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Proceedings of the 2nd International Conference on Microplastic Pollution in the Mediterranean Sea

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Contents

The Impact of Microplastics on Filter-Feeding Megafauna

Maria Cristina Fossi, Matteo Baini, and Cristina Panti(✉)

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1 Introduction

The Mediterranean basin, a worldwide biodiversity hotspot, as previously underlined, is one of the world seas most affected by marine litter, including microplastics [1–3]. Recent studies in the different regions of the basin suggest that some areas, including important MPAs and Specially Protected Areas of Mediterranean Importance (SPAMI) such as the Pelagos Sanctuary, are affected by important concentrations of microplastics and plastic additives, representing a potential risk for endangered species (baleen whales, sea turtles, filter feeder sharks) [4–10] living in this area and for the all Mediterranean biodiversity [11–14]. In this paper we reconstruct the scientific story of the invisible war between the charismatic megafauna (baleen whales, filter feeder sharks and manta rays) against the smallest marine debris (microplastics) and their potential toxicological effects.

2 The Impact of Microplastics on Filter-Feeding Megafauna

The first warning of this emergent threat in filter-feeding megafauna (baleen whales and filter feeder sharks) was reported by 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 sharks 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.

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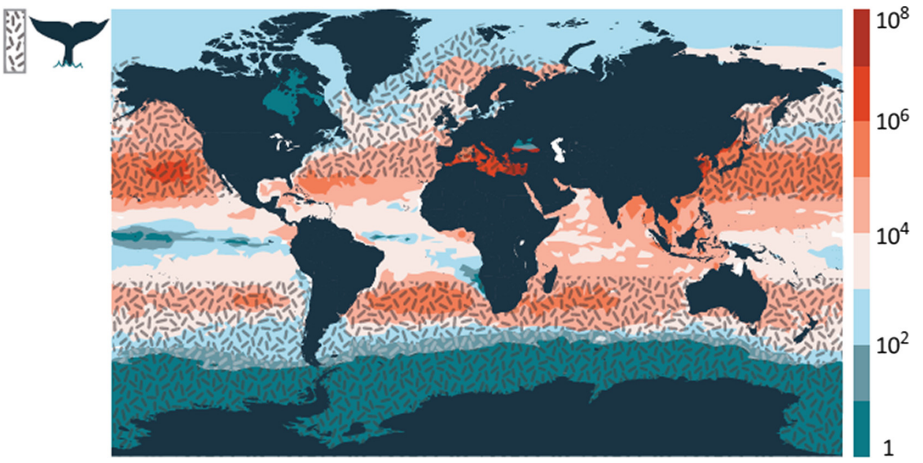

Fig. 1. Key Buoyant Microplastic Hotspots Overlap with Habitat Ranges of Filter-Feeding Marine Megafauna. The habitat ranges for *Balaenoptera physalus*, as indicated by thatched, lined, or dotted overlay, respectively, overlap with regions containing high levels of buoyant microplastic pollution. From Germanov et al. 2018 (Modified).

Recent studies suggest that debris, including micro-plastics and chemical additives (e.g., phthalates), tend to accumulate in pelagic areas in the Mediterranean, indicating a potential overlap between debris accumulation areas and endangered species' feeding grounds (*Balaenoptera physalus*) (Fig. 1). This fact highlights the potential risks posed to endangered, threatened and endemic species of Mediterranean biodiversity. In one of the most biodiverse area of the Mediterranean Sea, the Pelagos Sanctuary, cetaceans coexist with high human pressure and are subject to a considerable amount of plastic debris, including microplastics [4–10]. Therefore, filter-feeding megafauna resident in these area shave a high probability of ingesting microplastics, because they must filter hundreds to thousands of cubic meters of water daily to obtain adequate nutrition. They can ingest microplastics directly from polluted water or indirectly through contaminated planktonic prey. The high plastic: plankton weight ratios (0.5) in the Mediterranean might lead to a significant reduction in nutritional uptake for filter feeders, with animals feeding on the same quantities of particulate matter but receiving a lowered nutritional benefit. The estimated daily plastic ingestion rates for filter-feeding megafauna vary greatly, depending on location and feeding behavior, and range from as low as 100 pieces for whale sharks in the Gulf of California to as high as thousands of pieces for fin whales in the Pelagos Sanctuary (Fig. 1).

3 Conclusion

For these findings 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 by this "story" despite this subject at the beginning has been treated with great suspicion. This scientific topic is also developed in the Plastic Busters MPAs project, 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 [10].

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Microplastic Contamination of Sediment and Water Column in the Seine River Estuary

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1 Introduction

Nowadays, microplastic (MPs) pollution is well documented in marine ecosystems since the first publication alarming about marine plastic pollution in 1972 [1]. Similarly, continental contamination is more and more investigated. More recently, interest for estuarine systems is growing. Estuaries are considered as a suspected predominant pathway for microplastic pollution from continent to oceans. The specific conditions of estuaries, like salinity gradient, tides and hydrodynamics, could affect the repartition, settling and transfer of microplastics to marine systems.

This study aims to quantify levels of microplastics in water column and intertidal sediments in the Seine river estuary to investigate the impact on estuary specific conditions on microplastic pollution.

2 Materials and Methods

2.1 Study Site

The Seine river watershed is equivalent to 80 000 km^2 and accounts of 40% of national economic activity. The catchment of the estuary represents 11 500 km^2 and concentrate 40% of national economic activity [2]. The Seine river is heavily anthropized. It is under very strong pressure mainly induced by the agglomerations of Paris and Rouen. The Seine river estuary represents the last 160 km of the river. This estuary is delimited from the dam of Poses to the mouth of the river at Le Havre. It is characterized by semi-

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diurnal tides, and a strong tidal range reaching 7 m. Current speed can reach 2 $m.s^{-1}$ at the mouth of the river. Two petrochemical hubs are present in the estuary.

2.2 Samples Collection

Three sites were selected along the estuary: La Roque, Vieux-Port, and La Bouille (Fig. 1). Sampling trip was conducted in May 2017, during low flow period with a flow equal to 256 $m^3.s^{-1}$. Samples were collected during rising tide and ebb tide. At each location, two nets were towed, collecting surface (first 15 cm) and subsurface (50 cm) water. Both nets were plankton net 300 µm mesh, 50 cm diameter. The volume collected range from 10 to 90 m^3. All samples are transferred into glass bottles with aluminium cover.

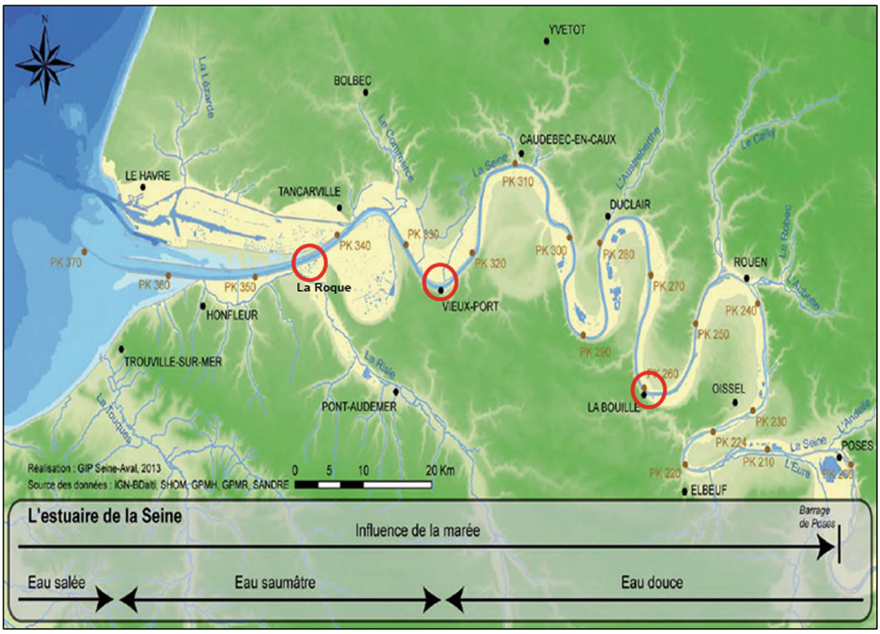

Fig. 1. Sampling sites location in the Sein river estuary

At each location, about 1.5 kg of sediment was sampled using a Van Veen grab. Sediment samples are also transferred in glass bottles with an aluminium cover.

2.3 Analytical Techniques

In the lab, water samples are subjected to a purification protocol. They are first sieved through 5 mm mesh sieve to remove all macroplastics and vegetal waste. Then, sodium dodecyl sulfate (SDS), and biozymes are successively added to denature all proteins, lipids and carbohydrates in the samples for 24 h at 40 °C each. Next, hydrogen

peroxide 30% (H_2O_2) is added to remove remaining organic fraction for 24 h at 40 °C. After this, sample is transferred in a separating funnel with sodium iodide (NaI, d = 1.65 g.cm^{-3}), and after a night of settling, MPs are recovered in the supernatant. Finally, supernatants are filtered through glass fiber filters 47 mm. Each filter is observed under a stereomicroscope and MPs-like particles are enumerated measured. MPs-like particles shape is noted as well.

Finally, about 25% of MPs-like particles is characterized using Raman spectroscopy to assess polymers proportions in each sample.

Sediment samples are also subjected to a purification protocol. First, 4 × 25 g of the samples are transferred in four separation funnels with NaI (d = 1.65 g.cm^{-3}). After a night of settling, supernatant is recovered and SDS is added for 24 h at 40 °C. Next, H_2O_2 30% is added also for 24 h at 40 °C. Between each step, samples are filtered on metallic filters (10 µm pore size) to remove all the solutions. As well as for water samples, filters are observed under stereomicroscope. Thanks to an image processing software, MPs-like particles were defined by length and shape.

Characterization FTIR micro spectroscopy is the final step to assess polymer proportions in each sample. As for column water samples, only 25% of MPs-like particle by sample will be analysed in the interest of time and efficiency.

3 Results and Discussion

3.1 Water Column

First results show that concentrations in MPs-like particles in the water column range from 1.7 particles.m^{-3} to 7.1 particles.m^{-3} (Fig. 2.). The lower concentrations are found at the upstream location, La Bouille. Levels of contamination in the Seine river estuary are higher than other concentrations found in France, 0.24 ± 0.35 particle.m^{-3} in the Bay of Brest [3]. Compared to the literature, these levels are higher than levels reported for other estuaries in the world, like Goiana river, Brazil, with 0.26 particle.m^{-3} [4] or for the Tamar river estuary with 0.74 particles.m^{-3} [5] Europe. There is a hot spot contamination at Vieux-Port, with 37.7 particles.m^{-3} and 8.6 particles.m^{-3} due to a point-source pollution of translucent microbeads. Indeed, these microbeads represented half of both samples.

Considering the samples in La Roque, most particles are lower than 1 mm; they represent respectively 81% and 87% of surface and subsurface water. A small part of particles was between 1 mm and 2 mm, and there were almost none between 2 mm and 5 mm. At La Bouille, most of particles were also smaller than 1 mm. They represent respectively 87% and 71% of surface and subsurface water.

During observation, particles were divided into four categories: fragment, sphere, film and foam. Particles were mostly fragment in shape. There were no differences in shape distribution between surface and subsurface water whatever the sample. Fragment shapes represent between 59% and 73% percent of the distribution, and films represent between 16% and 29%. However, the largest proportion of fragments were found in surface water.

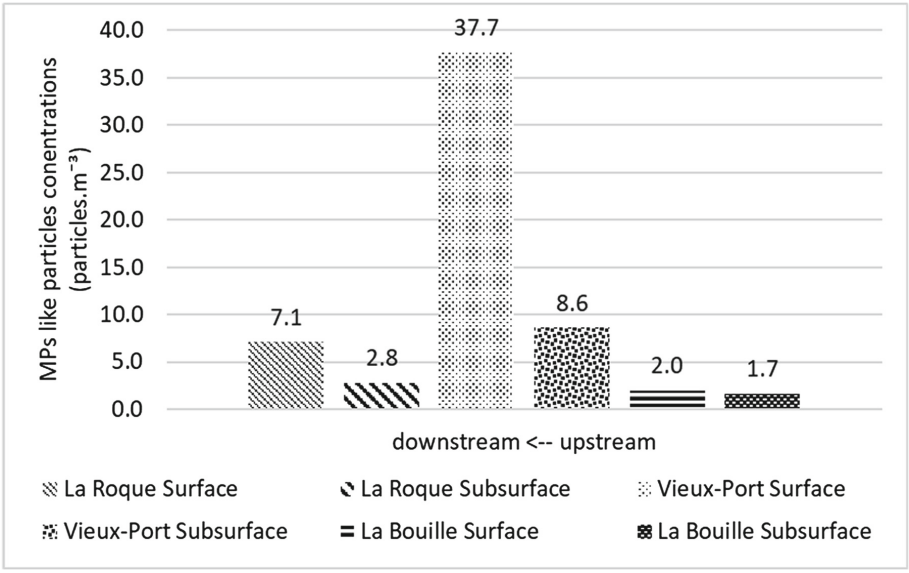

Fig. 2. MPs-like particles concentrations in the Seine river estuary

Characterization step using Raman spectroscopy of 25% of MPs-like particles in each sample showed a majority of polyethylene representing 28% of analyzed particles, then polystyrene with 22%, and polypropylene with 13%. After this, polyamide and polyethylene terephthalate represented 4% of analyzed particles. However, 51% of particles did not respond of spectrum was impossible to identify. Consequently, transformed Fourier infrared microspectroscopy (μFTIR) will be used in addition of Raman spectroscopy to identify the rest of refractory particles.

