomes. In the present work the EPS-producing marine cyanobacterium *Synechococcus* sp. PCC 7002 was modified to construct an EPS-depleted mutant, by targeted deletion of two genes involved in EPS synthesis and cellular export. Amino and carboxylic polystyrene NPs (PS-NH₂ and PS-COOH, respectively), bearing positive and negative surface charges, were used to study the mutual influence of plastic NPs and EPS on the growth (biomass production) and survival of *Synechococcus* sp. PCC 7002. PS-COOH NPs did not cause significant toxic effects on both wild type and EPS-depleted mutant of *Synechococcus* sp. PCC7002. On the contrary, the ecotoxicity of PS-NH₂ NPs was exacerbated towards the mutant strain compared to the wild type, as evidence by a significant decrease in cell viability and increase of intracellular reactive oxygen species (ROS, a biomarker of oxidative-stressed cells). Additionally, EPS secreted by wild type *Synechococcus* interacted with both NP types affecting their physical-chemical characteristics and colloidal behaviour. Our results indicate the key role of cyanobacterial EPS as determinants of plastic NP fate and biological effects in the marine environment and builds on the knowledge of nanoplastic ecotoxicology.



All that glitter is litter – the effect of conventional and biodegradable glitter on aquatic plants and invertebrates

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Glitter, traditionally made from polyethylene terephthalate (PET) coated in aluminium, is a popular decorative material used in arts and craft, cosmetics and in large quantities at festivals. Since recent public realisation that glitter is a type of microplastic, there has been mass phase out of PET glitter in favour of biodegradable alternatives. For example, in the United Kingdom alone 61 festivals have already pledged to switch to using biodegradable glitter instead of PET glitter. Glitter, similar to other types of microplastics, are washed down the drain ending up in aquatic habitats but the effects on the plants and animals in these habitats is unknown. This study used aquatic mesocosms to test the effects of PET glitter compared with biodegradable alternatives on the survival and reproductive rates of brine shrimp, *Artemia salina* and water fleas, *Daphnia magna* and of the growth and productivity of duckweed, *Lemna minor* and freshwater microalgae. After 30 days, there was a reduction of reproductive rates and an increase in mortality of *D. magna* when dosed with either type of glitter. On the contrary, the mortality of *A. salina* was reduced when dosed with PET glitter, but reproductive rates actually increased in response to biodegradable glitter. In freshwater mesocosms, the root length of *L. minor* and the chlorophyll content of the water column (indicating phytoplankton biomass) were significantly reduced when dosed with any type of glitter. This research indicates that glitter can have similar effects on aquatic organisms to other types of microplastics and that alternative types of glitter may not necessarily be better for the environment.

Ecotoxicological effects of microplastics in marine zooplankton

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In this study, carried out in the frame of the European JPI-Oceans Project Ephemare, the impact of microplastics (MPs) on marine zooplankton was evaluated. The ingestion and the ecotoxicological effects of polyethylene MPs were investigated in several models representative of taxa, including crustaceans and sea urchin early life stages (*Tigriopus fulvus*, *Artemia franciscana*, *Paracentrotus lividus*), rotifers (*Brachionus plicatilis*) and ephyrae jellyfish (*Aurelia sp.*) after a short term exposure (24-48 hour), by analysing MP build-up, lethal and sub-lethal (immobility, behaviour) responses. MPs were accumulated in all zooplankton species, without affecting survival. Conversely, sub-lethal effects were observed in all taxa: among them, immobility was only affected in copepod nauplii and jellyfish ephyrae, while changes in swimming behavior and frequency of pulsations were observed in all models. Taking into account these results, a simplified two level trophic chain, formed by the nauplii of the copepod *T. fulvus* as prey and the ephyrae stage of the jellyfish *Aurelia sp.* as predator, was selected to investigate MP trophic transfer. The latter was demonstrated in jellyfish ephyrae through MP-contaminated prey ingestion. Despite it, jellyfish immobility and behavior were not affected by MP transfer. This study contributes to expand knowledge on MP interactions with marine zooplankton, demonstrated that plastic particles can be easily ingested and transport along the food web.