3.2 Sediments

First results on sediment samples show MPs-like particle contamination about 300 particles.kg^{-1} of dry sediment in Vieux-Port. Fiber contamination was about 360 fibers. kg^{-1} of dry sediment. Most of the particles were films. Particles size range from 38 to 1 200 μm (Fig. 3). Some particles are found in both sediment and water column. This result involves the settling of particles from the water column to the river bed. Fibers size range from 126 to 4 260 μm (Fig. 4). Globally, most of fibers are longer than particles. Compared to the literature, concentrations in the Seine river are higher than concentrations found in South Africa, 20.0 ± 7.5 particles.m^{-3} to 46 particles.m^{-3} [6]. These high concentrations suggest that the Seine river estuary could be a sink for microplastics, but more results are required to go further. Analysis in the sediment are still ongoing.

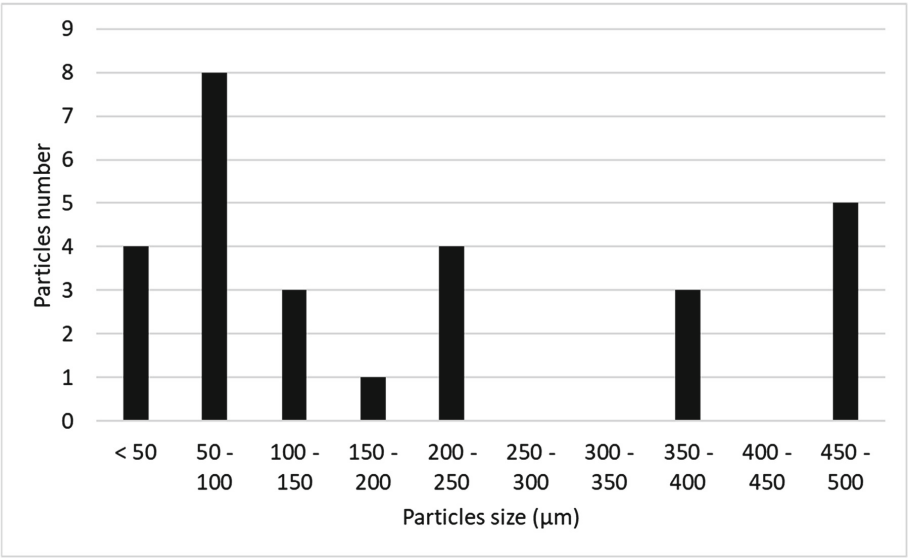

Fig. 3. Size distribution of MPs-like particles in sediment at Vieux-Port

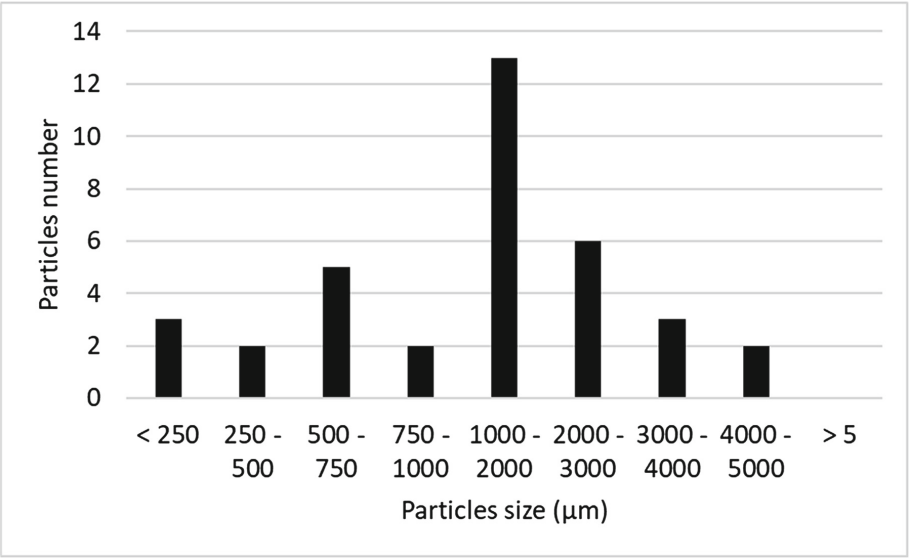

Fig. 4. Size distribution of fibers in sediment at Vieux-Port

4 Conclusions

Concentrations in MPs are high in the Seine river estuary water column, ranging from 1.7 particles.m^{-3} to 37.7 particles.m^{-3}. Same trend is found for sediment with 300 particles.m^{-3} and 360 fibers.m^{-3}. These concentrations show important contamination of the seine river estuary in MPs. These strong concentrations are not surprising as the estuary is subjected to a very strong anthropic pressure and important accumulation of plastic litter. Predominance of fragments indicates fragmentation of larger plastics as the major source of MPs in the estuary. Considering sizes of MPs, results showing most of MPs lower than 1 mm is consistent with the literature. Besides, since some results highlight the sink of particles from the water column to the sediment, consequently, this estuary is suspected to be a sink area for microplastics.

Nevertheless, because estuaries are not well documented, it is difficult to compare levels of contamination in MPs with other studies. Moreover, the lack of standardised protocols makes difficult the comparison of levels of contamination in MPs.

Moreover, characterization step is planned to identify polymer types in the sediment. Both Raman spectroscopy and μFTIR will be used to achieve this goal.

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Plastic Debris in 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 or in progress on plastic debris issue at the scale of the Seine Bassin catchment, combining a high population density and a strong anthropogenic pressure. These projects, illustrated in Fig. 1, 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.

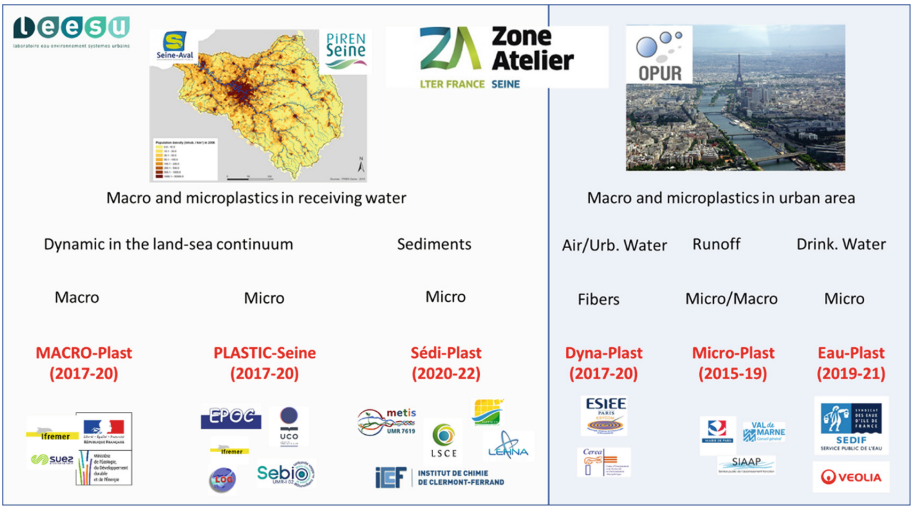

Fig. 1. Research projects launched and in progress on plastic debris issue at the scale of the Seine Bassin catchment

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A first part of this keynote was dedicated to macroplastic pollution and will present first levels found in urban water (Micro-Plast project). Based on tagged plastic litters and GPS-trackers, the fluxes of plastic litter in the Seine River were estimated (MACRO-Plast project). 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 was focused 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.

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

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1 Introduction

The Ephemare project was supported in the period 2015–2018 by JPI Oceans, as one of 4 sister projects in the joint action on ecological aspects of microplastics. Ephemare investigated several issues concerning the ecotoxicological effects of microplastics (MPs) in marine organisms. Ephemare included 16 European Institutions from 10 Countries and was organized into seven, highly complementary Work Packages (WPs) with the aim to elucidate adsorption and release of chemicals to/from MPs, coupled with MP ingestion rates, translocation in different tissues, trophic transfer and egestion, potential toxicological effects and mechanisms of action, as well as real distributions of MPs in marine organisms from several European areas. The project was also designed to raise public awareness through scientifically-sound and research driven results.

Ephemare tested several biological model organisms in laboratory experiments grouped according to their contact/ingestion pathway, comprising no feeders (algae, isolated cells, haemocytes or cell cultures), small and large filter feeders, and predators; these organisms were exposed under laboratory conditions to MPs of different sizes, shapes, polymer type, origins (commercially available vs field micronized) and contaminated with chemical pollutants. Likewise, a wide array of biological species was also collected in the field at coastal locations throughout Europe and analyzed for their content of MPs; as far as feasible, the sampled organisms included representatives from different trophic positions, feeding strategies and habitat preferences.

A suite of biological effects was evaluated at the individual, cellular, and molecular level to elucidate the potential toxicity of MPs and their mechanisms of action. At the individual organism level, the toxicity endpoints ranged from survival, growth rate, behavior, reproduction success, embryo and larval development to energetic physiology and performance. At the cellular and molecular levels, the main investigated pathways included immune responses, oxidative stress, neurotoxicity, biotransformation (particularly for MP-bound chemicals), genotoxicity and endocrine effects. The experimental conditions were designed to evaluate the direct effects of MPs, as well as their capability to modulate bioavailability and toxicity of sorbed chemical pollutants, in comparison with other particles in marine ecosystems.

A detailed description of the experimental set-ups and the obtained results have been published in a series of papers [1]. Herein we summarize the most relevant scientific “take home messages”. Among these, the first is that all the investigated species, from plankton to top predators, did ingest MPs both under laboratory and field conditions. Dynamic modeling confirmed the experimental observations that rate of MP uptake in different tissues, as well as the potential translocation between different tissues, and the egestion kinetics cannot be generalized in terms of “MPs” alone. Rather the involved processes are strongly influenced by the MPs’ size and shape, as well as composition.

Ingestion of MP is not only a direct phenomenon, since these particles can also be easily transferred through trophic chains. In this respect, the uptake of MPs in the jellyfish *Aurelia* can occur directly from water but also via feeding on nauplii of the copepod *Tigriopus fulvus* previously loaded with polyethylene fluorescent MPs (1–

4 μm in diameter, 10 mg/L) [2]. Other examples of simple artificial food chains confirmed that crustaceans and protozoa can efficiently transfer very small MPs (1–20 μm) to both adults and larvae of fish [3, 4]. In many cases, egestion of ingested MPs is rapid (4–6 h), although some particles can be retained within the mucus of intestinal villi and be taken up by epithelial gut cells. The uptake of chemical contaminants, however, is not necessarily increased by adsorption to MPs [5].

Moving the analysis of MPs from the laboratory scale to the field conditions places different demands on the protocols for the extraction and characterization of MPs in marine organisms from natural habitats. Two practical international training courses were held in Ancona to share different experiences among participants of both the Ephemare and Baseman consortia, resulting in a common JPI-Oceans deliverable on a harmonized protocol for monitoring MP in biota, including all the methodological details [6, 7].

The distribution of MPs in marine food webs was investigated in more than 1,200 specimens representative of almost 50 biological species, with different ecological and biological characteristics, sampled in different European areas, from the Mediterranean, the Atlantic Ocean and the North Sea [8, 9]. Just to give a snapshot on the Adriatic food webs, almost 500 organisms from 26 commercial species were sampled from the 3 main sectors, Northern, Central and Southern Adriatic. The overall results did not reveal marked differences in the number of particles extracted in different species and areas; however, the frequency of ingestion was significantly higher in organisms from Central and Southern compared to Northern Adriatic. This work also provided the first extensive characterization of textile microfibers (MFs) which documented more elevated numbers compared to MPs and confirmed the higher percentage of organisms ingesting MPs in Central and Southern Adriatic. Geographical differences were also observed in terms of size, shape and chemical typology of ingested MPs. Specifically, on the basis of frequency and characteristics of particles extracted in marine organisms, principal component analysis distinguished between the 3 Adriatic regions (North, Central, South) which correspond to 3 sectors of the Adriatic basin highly differentiated in terms of bathymetry, morphology and main currents circulation [9].

These field studies enabled several overall conclusions to be drawn. MPs ingestion is a widespread phenomenon, and the frequency of ingestion is a more appropriate index to highlight differences in exposure, rather than the number of particles. Textile MFs are more abundant than MPs, both in terms of numbers and frequency of ingestion. Frequency of ingestion typically ranged between 15 and 35% for MPs and between 50 and 90% for textile MFs. More than 32% of ingested MPs were smaller than 100 μm, 55% smaller than 300 μm, and 70% smaller than 500 μm. In contrast, widely used sampling methods for MPs in seawater typically only collect particles with dimensions greater than 300 μm; our findings highlight the need to quantify and characterize the smaller size fractions to properly evaluate potential biological effects. An unclear influence was found for trophic position, feeding strategy and habitat preference on MPs ingestion and, although local relationships could be observed, they were not easily generalized. Regional activities and hydrographic characteristics might influence the dynamics of the local exposure conditions and thereby the frequency of MPs ingestion and the differences in terms of size and typology of ingested particles. Finally, we found that biological species already used as indicators for biomonitoring

programs (such as those indicated by MSFD or national guidelines) should be also considered for MPs monitoring [9].

Concerning the toxicological effects caused by MPs at the organism level, standard ecotoxicological bioassays typically showed no effects, indicating that MPs *per se* are not acutely toxic under short term conditions [10, 11]. Lowest observed effect concentrations LOEC were typically greater than 30 mg/L, irrespective of particle size, shape, or polymer type. In general, concentrations of MPs causing acute toxicological effects in laboratory conditions were 5 orders of magnitude higher than typical environmental levels [12]. However, lack of acute toxicity does not necessarily mean lack of hazard: long-term and/or less acute MP exposure scenarios revealed significant biological effects in some cases. Virgin particles, those previously loaded with chemical pollutants, or field-collected and micronized MPs caused biometry abnormality and behavioral effects in medaka larvae [13, 14]; growth defects and decreased number of eggs appeared in adults of marine medaka after long exposures (3–4 months), and spawning success was decreased in zebrafish [15]. Some of these effects were more evident in organisms exposed to MPs previously loaded with different chemicals or to environmental MPs, compared to organisms exposed to virgin particles of commercial origin. These observations point to a non-negligible role of environmentally acquired contaminants on the overall toxicity exerted by MPs in aquatic ecosystems A significant decrease in predatory performance was observed in sandy goby juveniles [16], while two species of sediment-dwelling bivalves exhibited, with a different sensitivity, some changes in energy metabolism when MPs were present in sediments [17]. Overall, these data highlight that long-term and sub-lethal responses are needed to assess the effects of MPs in marine organisms, coupled with understanding of the uptake/release kinetics of associated contaminants [18, 19].

One of the major take-home messages from Ephemare is that the blanket definition of «microplastics» in biota an inadequate and too generic concept. Ingestion of these particles, excretion rate or potential translocation to different tissues, cellular compartmentalization and biological effects are strongly modulated by size and shape of MPs. Although they are still defined as particles smaller than 5 mm, the size classes of biological relevance are much lower, typically below 200 μm for ingestion, and below 20 μm for cellular compartmentalization. Likewise, shape modulates such phenomena, with spherules, fibres or fragments having different effects. Standard methods have been developed to test the toxicity of those “small microplastics” to zooplankton [12].

We also need to better address indirect effects that MPs might have in combination with other environmental stressors. Among these, chemical pollutants have received particular attention, for the capability of MPs to bind and release these compounds after ingestion, i.e. the so-called Trojan-horse effect. The sorption behaviour of pollutants to MPs has been evaluated during Ephemare under various experimental conditions: it appears to be a rather dynamic process, which depends on exposure conditions, typology of chemical, time of contact and characteristics of particles (size, shape, polymer type, virgin vs weathered). It is even more complex to generalize the release of contaminants from MPs, which is modulated by the particle radius, diffusion coefficient within the polymer matrix, polymer crystallinity, lipophilicity and gut conditions [19]. For amorphous polymers such as PE, affinity to hydrophobic chemicals is higher than that of biological tissues, and thermodynamic models point at diffusion within the

polymer as the rate-limiting process. Appreciable desorption dies occur for chemicals with relatively low lipophilicity, the accumulation of which on MPs is negligible in the environment. However, the role of surfactants should be considered further since these compounds appear to facilitate desorption of chemicals and are present in the digestive tract of many organisms [5].

Bioavailability of chemicals bound to MPs could be demonstrated in several experiments after ingestion but also merely by external surface contact. Larvae of *Artemia* could transfer very small MPs (1–20 μm) loaded with benzo[a]pyrene to zebrafish (BaP) [4]. Fluorescence tracking of BaP indicated that even a lipophilic chemical may be desorbed in the intestine of fish and be transferred to the intestinal epithelium and liver. Similar results were observed for the transfer of contaminants from MPs to fish larvae via *Paramecium* previously fed with BaP-loaded MPs. Although the majority of studies have investigated the transfer of chemicals after oral ingestion of MPs, transfer of BaP could also be shown after only superficial contact of MPs with gills of adult zebrafish [3]. Yet, there was no accumulation of particles on or inside the gills; most MPs remained trapped on the superficial mucus layer of the gill filaments and were thus excreted. However, BaP-borne fluorescence indicated the transfer of BaP to the cells of the gill filaments and arches after 6 and 24 h incubation, a phenomenon confirmed by gill EROD induction.

The transfer of a chemical from MPs to tissues does not necessarily mean that these particles should be considered as a major source of exposure for marine organisms, and BaP visualized by fluorescence microscopy under experimental conditions did not reach sufficiently high concentrations to induce toxic effects in the fish embryo [3, 4]. Compared to waterborne exposure, MPs certainly influence the tissues a chemical might be released to, and the timescale thereof. Mussels exposed to Hg2+, dissolved or sorbed onto particles (including MP and microalgae) accumulated the same amount of Hg independently of the exposure route but in different tissues, namely the digestive gland for particle exposure, and the gills for waterborne exposure. Approximately 70% of the Hg incorporated through MPs was quickly eliminated through biodeposits, while Hg2+ uptake via microalgae or water was translocated to other tissues [20, 21]. A different organotropism for chemicals released from MPs compared to waterborne exposure has been observed also for other compounds, such as Chlorpyrifos [22].

MPs do not appear to increase the load of bioaccumulated pollutants [23], nonetheless several lines of evidence showed that these particles can modulate the biological effects of chemicals [24, 25]. At the organism level, virgin MPs were shown to increase toxicity of chlorpyriphos to mussel larvae, while chlorpyriphos-spiked MPs were less toxic than the combination of MPs and dissolved chlorpyriphos. Synergistic effects of PFOS and MPs were observed on the 21d chronic assay with *Daphnia magna*, while both synergistic and antagonistic effects were caused by gold nanoparticles (5 nm) and MPs (1–5 μm) on mortality and reproduction success of *Daphnia magna* [26].

Molecular and cellular mechanisms by which MPs can modulate biological effects of chemicals have been further addressed in experiments with invertebrates and fish, exposed to various combinations and typologies of MPs dosed alone and in combination with chemicals. Beside the ingestion of particles, translocation and possible bioaccumulation of chemicals, a wide array of biomarkers including immune

responses, oxidative stress, neurotoxicity, lipid metabolism, peroxisomal proliferation and genotoxicity have been investigated at the functional cellular level, proteomic profile and gene expression [27–30]. The main overall results confirmed the ingestion of MPs both *via* both water and diet, an uncertain translocation of MPs to different tissues depending on their size, some typical inflammatory responses at histological and gene expression levels, accompanied by the confirmation that MPs can also act as vehicles of associated contaminants which are desorbed and accumulated by organisms, even though concentrations were not particularly elevated [31, 32]. The analyses of several biomarkers confirmed a certain involvement of oxidative pathways and cholinesterase inhibition, but immunological parameters were generally those revealing more frequent and rapid variations. When the overall biological significance of cellular variations was summarized using weighted criteria based on toxicological relevance and magnitude of observed variations, the elaborated level of hazard generally ranged between slight and moderate, confirming a general lack of acute effects in the medium-term. At the same time, however, the overall results highlighted a clear shift from a physical to a chemical toxicity in mussels exposed to BaP-contaminated MPs [30]. At the beginning of the exposure, the main effects were induced by MPs (possibly reflecting a physical challenge), followed by effects ascribed to a combination of MPs and BaP; only after prolonged exposure, effects of BaP prevailed over those induced by MPs (chemical impacts dominant).

The main conclusions on biological effects of MP ingestion in marine organisms can be summarized as follows: standard ecotoxicological assays do not reveal acute toxic effects after short-term exposure, whereas sublethal effects may appear at longer exposure times; MPs can bind and release pollutants to organisms in a way that depends on the physicochemical features of the MPs and the physiological features of the organisms, and they do not represent a major source of chemical exposure in absolute terms; MPs can modulate the effects of chemicals and may cause interaction between chemical and physical challenges; effects at the cellular level were moderate, but the observed susceptibility of the immune system points to potential subtle effects on organisms' health status under chronic exposure conditions; the possibility of MPs to modulate organismal responsiveness towards other stressors including climate change variables deserves attention.

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What Can Model Polystyrene Nanoparticles Can Teach Us on the Impact of Nanoplastics in Bivalves? Studies in *Mytilus* from the Molecular to the Organism Level

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1 Introduction

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 (from 1 to 1000 nm) under abiotic conditions. However, as for microplastics, the use of synthetic nanopolymers may provide an initial estimate of their toxicity and mechanisms of action on marine organisms under laboratory conditions. Engineered polystyrene nanoparticles (Amino modified-PS-NH_2 and Carboxy modified- PS-COOH), has been used to study effects at different level of marine invertebrates and reported tissue accumulation and interactions with cells [1–4].

The edible marine bivalve *Mytilus galloprovincialis*, represents a good study model with large background knowledge on biochemical and physiological responses to environmental stressors, and it combines ecological importance with economic and commercial value and societal interest [5]. Moreover, it was demonstrated that mussels are sensitive target for different types of nanosized particles that have been shown to trigger immunomodulatory effects in *Mytilus* upon laboratory exposure [6]. Mussel immune system relies only on innate immunity, which mainly involves circulating hemocytes acting in collaboration with other soluble factors present in hemolymph serum [7]. This system responds very fast upon encounter with foreign particles, which makes it a suitable tool for studying also the effects of nanoplastics on *Mytilus*. Moreover, agglomeration of nanosized polystyrene in seawater facilitates their ingestion in suspension feeding bivalves, and their potential translocation from the gut to the circulatory system [8].

In this line, the effects of two types of commercial nanopolystyrene-NPs (PS-NH_2 and PS-COOH, 50 nm) have been investigated both *in vitro* and *in vivo* on *M. galloprovincialis*. Short-term *in vitro* exposure to NPs allowed to screen for general concentration effects and immune response of *Mytilus* hemocytes. Further PS-NH_2 *in vivo* experiment, with more realistic environmental exposure, contributed to study

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the effect at the whole organism level with different exposure pathway. Moreover, the effects were investigated in early larval stages, known to be more susceptible to environmental factors. Finally, in an attempt to understand if the impacts observed are common to several species of bivalves, the effects of these NPs were also studied in the Antarctic mussel *Laternula elliptica.*

2 Experimental

2.1 Materials

Unlabeled 50 nm amino polystyrene NP - PS-NH_2 (Bangs Laboratories), and the 40 nm nano-polystyrene, carboxylated - PS-COOH (Invitrogen), were characterized in different media (MilliQ water, artificial sea water-ASW, *Mytilus* hemolymph serum-HS) by dynamic light scattering-DLS analysis [6, 9–11] and the results are summarized in Table 1.

Table 1. Physico-chemical characterization of nanopolystyrenes (PS-NH_2 and PS-COOH) behaviour in exposure medium obtained by DLS analysis [6, 9–11].