Impact of microplastic beads and their associated microbial communities on the sea urchin *Paracentrotus lividus* off the Canary Islands (subtropical NE Atlantic)

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In the marine environment, microplastics (< 5 mm) are colonized by a microbial biofilm, known as “plastisphere”, which represents a new habitat with distinct biological communities. Microplastics thus become a potential vector of microbes, which in turn might affect their degradation rate, increase their size, influence their fate and behaviour and ultimately their interaction with marine organisms (ecotoxicity). Although the plastisphere plays a key role in the environment, little is known about the impact of microplastics colonized by microbes on marine species. To this aim, specimens of adult sea urchin (*Paracentrotus lividus*) were exposed for 48h to 45 µm polystyrene fluorescent beads previously incubated (1 week) in natural seawater collected from polluted coastal sites to test the hypothesis that microbial communities can modulate the effect of plastics on the sea urchin stress responses. Parallel control experiments were performed exposing animals to virgin beads or to polluted sea water without microplastics. The composition of the microbial community adhering to polystyrene beads was characterized using Scanning Electron Microscopy (SEM). Number and phenotypes of sea urchin’s immune cells were characterized as well as markers of oxidative stress, including reactive oxygen species and reactive nitrogen species, enzymatic activities and general antioxidant responses analysed both in immune cells and in tissues (digestive and water vascular system). The results show that microbes colonization can play a significant role on polystyrene microbeads impact on marine biota.

Occurrence of microplastics in gastrointestinal tracts (GITs) of *Coryphaena hippurus* from Mediterranean Sea

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One of the main impacts of marine litter is plastics ingestion by marine organisms. Even if the occurrence of plastics in fish of the Mediterranean Sea was already reported in several studies, the information regarding some pelagic species was limited, especially, regarding microplastics. The common dolphinfish, *Coryphaena hippurus* Linnaeus, 1758, is an opportunistic and voracious pelagic predator and it is a species of commercial value. So far, major reports on this species from different areas were mainly focused on its diet showing the occurrence of meso- and macroplastic fragments. In this framework, this study aims to highlight for the first time the occurrence of plastic debris in the gastrointestinal tracts (GITs) of *C. hippurus* from Mediterranean Sea. This species was also chosen as medium-scale (Mediterranean UN Environment/M-PA sub-regions) bioindicators of micro and macro litter in open waters in the project "Plastic Busters MPAs: preserving biodiversity from plastics in Mediterranean Marine Protected Areas". In this way, a new experimental digestion method basing on a combination of basic-acid agents was applied due to the complex diet of this species. Two digestion steps, including potassium hydroxide (KOH) and nitric acid (HNO₃), allow removing most of the organic and inorganic material, respectively. The method was applied to 27 fish sampled from Sicilian "Fish Aggregating Device" (FAD) Fisheries in the central Mediterranean Sea. The frequency of occurrence of ingested plastics was 63% and most of them were microplastics. Polymers will be identified by Fourier transform infrared (FT-IR) spectroscopy technique and, also, plastic additives will be detected. Moreover, these results confirmed the newly developed digestion method as a reliable approach to detect microplastics in pelagic fish.



Microplastics in the River Thames water column: levels comparable to some of the highest recorded in the world

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Studies of microplastic contamination in UK rivers remain few, despite an increasing concern over its ecological effects on aquatic biota and the role of freshwater inputs in contributing to plastic pollution in marine habitats. This study focussed on the quantification of microplastics in the water column of the River Thames, the catchment responsible for draining Greater London. The main aims of this study were to quantify and identify microplastics in the water column at two sites on the Thames Tideway, namely Putney and Greenwich, and to test the hypothesis that there will be a net accumulation at the latter, downstream site. Water column samples from the two sites were collected from June through to October 2017 and were taken on the ebb and flood tides at both the surface and at a depth of 2m. These samples were sieved, and digestion methods were developed for isolating plastic fragments into different size fractions. All 71 samples from the Thames were found to contain microplastics, ranging from 32µm-5mm in diameter; these were counted and categorised by type and colour. FTIR analysis showed that polyethylene and polypropylene were the most common polymers in the microplastic samples from the River. It was estimated that, excluding microfibrils, there were 25.1 microplastics m⁻³ at Putney and 14.6 microplastics m⁻³ at Greenwich. These levels are comparable to some of the highest recorded in the world

Do microplastics affect the freshwater blackworm *Lumbricus variegatus*?