Nanoparticle	Medium	Z-average (nm)	PDI[a]	ζ-potential (mV)[b]
PS-NH2	MilliQ water	57 ± 2	0.07 ± 0.02	+42.8 ± 1
	ASW	200 ± 6	0.3 ± 0.02	+14.2 ± 2
	HS	178 ± 2	0.37 ± 0.01	–
PS-COOH	ASW	1764 ± 409	>0.4	−7 ± 5

[a]Polydispersity Index (PDI),
[b]ζ = zeta potential

Mussels (*Mytilus galloprovincialis* Lam.), 4–5 cm long, purchased from an aquaculture farm (La Spezia, Italy), were transferred to the laboratory and acclimatized for 24 h in static tanks containing aerated artificial sea water-ASW [12], pH 7.9–8.1, 36 ppt salinity (1 L/animal), at 16 ± 1 °C. Hemolymph was extracted from the adductor muscle of 4–5 animals, using a 1 ml syringe with an 18 G1/2″ needle, filtered with gauze and pooled. All procedures were carried out at 16 °C.

2.2 Methods

For *in vitro* experiment, hemocytes monolayers were prepared; 20 µl of hemolymph was dropped onto a glass slides and incubate 20 min to let the cell to adhere to the support. Hemocyte monolayers were incubated with NPs suspension in filtered ASW or HS for 30 min to reach the desired final concentrations 50 µg/mL. Lysosomal membrane stability (LMS) was evaluated by the NRR (Neutral Red Retention time) assay.

Phagocytic activity was evaluated as uptake of Neutral Red-conjugated zymosan particles in hemocyte monolayers. For other experiments, the whole hemolymph was incubated with NP for 30 min. Lysozyme activity serum was measured by the lysis of *Micrococcus lysodeikticus*. Extracellular oxyradical production (ROS production) was measured by cytochrome c reduction (for detailed method see Canesi et al. [9]).

For *in vivo* exposure, mussels were exposed 24 h to NPs (10 μg/L/mussel) and a parallel group of control (untreated) mussels were kept in clean ASW. ASW was changed each day before addition of the NPs. At the end of the exposure, several hemocyte functional parameters were measured (as for *in vitro* test) and tissues (gills and digestive gland) were dissected for antioxidant and biotransformation enzymes activities measurement.

3 Results and Discussion

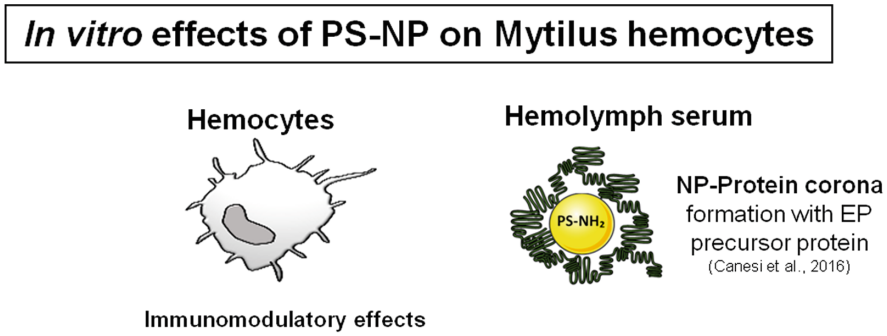
3.1 Results

The behavior of NPs varied according to the media and the charge type (Table 1). In ASW, PS-COOH formed bigger agglomerates ($\sim$1000 nm) compared to PS-NH_2 ($\sim$200 nm). Moreover, they retain different charge, with a positive zeta potential for PS-NH_2 (+14 mV) while PS-COOH has a slightly negative value (−7 mV).

NPs have shown to affect the functional immune parameters of *Mytilus* hemocytes *in vitro* (Fig. 1). Amino modified PS-NH_2 caused a decrease in hemocyte LMS for both media suspension ASW and HS, with suspension in HS displaying stronger lysosome destabilization. Phagocytic activity was decreased at similar level in both media. Lysozyme release and ROS production by hemocytes was increased in presence of PS-NH_2 suspended in ASW, and the effect was stronger in HS. Moreover, upon exposure, some pre-apoptotic signs evaluated in mitochondria were observed. Exposure to the other type of NP, carboxylated - PS-COOH showed opposite effects in *in vitro* test, with general stronger effects in ASW compared to HS. PS-COOH caused LMS disruption when in ASW, while it was ineffective in HS. Lysozyme release after PS-COOH exposure was higher in ASW compared to HS medium.

Moreover, *in vitro* study with PS-NH_2 and hemolymph serum of *Mytilus* the formation of a hard corona with the extrapallial precursor protein-EPp (*or* putative C1q domain containing protein-MgC1q6) [6, 9]. However, no hard protein corona was observed with PS-COOH and hemolymph serum of *Mytilus*.

PS-NH_2 have also shown to impact *Mytilus* early embryo development, especially on shell formation. At lower concentrations (<1 mg/L) PS-NH_2 affected the development of normal D-shaped larvae at 48 h post fertilization Moreover, dysregulation of transcription of genes involved in early shell formation (Chitin synthase, Carbonic anhydrase, Extrapallial Protein) at both 24 and 48 h post fertilization was observed [11]. Higher concentrations (5–20 mg/L) resulted in high embryotoxicity/developmental arrest at.



		Medium	PS-NH_2	PS-COOH
In vitro exposure (50 µg/ml; 30 min- 1h)	Lysosome membrane stability	ASW	↓	↓
		HS	↓↓	=
Functional hemocyte and hemolymph parameters	Phagocytosis activity	ASW	↓	=
		HS	↓	=
	Lysozyme release	ASW	↑	↑↑
		HS	↑↑	↑
	Extracellular ROS production	ASW	↑	**nd**
		HS	↑↑	**nd**
	NO production	ASW	↑	**nd**
	Pre-apoptotic signs	ASW	↑	**nd**

Fig. 1. Summary of the immunomodulatory effects of NP exposure in *Mytilus galloprovincialis*, (nd-not determined).

In vivo exposure of adult *Mytilus* to PS-NH_2 (10 µg/L, 24 h) resulted in a general immunomodulatory response in hemolymph (Fig. 2). LMS and phagocytosis activity were decreased, and the exposure enhanced the lysozyme release and production of ROS by hemocytes. Moreover, general inflammation and oxidative stress in tissues (gills and digestive gland) was recorded with antioxidant enzymes activity slightly enhanced after exposure compared to control.

For the Antarctic mussel, *Laternula elliptica,* after *in vitro* exposure of hemocytes to both PS-NH_2 and PS-COOH, neutral red uptake, a marker for cell toxicity, was measured. No effects were observed at different concentration (50 and 100 µg/mL) and exposure times tested (1–4 h).

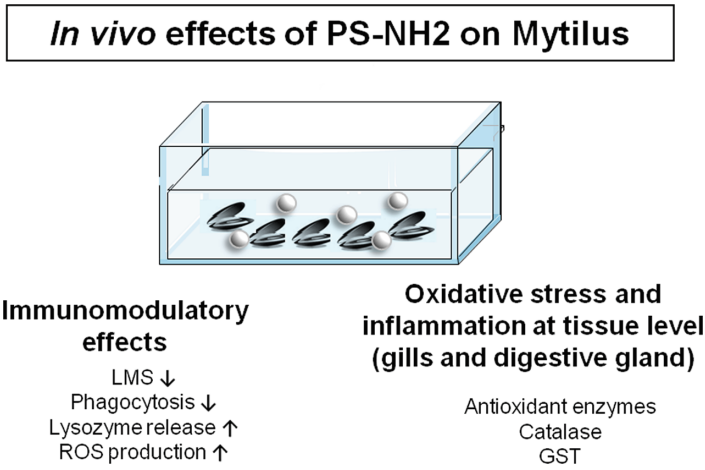


Fig. 2. Summary of the effects of PS-NH_2 (10 µg/L) on 24 h *in vivo* exposure of *Mytilus galloprovincialis*

3.2 Discussion

The results summarize the *in vitro* and *in vivo* data so far obtained on the effects of model polystyrene nanoplastics in *M. galloprovincialis*, at different levels of biological organization, from cell to whole organism level.

Short-term *in vitro* exposure of *Mytilus* hemocytes to NPs triggered immune responses, which were accentuated in HS suspension for PS-NH_2 with respect to ASW medium. The EPp that showed to coat the PS-NH_2 surface, one of the most abundant serum protein in *Mytilus* hemolymph, likely plays a role in the recognition of PS-NH_2 and immune response by hemocytes, [6].

An opposite trend was observed for PS-COOH in different media. No hard and stable protein corona was observed in HS, which could explain the absence or lower effects observed in HS with respect to ASW medium. Moreover, PS-COOH have shown to form larger aggregates ($\sim$1000 nm) that likely reduce the chance of interactions with hemocytes.

All together, the *in vitro* results confirm the importance of physico-chemical characteristics of NPs behaviour in exposure media, in which the role of surface charges, the aggregation state, and the protein corona formation in nanoplastic determine the effect hardness on biological model.

In vivo exposure to PS-NH_2 showed to alter general immune homeostasis of adult *Mytilus* and trigger inflammatory process in tissues over 24 h exposure at very low concentrations (10 µg/L). Similarly, Brandts et al. [3], have shown that 96 h exposure of NP (110 nm) potential toxic effect to hemocytes of *Mytilus*. In tissues, in line with the present study, increased in total antioxidant capacity was recorded. Moreover, NPs have shown to alter the expression in gills of genes associated with biotransformation and immune function [3]. In the scallop *Pecten maximus*, exposed to NP (25 and 250 nm), a rapid uptake of NP occurred, accumulated in hepatopancreas and gills.

Moreover, NPs were detected in other tissues such as muscle, that further suggested translocation of NPs across the epithelial membrane [2]. The results suggested that even at low concentrations, bivalves are able to uptake and accumulate NPs in digestive tract and can be further translocated to hemolymph and other tissues.

The effects of PS-NH_2 were further studied in embryos, suggesting strong impairment of their development and total arrest at higher concentration. Similar observations were made in the sea urchin showing developmental defects in embryos [10].

In few words, they are observations that could imply high environmental impact, with increasing nanoplastic pollution, population of invertebrate could be affected. Recently, plastics residues have been also encountered in remoted areas like Antarctic. Even though, early *in vitro* exposure experiment to NP using *L. elliptica* hemocytes did not elicit toxicity, it is not excluded that seawater contamination will not affect whole animal physiology or fitness.

4 Conclusions

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.

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

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In Vitro Effects of Mercury (Hg) on the Immune Function of Mediterranean Mussel (*Mytilus Galloprovincialis*) Are Enhanced in Presence of Microplastics in the Extracellular Medium

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1 Introduction

There is a great concern that physical and chemical properties of microplastics (MPs) may facilitate the sorption of toxic metals and organic contaminants to the particle surface, serving as a enriched vector (Trojan horse) of contaminants to marine organisms following ingestion [1]. Yet the extent to which sorption of contaminants onto different types of MPs enhances or mitigates the effects of these pollutants remains unclear. Apart from direct action on specific body tissues, contaminants may exert toxic effects by influencing homeostatic mechanisms, such as the immune system. Immune defence in mussels is comprised of cell-mediated and humoral mechanisms, in which hemocytes play a key role [2]. Phagocytosis is performed by specialized cells such as macrophages, and it plays a role in the clearance of particles having a diameter greater than 0.5 μm. Particle size, shape and surface properties affect efficient entrapment and subsequent uptake by macrophages [3]. Mussels have highly developed phagocytosis processes for the cellular internalization of microscale particles (0.1–100 μm) and they represent a suitable model for investigating the effects and modes of action of micro and nanoparticles in the cells of aquatic invertebrates. Polystyrene microspheres filtered by the blue mussel *Mytilus edulis* are bioaccumulated in gut and digestive tubules and subsequently translocated in hemolymph and hemocytes [4]. The capability of microplastics to adsorb chemical pollutants from the environment is a potential risk that has motivated research. Polyethylene generally exhibits a greater sorption capacity than other plastic types [5]. To distinguish between the potential adverse effects caused by exposure to MPs, absorbed chemicals, and their combined effects on marine life, controlled laboratory experiments are necessary. The use of freshly isolated primary cultures has the advantage that they are more differentiated than cell lines, contain

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different types of cells and are thus thought to respond more similarly to a living animal, while at the same time reducing the amount of work and animals required for *in vivo* testing.

In this study we tested the hypothesis that the simultaneous presence of Hg and polyethylene MPs in the extracellular medium may increase the toxicity associated with Hg in an individual way, altering the immune function in mussel hemocytes. To this end we investigated the *in vitro* effects caused by individual and co-exposure of Hg and polyethylene MPs (size range from 15 to < 1 μm) on phagocytosis efficiency and lysosomal membrane stability in freshly primary cultures hemocytes of the Mediterranean mussel (*Mytilus galloprovincialis*).

2 Experimental

2.1 Mercury and Microplastics

A working solution of mercury ($HgCl_2$) with a nominal concentration 10^{-8} M in filtered sea water (FSW) was used in the individual exposure (Hg) and co-exposure (Hg and MPs) experiments. The reason behind this choice was that previous results (data not shown) demonstrated that *in vitro* exposure of mussel hemocytes to $HgCl_2 \leq 10^{-7}$ M caused maximum phagocytic efficiencies and high lysosomal membrane stability prior to inhibition and membrane destabilization phase. Total Hg concentration in working solutions was quantified (AMA254 LECO Mercury analyzer) immediately after its preparation and after exposure experiments were conducted.

Three commercial polyethylene microplastics (PE-MPs) were used in the experiments: MPP-635 XF (δ = 0.96; <6 μm), AQUATEX-325 (δ = 0.99; <11 μm; oxidized PE) and AQUAMATTE-26HD (δ = 1.07; <8.5 μm; oxidized and modified PE) The processes followed to oxide (AQUATEX-325 and AQUAMATTE-26HD) and to modify (AQUAMATTE-26HD) polyethylene microparticles were not provided by the supplier (www.micropowders.com).

Suspensions of each type PE-MP were prepared at two nominal concentrations *Low* and *High* (1 and 100 $\mu g \cdot mL^{-1,}$ respectively) to be used in the exposure (MPs) and co-exposure (MPs and Hg) experiments. Number of particles per mL (mean) and size (90th percentile of the particle diameter (PD90)) in working solutions were estimated by means of a Coulter Counter (Multiziser 3, Beckman Coulter; MSIII) using a quantification limit from 1.4 to 42 μm. Before quantification in MSCIII, MP working solutions were diluted (1/10 and 1/20) due to technical limitations (saturation reading). Immediately before use, the MPs suspensions were sonicated. All the testing solutions containing PE-MPs and Hg (10^{-8} M) were similarly prepared.

2.2 Characterization of Microplastic Working Suspensions

Number of MP particles per milliliter ($pp \cdot mL^{-1}$) and particle size (PD90) in *Low* and *High* working suspensions for each MP type were estimated from their corresponding regression function ($R^2 > 0.8$). Data ($pp \cdot mL^{-1}$; PD90) from serial dilutions of each

working solutions were used to obtain the function models (Microsoft® Excel v16.0 for Windows, EE. UU).

2.3 Animals and Hemocyte Monolayer Preparation

Mussels (*Mytilus galloprovincialis*) from natural populations were randomly sampled within the size range of 35–45 mm shell length. Hemolymph samples were obtained of a least 12 mussels for each experiment/treatment. Hemolymph was withdrawn from the anterior adductor muscle and aliquots of 40 μL were dispensed onto glass slides, placed in dark wet chambers and incubated at 16 °C for 30 min. Slides were pretreated with Poly-L-lysine and non-adherent hemocytes were subsequently removed and hemocytes monolayer samples were added with 40 μL of each working solution (Hg, PE-MPs suspensions and PE-MPs + Hg), and incubated for further 30 min at 16 °C. Untreated hemocyte samples, incubated with filtered seawater (FSW) were run in parallel.

2.4 Lysosomal Membrane Stability and Phagocytic Efficiency Tests

Lysosomal membrane stability (LMS) was measured by the neutral red retention assay (NRR) in haemocyte cells following the procedure described by [6], with modifications as described in [7]. Results of LMS were expressed as the neutral red retention time (NRRT), which corresponds to the last time period recorded when there was no evidence of dye loss or lysosomal abnormalities in more than 50% of the cells.

Phagocytic efficiency (PhE) was evaluated using fluorescent microscopy. Briefly, control and pre-treated samples were gently washed three times with FSW before incubating for further 30 min with Fluorescein-labelled zymosan A bioparticles (Invitrogen), added at 30:1 (target: hemocytes) ratio. After incubation with zymosan, uninternalized particles were removed by washing with FSW and slides were finally fixed in Beker's fixative during 20 min (+2.5% NaCl), rinsed with FSW and immediately stained with Giemsa solution during 8 min. Coming up next, samples were embebbed and rinsed in distilled water, dry on air and finally mounted in Mounting Medium for substitutes of xylene DC (Panreac). PhE was expressed as percentage of cells that internalized at least 3 fluorescent particles (positive cells), observed under a fluorescence microscope at a magnification of x400 (Olympus BX43), after counting at least 300 cells for each sample.

3 Results and Discussion

3.1 Suspensions of Microplastic Particles in Sea Water

Notably, the results showed quite different number of particles per mL between suspensions of different PE-MP types that had been prepared at the same concentration (up to an order of magnitude). Positive correlations were found between number of PE-MP particles per mL and dilution factor of working suspension (dilution factor correction was included in the quantification by the MSCIII) (Fig. 1).

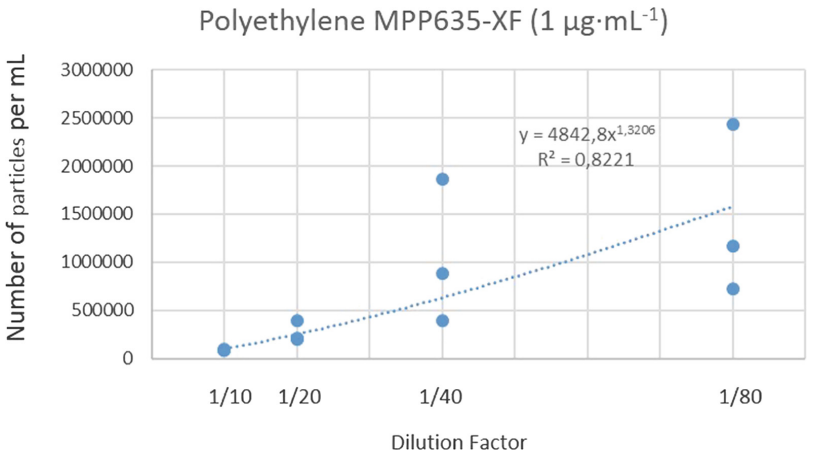

Fig. 1. Number of polyethylene microparticles MPP635-XF in a dilution series of a working suspension prepared at a nominal concentration of 1 $\mu g \cdot mL^{-1}$.

Overall, the number of particles per mL was lower in working suspensions of *Low* concentration (4000–20000 $pp \cdot mL^{-1}$) than of *High* concentration ($200 \cdot 10^3$–$2.5 \cdot 10^6$ $pp \cdot mL^{-1}$) for all three PE-MP types tested (specific data not shown). Particle size was lower (<4 µm) in the most diluted suspensions and this value was used as proxy to the existing in the working suspensions.

3.2 *In Vitro* Effects of Individual Exposure to Hg and MPs

Overall, LMS hemocytes was consistently lower in samples exposed to Hg during 30 min than in control samples, even at the lowest tested concentrations (0.1 $\mu g \cdot L^{-1}$). Concentrations of Hg $\geq 10^{-6}$ M initiated acute destabilization of the lysosomal membrane (NRRT Median $\leq$ 50 min) (Mann Whitney U test, p-value $\leq$ 0.05) (data not shown). Similarly, the PhE was significantly reduced in samples exposed to extracellular Hg $\geq 10^{-6}$ M (3.9%) (ANOVA p-value = 0.00; Dunnet test p-value = 0.00). However, a light increase of PhE (14.2%) (12.9%) was observed in samples exposed to 10^{-9} M Hg, compared with control samples.

There were no significant differences in hemocyte LMS and PhE noted between *Low* and *High* concentrations of MPs. As previously observed after exposure to Hg, LMS was consistently lower in hemocytes exposed to PE-MP than controls. This pattern was observed for the three types of MPs at the concentration range tested (data not shown). Concerning PhE, no significant inhibitory effects were found for any type/concentration of MPs (one-way ANOVA, Dunnet T test p-value > 0.05), although a PhE stimulation pattern was found after exposure, with particular reference to MP *Low* concentrations (data not shown).

3.3 *In Vitro* Effects After Co-exposure to Hg and Polyethylene MPs

Hemocytes co-exposed to PE-MP particles and 10^{-8} M Hg displayed a similar pattern of LMS than hemocytes exposed only to MP-PE, but with significant lower values than

control in samples treated with MPP-635XF and AQUAMATTE-26HD (1-way ANOVA; Dunnet test p-value < 0.05). Concerning PhE, a significant inhibition was demonstrated in samples co-exposed to 10^{-8} M and *High* concentration of the three investigated types of MP-PE with particular reference to AQUAMATTE-26HD

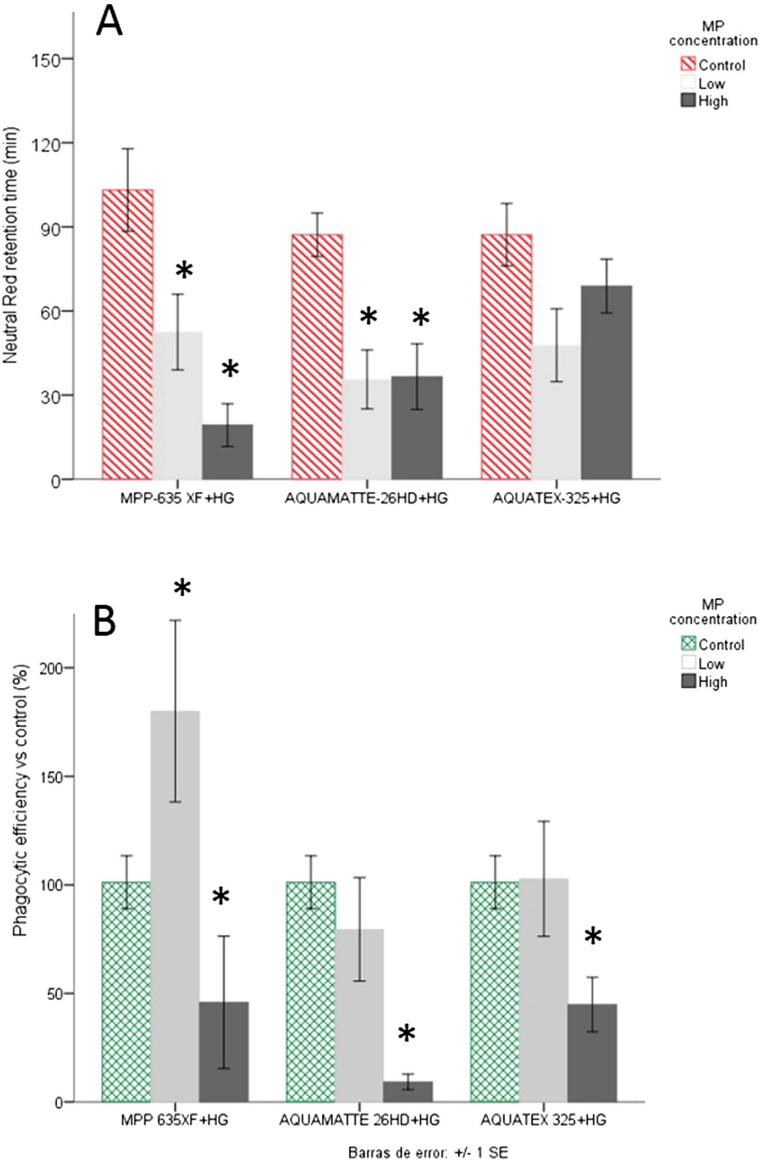

Fig. 2. Lysosomal membrane stability (A) and phagocytic efficiency (B) (mean ± standard error) in hemocyte samples co-exposed to Hg (10^{-8} M) and different polyethylene MPs suspensions in seawater (Low = 1 μg·mL^{-1}; High = 100 μg·mL^{-1}). Asterisks indicate significant differences against control (Dunnet test; p-value < 0.05).

(ANOVA; test de Dunnett, p-valor < 0.05). Chemical analysis confirmed that total Hg concentration were not significantly different between suspensions *Low* and *High* for each type of MP tested (t test for the Mean; p-value > 0.05) (data not shown) (Fig. 2).

3.4 Discussion

Exposure conditions showed to be very different for the three PE-MP types, even when prepared at same concentrations. Aggregation is largely determined by the ionic strength (IS) and valence of the electrolytes in the medium [5]. However, when these factors are attempted to keep constant, as in our study, characterization of different PE-MP types in seawater suspensions suggested that disaggregation and aggregation processes of microparticles occur at different degrees as seawater dilution increase and decrease, respectively. These results underline the need and importance of quantifying number and size of MPs in working suspensions for interpreting, assessing and comparing laboratory results.