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Plastics in rivers and lakes are an emerging issue for science and regulation. So far there is little knowledge about the pollution and ecological risks of freshwater environments with micro-, meso-, and macroplastics, whereas the occurrence of plastics in the marine environment has already been identified as a critical environmental concern. On this account, numerous studies on microplastics (MP; < 5 mm) in the marine environment have been published. Although MP ingestion has already been documented also for a small number of freshwater species, only few studies investigated resulting biological effects. The aim of our study is to investigate the potential effects of polyvinylchloride (PVC), polyethylene terephthalate (PET), Polymethylmethacrylat (PMMA), Polystyrol (PS), and Polylactide (PLA) particles on the benthic freshwater species *Lumbricus variegatus*. The effects of direct and indirect routes of exposure to MP in the size range of 63-200 µm were assessed using a sediment-water toxicity test design under static conditions over a time period of 28 days (modified test method based on OECD (2007) Test No. 225). Investigated endpoints include surviving individuals, biomass and fitness parameters (Glycogen, triglyceride, adenylate energy charge) as well as in-vitro testing of estrogenicity and the analysis of leached chemicals. A general uptake and excretion of MP particles (< 200 µm) by *L. variegatus* were observed. Effects of MP particles on *L. variegatus* vary between plastic materials and only occurred after direct-contact exposure. For example, a dose-response relationship of surviving individuals could be derived for PVC, but not for PET. For the other three materials, the results will be obtained within the next weeks and presented at the conference. The analysis of leached chemicals indicated that there is no direct causal link between inhibition of surviving individuals and present diethylhexyl phthalate (DEHP) or estrogenic effects.



Effects of polymethylmethacrylate nanoplastics on lipid metabolism in *sparus aurata*

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Nanoplastics (NPs) are considered emerging pollutants, and this has motivated recent research to establish their ecological and environmental consequences. At present, the study of the effects of NPs in aquatic organisms is still scarce, especially in organisms of higher trophic levels, such as fish. The Mediterranean Sea is considered a large region of high plastic accumulation, due to the high plastic load that enters the coastal areas and the limited outflow to the Atlantic Ocean. Gilthead sea bream (*Sparus aurata*) is a common fish species in the Mediterranean Sea and one of the most cultivated species for human consumption in this area. Due to this, it is highly relevant to investigate the effect that these emerging contaminants may have on this fish species. Therefore, the objective of this study was to evaluate the effect of polymethylmethacrylate (PMMA) NPs on lipid metabolism in *S. aurata*. For this purpose, juvenile gilthead sea bream (8.7 ± 2.5 g) were exposed to increasing concentrations of 61 nm PMMA NPs (0.001, 0.01, 0.1, 1 and 10 mg / L) and liver samples were taken after 24 and 96 h. Lipids have great relevance in this context, since they are the main energy supplier in fish. We evaluated gene expression levels (*ppar- α* , *ppar- β* and *ppar- γ*) and biochemical biomarkers (cholesterol, triglycerides and glucose) related to lipid metabolism, significant to analyse the potential effects of NPs' exposure in a fish destined for human consumption.