Although no studies report on the presence of environmental loads of MPs $\leq$ 6 μm in marine waters, weathering of macroplastics and large microplastics is expected to yield MPs below this size [8]. The simultaneous presence of Hg at concentrations $\geq 10^{-8}$ M and MPs of polyethylene at extracellular concentrations above $200{\cdot}10^{3}$ pp·mL^{-1} may cause negative interactive effects on PhE and LMS in mussel hemocytes. However, toxicity seems to be associated also with other factors such as the functionality and surface load of the plastic particles. In our study, co-exposure *in vitro* experiments were conducted using filtered seawater. First evidences of the formation of a biocorona complex around nanoplastic particles in marine invertebrates have been described [9, 10] and LMS of hemocytes appear to result more sensitive to nanoplastic exposure when cells are incubated in hemolymph serum than in seawater [11]. In our study, the presence of polyethylene MPs (3–6 μm) in the extracellular medium at concentrations between 4000 and $2.5{\cdot}10^{6}$ pp·mL^{-1} "*per se*" did not cause significant effects on hemocyte LMS and PhE. However, further research on "*in vitro*" exposures should be conducted using hemolymph serum as extracellular matrix to confirm our results.

4 Conclusions

The present work confirms the hypothesis that the simultaneous presence of Hg and polyethylene MPs (<6 μm) in the extracellular medium at concentrations above $200{\cdot}10^{3}$ pp·mL^{-1} may increase the toxicity associated with Hg in an individual way, altering immune function in mussel hemocytes (*Mytilus galloprovincialis*). The interactive effects of MPs and Hg causing toxicity seems to be associated not only with the number of particles present in the extracellular medium but also with other factors such as the functionality and load of the surface of the plastic particles. Our findings should be a basis for further research (or work).

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Study of Chemical Pollutants over Marine Microplastics Based on Their Composition and Degradation Rate

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1 Introduction

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) [1, 2]. But this affinity and preconcentration is not the same for all kind of plastics.

These POPs have much more affinity for the plastics than seawater and therefore they tend to accumulate over microplastic surface, this is due that POPs are soluble in lipophilic compounds [3]. The ability of plastics to transport persistent chemical pollutants around ocean is well documented [4–6], even the bio-accumulation and biomagnification in the food chain of small microplastics and their associated POPs [7], but there is little evidence of the influence of the kind of plastic and its degradation on the adsorption rate of these pollutants over microplastic [8].

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 [9], 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.

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2 Experimental

2.1 Materials

Were used six kinds of the main plastics according to the international classification [8, 10] for the study of the POPs adsorption rate in the different plastics. All the samples of plastics treated had similar characteristics from the point of view of colour, size and shape. To such end, only plastics with no artificial colouring were used and these were diced into 5 × 5 × 5 mm pieces.

In the study to assess of the POP adsorption rate depending on degradation of the microplastics, were worked with eight pellets high density polyethylene (HDPE) samples; four samples for physical degradation and four for chemical degradation (Fig. 1).

Fig. 1. Pellets samples studied: with physical degradation: virgin pellets (a), artificially degraded (b), artificially degraded by friction with sand (c) and natural degradation (g). With chemical degradation; degraded with the sun (d) and marine degradation depending on the yellowness grade (e, f, g).

To determinate the concentration of POPs was needed an equipment of gas chromatography with mass spectrometry (GC-MS), model 7820A and 5977 MSD with an HP-5MS Ultra Inert 19091S-433UI column of the brand of the Agilent Technologies®, following its multi-residual analysis methodology [11]. 1 ul n-hexane is injected with IS in the following conditions of oven temperature: 60 °C (2 min), 20 °C/min, 175 °C, 5 °C/min, 250 °C, 10 °C/min, 325 °C (5 min). The transfer line set at 280 °C.

And a Selecta® rotaterm orbital shaker was used to study the adsorption rate of POPs on microplastics in laboratory.

2.1.1 Reagents

The compounds analysed were 15 organochlorinated pesticides (OCPs), 8 polychlorinated biphenyls (PCBs) and 6 polycyclic aromatic hydrocarbons (PAHs).

The patterns of reference of the POPs analysed were obtained from an EPA pesticide mix (Supelco®), a mix of PCB 32 (Sigma Aldrich®) and an assortment of PAHs (Supelco®).

The reagents used to determine the POPs were: methanol (Merk®), n-hexane SupraSolv (Merck®) and Milli-Q water (Millipore). The internal standards (IS) used were: Chrysene D12, Acenapthene D10, Penanthrene D10, Perylene D12 (Supelco®), at a concentration of 2.5 ng· mL^{-1} and Telodrin for analysing OCPs from Dr. Ehrenstorfer GmbH®, at a concentration of 25 ng·mL^{-1}.

2.2 Methods

Were used 1 g of each the different plastics were submerged in 100 mL of sea water at a concentration of 2 ng·L^{-1} of the POP mixture to be studied, and they were left in an orbital shaker for 24 h at 100 rpm to simulate the conditions of ocean movement. After adsorption, desorption was carried along with a detailed analysis in points 2.2.2.

2.2.1 Solid-Liquid-Liquid Microextraction (μSLLE)

The POPs were extracted from the micro using 10 mL of methanol for 1 g of sample, submerged in an ultrasound bath for 4 min. After the solid-liquid extraction, 100 μl of n-hexane were added with the IS. This produces a liquid-liquid micro-extraction of the POPs from the methanol to the n-hexane.

In order to force the separation of the n-hexane phase on the surface, 8.5 mL of Milli-Q water are added in a 20 mL vial with a screw top. Adding water generates a reduction in the solubility of the methanol in n-hexane, enabling the two solvents to be separated [12]. This is centrifuged for 5 min at 3000 rpm and 10 °C to prevent the extractant from evaporating. After centrifuging, the surface micro-layer that contains the n-hexane is removed and put in a flat-based micro-insert for separating the supernatant layer properly. 40 μL of n-hexane are isolated, thus ensuring that no methanol is carried over from the liquid phase immediately below, and this is put in a conical micro-insert for GC-MS analysis.

3 Results and Discussion

3.1 Study of the POP Adsorption Rate Depending on the Kind of Plastics

The highest adsorption rates for organochlorinated pesticides are found in PET (polyester), HDPE (High-density polyethylene), LDPE (low-density polyethylene) and PS (polystyrene). PET, LDPE and PS are the most widely used single use plastics, especially as containers for food and drinks [8, 10].

All in all, the plastics that show the highest adsorption rate of the three families of POPs is PVC and LDPE, followed by PS (Fig. 2).

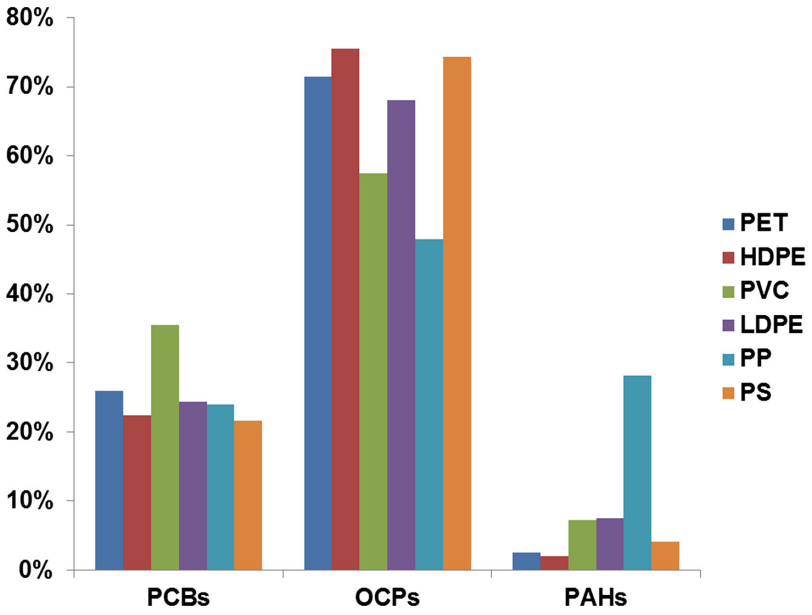

Fig. 2. Percentage of POPs adsorption rate in the different plastics studied

3.2 Study of the POP Adsorption Rate Depending on the Physical and Chemical Degradation of the Microplastics

The greater the surface/volume ratio, the greater the adsorption rate for all the compounds, which is more significant in the analyses of POPs. But the samples with the highest adsorption rate are those that have undergone a natural degradation process, as the micro-fissures in the samples are far more abundant. This also happened with the old samples, which showed the highest level of yellowness.

4 Conclusions

The differences between the different kinds of plastics, along with the physical and chemical degradation that these may suffer, are directly related to their pollutant adsorption rate. There is a proportional relationship between the degradation of microplastics and the concentration of pollutants adhered to it, related to the greater surface/volume of these microplastics.

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Marine Litter: Are There Solutions to This Environmental Challenge?

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1 Introduction

Between 1950 and 2015, it is estimated that 6300 Mt of plastic waste have been produced. Of this, around the 80% ended up in landfills or in the natural environment [1]. The combination of this type of waste disposal and of the durability and resistance to degradation of plastics, has led to the current ubiquitous and abundant presence of plastic debris in the environment. The greatest warning signal of this plastic pollution problems has come from marine environment, where it is estimated that 75% of all marine litter is plastic and this debris has been reported to be accumulating at the sea surface [2], on shorelines of the most remote islands [3], in the deep sea [4] and in arctic sea ice [5]. Despite first reports on marine plastic litter dates back to the 1960s only recently it has been recognized as a pervasive global issue [1].

There is a range of evidence on the harm caused by marine litter; with negative impacts on commercial fisheries, maritime industries and infrastructures, as well as on a wide range of marine organisms as a consequence of entanglement and ingestion [6].

Plastic debris can be defined and described according to different characteristics including origin, polymer type, shape, size, colour or original use. However, the main classification used is about the size: macroplastic (>20 mm diameter), mesoplastic (5–20 mm) and microplastic (<5 mm) [7]. Since macroplastics are more visible, they have been for long time considered as one of the most concerning forms of plastic pollution. In fact, these items can be more easily recognized and categorised according to their original usage (i.e. fishing, packaging, or sewage related debris). More subtle and complicate is instead the pollution related to the presence of microplastics that, with accumulating data on the impact and consequences of such debris, has received increasing research interest and currently represents one of the greatest challenges in the fight against plastic pollution.

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2 Microplastics: Definition and Sources

The presence of small plastic fragments in the open ocean was reported for the first time in the 1970s in the North Atlantic, during sampling campaigns with plankton tows [8]. However, it was not until 2004 that the term 'microplastics' was coined in a paper describing the long-term accumulation of plastic fragments with dimensions of few microns in beach, estuarine and subtidal sediments in the UK [9]. This terminology was subsequently considered during the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris in 2009, where scientific experts agreed on using the name "microplastics" to define plastic particles smaller than 5 mm. The workshop also distinguished microplastics according to their sources [10]. Microplastics are classified as "primary" if they are intentionally produced in such micro dimensions for direct usage or as precursors for other products, for example in cleaning products, cosmetics [11] and as air-blasting media. Instead, they are defined as "secondary" if they derive from the fragmentation of larger plastic debris, as a consequence of ultra-violet (UV) radiation and oxidation and/or physical forces from abrasion, wave-action and turbulence [12]. Secondary microplastic can also be generated as a consequence of items such as tyres and textiles becoming abraded during life in service [13, 14]. The occurrence of these pollutants not only in marine environment, but also in freshwater systems [15].

Microplastics cannot be cost-effectively detected, collected for recycling or successfully removed, causing a range of negative economic and environmental concerns. Microplastics are able to interact with a very wide variety of marine organisms, from zooplankton to marine mammals [6]. Moreover, they may also present a toxic hazard to marine organisms, by accumulating persistent organic pollutants (POPs) already present in water, or by leaching additives added to plastics during their production or residual monomers or oligomers [16]. However, recent modelling work suggests that the importance of microplastics as a transport vector for sorbed contaminants is likely to be minimal in most scenarios [17].

To understand correctly the occurrence and impact of microplastic pollution, the EU [18] is directing efforts to compare and harmonise monitoring protocols, including those used for microplastics, with the scope of ensuring greater inter-comparability among data.

3 Solutions and Future Challenges

The success of plastics is mainly due to four key properties being light weight, durable, versatile and inexpensive. They have made plastics suitable for the most disparate applications, shaping modern society with numerous societal benefits in healthcare, agriculture, transport, construction and packaging [19].