Measuring the size and the charge of microplastics in aqueous suspensions with and without microorganisms using a zeta-sizer meter

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To simulate the marine environment in the laboratory, a 1.5-L glass beaker was used as bioreactor. The total suspension volume in the reactor was 1 L. An air pump was providing constant air flow. On the first day of the bioreactor set up, 1 L of seawater was used directly from the marine environment (Patras Marina, Greece) into the reactor. Every five days, Synthetic Seawater (SSW) was used to refresh the aquatic solution (supernatant liquid 300 MI) and then SSW with 320 mg L⁻¹ C₆H₁₂O₆ was added as energy source. 1 g of microplastics was added in the bioreactor on the first day [the polyethylene (PE) powder (~ 1 μm) was provided by Greek plastic industry (www.tsianakas.gr)]. A magnetic stirrer was also used for stirring the liquid in the bioreactor. The process lasted for a month. After the virgin samples of PE powder were coated with carbon, Scanning Electron Microscopy (SEM) was used to visualize them. Zetasizer Nano ZS was used to calculate the size and the charge of the samples: (a) SSW without microorganisms using only virgin PE microplastics and (b) supernatant liquid from bioreactor. In both cases, ethanol was added before the measurement to decrease the neutral buoyancy of microplastics.

In conclusion a) Virgin PE microplastics agglomerate in larger particles and have neutral surface charge in suspensions, whereas b) suspensions with PE microplastics and microorganisms also include agglomerates of both PE and microorganisms that demonstrate high surface charge.

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Microplastic release from plastic bottles - comparison of two analytical methodologies (SEM-EDX and μ -FTIR)

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Plastic debris and microplastics (MPs) evolve into an urgent problem for environmental contamination; any ecosystems are subject to a sort of “plastic invasion”. Until now, many sources have been identified and nearly all the environmental compartments are subject to MP contamination. The detection of MPs is, in principle, relatively simple. However, due to their size, and especially when small sizes are considered (around 1 μm), it may be problematic. In literature, different analytical approaches such as micro-Fourier transform infrared spectroscopy (μ -FTIR) and RAMAN spectroscopy, were proposed. In this work, we compared μ -FTIR with Scanning electron microscopy coupled to Energy dispersive X-ray spectroscopy (SEM-EDX) for analysing MPs concentrations in single-use polyethylene terephthalate (PET) mineral water bottles. We further considered the release of MPs from the bottle upon exposure to mechanical stress (squeezing treatment; none, 1 min, 10 min). After bottles were exposed to the three treatments, water was filtered on a silver membrane. Subsequently, the filters were analysed by SEM-EDX and μ -FTIR. The first methodology showed the presence of an increasing number of particles in relation to the treatment, but this increase was not statistically significant and, in addition, most of them were not of plastic origin. In the water samples, only few particles of small size ($<10 \mu\text{m}$) were identified by their elemental composition as PET. In contrast, μ -FTIR analyses of replicates of samples analysed by SEM-EDX, showed a lower number of particles and none could be identified as PET ($>10 \mu\text{m}$). SEM-EDX technique showed a high analytical potential because of its resolution for small size MPs. It should be considered as additional analysis to support spectroscopic methods.

Following the fate of microplastic along the Ticino River, Italy

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The Ticino river is known to be one of the cleanest and natural rivers in northern Italy. However, little is known about its microplastic (MP) pollution. Unlike marine MP pollution, freshwater studies are still few and studies on freshwater organism are very scarce. In this study we aim to identify and report the abundance, composition and fate of MPs along the Ticino river by sampling surface water, subtidal sediment, macroinvertebrates and fish (the wels catfish *Silurus glanis*). Sampling sites are situated between Lago Maggiore and the confluence with the Po river and include sampling directly downstream of waste water treatment plants (WWTP) locations as potential main input of MP. By analysing the size, shape, abundance, distribution and polymeric composition of the sampled MP from different environmental matrices, this research will contribute to demonstrate predictable patterns of MP contamination along the river. This study represents one of the first in Italy investigating the occurrence of MP over the flow of a river, not only from water and sediment samples, but also related to biota. First results of MP concentration in sampled media will be presented in the conference. Preliminary experiments already proved the presence of fibres in wels catfish stomach. In addition to the implications of the results, also the applicability of the used sampling, extraction and analytical technique will be assessed.



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