However, the accumulation of plastic litter in the oceans is actually a symptom of a much wider problem – the accumulation of plastic waste. It is clear we have buried beneath the ground for future generations to deal with far more plastic than has accumulated in the oceans. The underlying issue is our linear use of plastics through

short-lived applications to persistent waste. The combination of the growing human populations and the improvement of living standards, along with the increasing plastic production and a lack of consideration at the product design stage of a product fate after use, have led to the culture of a disposable convenience driven society with associated consumer behaviour unconcerned about the consequent environmental implications. By comparison with many other current environmental challenges, the benefits resulting from the use of plastics are not directly linked to the emission of plastic debris to the environment or to degradation of the environment. Hence, in theory at least, it is possible for society to retain the benefits of plastic products and at the same time reduce the quantity of plastic litter entering the environment [20].

In order to establish efficient prevention and mitigation strategies, the identification and comprehension of the different sources of marine plastic pollution is important to gain an accurate assessment of the quantities of plastics and microplastics entering the ocean, to provide an indication of regional or local 'hot spots' of occurrence, and to determine the feasibility of introducing management measures to reduce these inputs [21]. In general, the sources of marine plastic litter are quite well known, but what is still missing is consistent data on the relative importance of the different sources, mainly due to the lack of standardised protocols for replicable measurement of waste generation, collection rates, classification and waste disposal methods for rural areas and urban centers in countries around the world [1]. Therefore, potential solutions to mitigate this problem are widespread and complex and needs joint efforts from industry, governments, society and scientific research.

Starting from product design, disposal pathways for a product need to be considered right from the beginning. Although most plastics are inherently recyclable, many single-use items are not currently designed to be widely compatible with recycling. Long-term sustainable solutions require moving from a linear economy towards a more circular economy that takes into account the end-of-life of the product, leading to its recycling (when possible) or correct disposal [22]. In this respect, waste management frameworks should be improved: this is not just a problem for developing nations, even in industrialized nations with good waste management infrastructure there are generally very low levels of recycling and little evidence of product design being linked to the waste management options that are available.

Governments also have a key role since they can create a legislative framework to stimulate mitigation actions of plastic waste at its sources. For instance, policies from governments in many nations to either ban the sale of single-use plastic bags, charge customers for their use and/or generate taxes from stores who sell them, resulting in a substantial reduction of their use [23]. Currently, there is no consistency of policies in this topic between countries, so a more effective and pragmatic global cooperation among the country governments is of striking importance. There is also a key need to government policies to help create a level and fair playing field for industry so that more reputable companies are not undermined by the less environmentally scrupulous.

Education, outreach and awareness raising are also important ways to address marine litter [24]. It has been suggested that marine litter can be used as a vehicle to inspire and promote more sustainable economies and lifestyles [25]. Improving and spreading public awareness of the problems caused by plastic pollution is the first step towards changing people's behaviour on plastic consumption.

Finally, advances in academic research from material science to waste treatment, are likely to be pivotal in optimising and evidencing new solutions or alternatives to our current approaches to design, use and dispose of plastics. For example, biodegradable or compostable polymers could perhaps replace traditional plastics for some applications, like single-use plastic items, and are sometimes promoted as way to reduce pollution also in marine environment. However, more studies are needed to assess the degradation rate of biodegradable or compostable polymers in a range of natural environments. In most cases, the potential advantages of these novel materials can only be reached in dedicated and specifically managed waste collection systems that provides conditions suitable for degradation. Yet there is often failure to communicate this to the consumer since labelling to facilitate appropriate disposal is lacking [26].

Microplastics represent a more specific issue that requires more complex and challenging actions. Preventing the emission of macroplastics in marine environments will certainly reduce the generation of microplastics by fragmentation. However, for other sources the best approach could be removal at source, for example by banning of microbeads in personal care products [23], or at the design and production stage by for example improving synthetic textile design so that garments release fewer fibres and last longer in service – such that they are on the whole more sustainable [27–29].

4 Conclusions

Since the mass production of plastics commenced in the 1950s, global plastic production has increased almost exponentially. At the same time, plastic pollution and the generation of waste has increased accordingly, hence there is now an urgent need for prevention, mitigation and to a lesser extent remediation actions.

Key solutions to address plastic pollution are already available but there is a need for clear and independent evidence to guide the most appropriate choice of interventions as well as for coordinated action among the international community and several sectors/stakeholders. A synergistic approach should involve dedicated policies and regulations to prevent unnecessary plastic emissions in the environment, changes in product design and production to promote circular economy, and social science to better understand attitudes and perceptions about the issue and the solutions as well as to help raise awareness.

A key and largely missing element is support for academic research and collaboration across the disciplines, bringing together environmental and material scientists, waste managers, product designers, legal expertise as well as social scientist. Only by considering both the challenge and the solutions in a holistic manner, we can hope to reach optimal solutions and minimise the (currently high) risk of taking uniformed knee jerk reactions that may have far reaching negative consequences.

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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 Boknafjord (Norway)

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1 Introduction

Plastic waste is of increasing concern in the aquatic environment. A large portion of this 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 biofilm formation they eventually sink and accumulate on the seafloor together with non-buoyant by design plastic particles, posing a risk to benthic communities. There is, however, a considerable lack of standardized methods to characterize microplastic particles occurrence and composition. Current efforts are underway to develop standardized methods to characterize and quantify the occurrence of microplastic in different environmental matrices using vibrational microscopy such as Fourier Transformed Infra-Red (FTIR) or Raman techniques. However, the processing and interpretation of complex datasets hampers their use within monitoring programs. As an alternative, a thermal degradation method based on a gas chromatographic mass spectrometer coupled with pyrolysis represents a promising method for qualitative and quantitative polymer analyses [1, 2]. A technique has been developed that combines sample preparation and thermo-analysis for identifying microplastics in samples of marine sediment. In the present study, a recently validated thermal degradation method is used to characterize the microplastic occurrence and polymeric composition in marine sediments collected in some marine areas in the northern Adriatic Sea and in an urban fjord located in the south-west coast of Norway.

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2 Materials and Methods

2.1 Study Areas

Boknafjord is a 92-km long fjord system in Rogaland county, on the south west coast of Norway. The fjords within the system are generally very steep sided and deep, frequently attaining depths of more than 200–400 m. The fiord system hosts the cities of Stavanger and Sandnes which combined have a population of approx. 221,000 inhabitants. The dominating activities in the area include transport, tourism, fisheries, oil & gas related services as well as aquaculture. Furthermore, the coastline is dominated by several small and medium sized rivers which collect run off water from pristine to industrialized areas and construction sites contributing to increasing anthropogenic pressure through the release of organic and inorganic chemicals, nutrients and wastes, including plastic litter.

The Adriatic Sea is a semi enclosed basin dominated by a peculiar combination of oceanographic currents, with one flowing towards the SE along the western coast and a second flowing NE along the eastern coast. Strong periodic winds contribute to wave formation and surface water circulation. The Adriatic Sea has been divided into three distinctive sub-basins [3, 4]. The investigated area spreads from the northern section with an average depth of about 45 m to the central sector characterized by an average depth of about 140 m. In the area, several anthropogenic activities coexist such as heavy marine traffic from commercial and tourist vessels, offshore activities, ferry boats and trawl-fishing. Furthermore, the Italian coastline is dominated by several rivers which collect wastewater and rainwater from one of most heavily industrialized area of Europe thus contributing towards the anthropogenic pressure through large loadings of organic and inorganic chemicals, nutrients and garbage, including plastic litter.

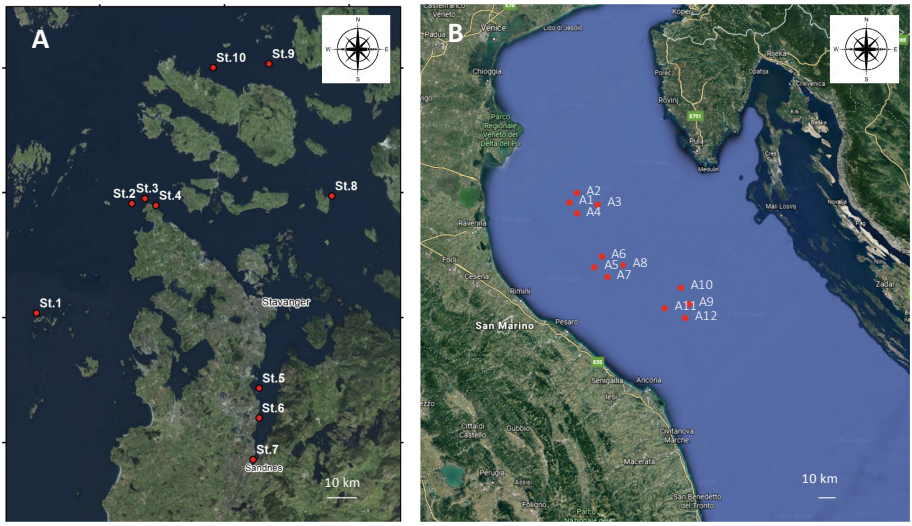

Fig. 1. Boknafjorden (A), and Northern Adriatic Sea (B), location of the sampling sites.

2.2 Field Sampling

The surveys in the addressed case studies were performed during two coordinated sampling cruises; the first operated during summer 2016 in Norway and the second in the Adriatic Sea in winter 2017. Ten sampling sites were selected to investigate any potential geographical and/or input source related distribution patterns of plastic microlitter in Bokna Fjord, while 12 sampling sites were selected in the northern and central Adriatic Sea (Fig. 1). Wet sediment (8 kg) was sampled from the top layer (0–5 cm) from each of the sites using a Van-Veen grab (surface, 0.095 m^2). Samples were placed in a 15 L bucket, roughly homogenized by a medium sized steel spade and stored at 4 °C in metal cans prior to analysis of plastic levels and composition. All devices used in the homogenization phase were of stainless steel.

2.3 Laboratory Analysis

Microplastics in collected sediments were extracted by applying a multi-step procedure based on combined enzymatic and oxidizing treatments followed by a density separation phase. The 8 kg bulk samples from each of the sampled sites were homogenized by means of a standard stainless-steel orbital food mixer (approx 20 rpm, 10 min at RT) using the K-beater knife. After homogenization, each of the sediment samples was split into three replicates of 2 kg. The remaining material was stored as back up material. The obtained replicates, three per site were then transferred to pre cleaned pyrex beakers and gently mixed by a stainless steel jar test instrument (VELP Scientifica, Milan, Italy) with 1:1 (w/v) glycine buffer, with Protease (P3111 Sigma-Aldrich, Germany), and then incubated for 24 h. The enzymatically digested samples were further oxidized to reduce the interference of organic matter by adding 30% H_2O_2 and incubated for 36 h. Plastic particles in the digested sediment samples were extracted from the matrix by a density-based separation step adding zinc chloride ($ZnCl_2$) powder to reach a final density of the mixture of 1.75–1.80 g/cm^3. The mixture was then thoroughly stirred for 30 min before being left to settle for 24 h. The supernatant containing the floating plastic particles was collected and size fractionated through a set of certified 250, 100, 40 and 10 μm mesh stainless steel sieves. Four size fractions were obtained: microplastic particles > 250 (D1), 250 μm < D2 < 100 μm; 100 μm < D3 < 40 μm and 40 μm < D4 < 10 μm. In this work the selection of size fractions was based on recent findings reporting microplastic particles smaller than 250 μm accounting for more than 75% of the total observed microplastic in similar fjords and marine environments in the North sea [21]. The fractionated material was washed by rinsing sieves with 100 ml of filtered MilliQ water to remove inorganic salts. Plastic microlitter debris >250 μm was manually picked, weighed and placed in the pyrolysis tin cups either individually or in groups of particles, dependent on volume. For all other fractions the content of each sieve was wet transferred from the sieve surface to pre-weighted and pre-burned GF/C fiberglass filters by means of a glass microanalysis vacuum filtration unit. In this work results are presented as sum of all size fractions The

obtained filters were then dried overnight (55 °C) and weighed to estimate the total amount per fraction. Procedural blanks represented by MilliQ water and all involved reagents were run within the same procedure to exclude plastic contamination in the analytical processes. Dust trap collectors were used to evaluate possible sample contamination from atmospheric fall-out. Eight of the most commonly used plastic polymers were used to set up the calibration and quantification curves: polyethylene - PE, polypropylene - PP, polystyrene - PS, polyvinyl chloride - PVC, polyamide - PA6, polymethyl methacrylate - PMMA, Polycarbonate - PC and polyethylene terephthalate – PET, of purity >99%. Pyrolysis GCMS measurements were performed by a Shimazu Optima 2010C GCMS controlled by GCMS solution V 4.45 and coupled with Frontiers lab's Multi-Shot Pyrolizer EGA/PY-3030D with auto-shot sampler. Pyrolysis was performed at 590 °C on a tin pyrolytic target cup. Calibration curves, proficiency test and routine operational conditions were performed following Gomiero et al. [9]. In order to provide a comprehensive outlook of microplastics distribution in analysed sediments results are presented in this work as total amount of each polymer per site.

3 Results

Outcomes of the Norwegian case study showed that the most abundant polymer characterized, present in all investigated sites was PE, with values ranging from 32.3 μg/kg (site 3 N) to 139.2 μg/kg of sediment DW (site 10 N) as sum of all the size fractionated sections, followed by PVC and PET which ranged from 9.0 (site 1 N) to 120.0 μg/kg of sediment DW (site 4) and from 12.0 μg/kg (St.1) to 136.5 μg/kg of sediment DW (site 2), respectively. Both PP and PA66 were observed in 80% of all the investigated sites with concentrations ranging from 10.0 μg/kg (St. 10) to 78.4 μg/kg of sediment DW (St. 6 N) for PP and 16.0–73.1 μg/kg of sediment DW of PA66, respectively. PS and PMMA were only detected in 60% and 40% of the investigated sites, respectively. PC was not found in any of the analysed samples. Furthermore, the most contaminated sampling site was St. 7 N with 495 μg/kg as sum of the total quantified plastics while the least impacted site was St.1 N (40.8 μg/Kg of sediments DW).

Similarly to the Norwegian samples, the characterization in the northern Adriatic sea case study showed that the most abundant polymer characterized, present in all investigated sites was PE, with values ranging from 84.3 μg/kg (site 10A) to 242.2 μg/kg of sediment DW (site 3A) followed by PET and PP which ranged from 46 (site 5A) to 167 μg/kg of sediment DW (site 2A) and from 29.0 μg/kg (site 12A) to 111 μg/kg of sediment DW (site 4A), respectively. PA66 was observed in 87% of all the investigated sites with concentrations ranging from non-detectable (site 8A and 11A) to 89.4 μg/kg of sediment DW (3A). PMMA and PC were only detected in 60% and 40% of the investigated sites, respectively with values ranging from 32 to 82 μg/kg and from 8 to 10 μg/kg, respectively (Figs. 2 and 3).

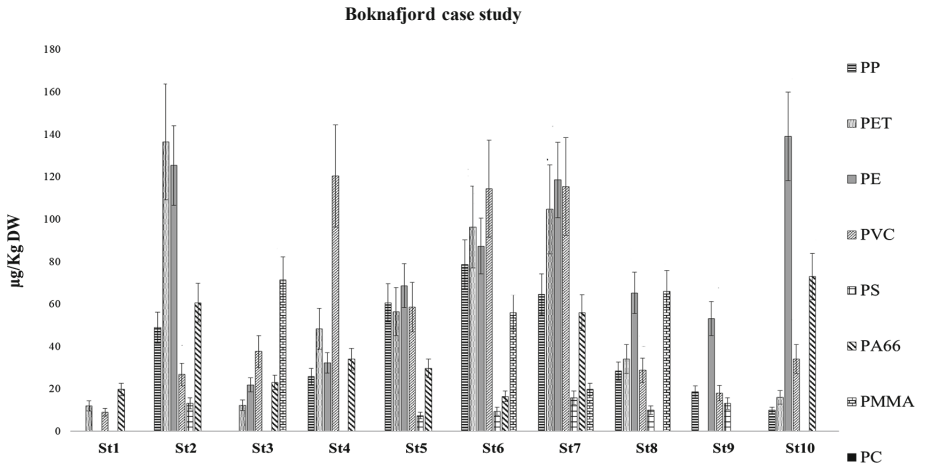

Fig. 2. Overview of the total content of Polypropylene (PP), Polyethylene Terephthalate (PET), Poly Vinyl Chloride (PVC), Polystyrene (PS), Polyamide 66 (PA66), Poly-methyl methacrylate (PMMA) and Polycarbonate (PC) in analysed sediments (µg/Kg DW) collected in the Boknafjord. Average values of three replicates ± std.

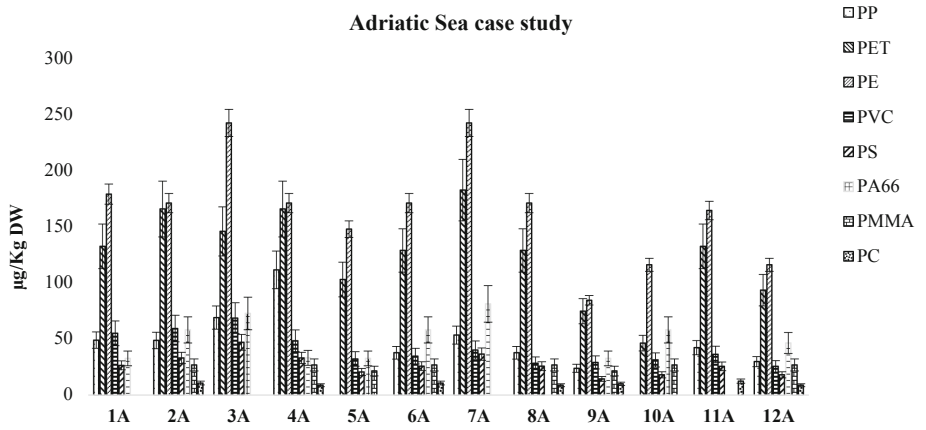

Fig. 3. Overview of the total content of Polypropylene (PP), Polyethylene Terephthalate (PET), Poly Vinyl Chloride (PVC), Polystyrene (PS), Polyamide 66 (PA66), Poly-methyl methacrylate (PMMA) and Polycarbonate (PC) in analysed sediments (µg/Kg DW) collected in the Boknafjord.

4 Discussion

During this work, a novel thermal oriented degradation method has been used to characterize the occurrence, levels and polymer composition of microplastic in marine sediments in two areas dominated by different oceanographic conditions and input

sources. The accumulation of plastic litter in marine sediments has been well documented world-wide for coastal and marine areas submitted to moderate to high anthropogenic pressure [5]. More recent papers report the occurrence of plastic in shallow and deep-sea sediments collected in remote areas far from any significant industrial, or domestic influence, pointing towards the significant role of global seawater circulation in the actual distribution of plastic litter, both in the water column and in the sediment environment [6]. The occurrence of MPs was therefore expected in the sediment samples collected in both areas. For the Norwegian case study, the extent of microplastic contamination in this area has not previously been investigated. Boknafjord is an urban fjord system close to Stavanger, the fourth most inhabited city in Norway. Areas with different oceanographic conditions and with moderate to high expected levels of plastic contamination were selected for preliminary characterization of the fjord area. Almost all the investigated polymers were characterized in the digestates of the processed sediments, with PVC followed by PE, PET and PP being the most accumulated compounds. These four compounds also represent the polymers with highest production, accounting for more than 70% of the plastic demand in Europe [7]. Each of the addressed sites showed a distinctive polymer composition in terms of relative and total abundance as well as a polymer related particle size distribution. The observed relative and total abundance of polymers in the investigated sites reflects the composition of microplastic litter emissions typical of coastal urban areas. A similar trend is reported by Vianello et al. [8] analysing the occurrence of MPS in the Venice lagoon. Higher levels were detected within the Adriatic Sea case study. With some of the most significant amounts of solid waste generated annually per person (208–760 kg/year), the Mediterranean Sea is one of the areas of the world that is most affected by litter [9]. Litter enters the seas from land-based sources, ships and other infrastructure at sea and can travel long distances before being deposited on the seabed or along the coasts resulting in an observed mean density of floating microplastics in the Mediterranean Sea of more than 100,000 items/km^2. In this context, the Adriatic Sea represents a hot spot for plastic litter both because of peculiarities in its oceanographic conditions as well as the high degree of anthropogenic pressure related to tourism, artisanal and industrial activities coexisting in a narrow area. Few studies have addressed the occurrence of floating plastic debris in the surface water of the Adriatic Sea. Suaria *et al.* [10] reported by a larger study addressing the Mediterranean Sea and partially the Adriatic sector a clear prevalence of smaller particles. Quantitative estimations collected by a 400 μm net mesh pointed out values ranging from 0.4 ± 0.7 to 1.0 ± 1.8 items/m^3.

On the other hand, the observations through six years survey performed by Strafella *et al.* [11] about macro and meso litter on the Adriatic sector seafloor point out that 43% the total litter collected was identified as plastic litter resulting on 44.34 kg/km^2.

Lost fishing nets and mussel culture debris accounted for 50% of the overall plastic litter collected over the investigated period while the remaining plastic comprised a wide range of objects such as garbage bags, shopping bags, cups, bottles, food packaging, dishes, other kitchen items and industrial packaging. Results of this study indicated that the largest amount of mussel culture debris was found close to the coast and its distribution was constant over the years. It is already well known that plastic is extremely dangerous for marine life and human health, being a source of toxic

chemicals such as PBCs or dioxins [12, 13], furthermore plastics on the sea bottom, being affected by different chemical and physical conditions, may degrade becoming microplastic which is considered a multiple stressor in aquatic habitats and may enter in the human food through their ingestion by fish or other sea food and, in addition, it may result in the risk of chemicals bioaccumulation and biomagnification [4, 14–20].

5 Conclusions

Increased knowledge on microplastic occurrence within marine sediments requires accurate measurement and distribution analysis of plastics in natural systems. The capability to carry out this work is still limited at present. In the research presented here a method to characterize and quantify microplastics from sediments is applied to investigate the occurrence and the polymeric composition of microplastics extracted from sediments collected in two coastal areas located in the North Sea and in the Adriatic Sea dominated by multiple and diversified input sources. Taking into account the relatively scarce information available on marine litter and that marine litter is one of the descriptors of the Marine Strategy Framework Directive (MSFD), the present study reports the data on marine micro litter collected at two different expected hot spots of plastic pollution. The aim of the present work was to provide information on the composition, weight and spatial distribution of benthic anthropogenic debris occurring in these areas, as well as to address the gap in knowledge and to serve as a baseline for future comparisons.

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Microplastics Extraction and Counting from Wastewater and Sludge Through Elutriation and Hydrocyclone

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1 Introduction

Microplastics are defined as plastics whose size is smaller than 5 mm [1, 2]. According to Correira (2018), around 35% of the microplastics present in the ocean, correspond to fibres from synthetic textiles [3] detached from clothes in washing machines [4]. Moreover, other products such as contact lens cleaners [5] or personal care products are found as well [6].

The critical issue of microplastics is that they are directly released from the wastewater treatment plants (WWTP) to the oceans and rivers [7] due to their resistance to biodegradability. Hence, they cannot be removed totally from this WWTP. The retention of these solids on WWTP is more than the 88%, showing a final concentration on the effluent between 3–12% of the initial concentration [8]. Microplastics poured into these channels are consumed by marine species, changing the food chain and affecting human health due to its toxicity and carcinogenic properties [9, 10]. Therefore, the development of technologies that reduce the contamination by microplastics is nowadays a need.

Fenton (1894) reported the Fenton reaction for the first time [11]. The oxidant power of H_2O_2 is used, in conjunction with an iron catalyst, to degrade the organic matter in acidic conditions (pH 2.8 as an optimum condition). Fenton process is considered the best option for organic matter degradation [12, 13]. The whole process is described for Ameta *et al*. [14].

Currently, some studies are focusing on the microplastics separation through elutriation. Elutriation uses particle density as separation criterion, separating dense particles from the light ones using an upward stream of tap water [15–17]. Kedzierski et al. described an extended study of this process. The key parameters determination for designing an elutriation column is explained extensively [18].

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1.1 Elutriation

The elutriation process consists in the separation of light and heavy particles using a gas or liquid stream circulating upwards. Particles separation is done according to density differences, which is related to terminal falling velocity [15, 19].

This process is carried out in an elutriation column. The working mechanism consists on the injection of tap water from the bottom. Thus, microfibers are pulled and extracted at the top of the column; meanwhile, the heavier portion is settled on the bottom. It should be considered operating in isothermal conditions to avoid particle hydrodynamic variations related to viscosity or density [19].

The elutriation column modelling was done according to Kedzierski et al. [20].

1.2 Hydrocyclone

Hydrocyclones are devices used in solid-liquid phase separations. They consist of a conic end linked to a cylindrical body. The initial suspension is feed tangential to the cylinder. Dense particles will enter the system describing a rotational movement descending to the underflow drainage. Light particles will be pushed to the upper tube. As a result, two suspensions are obtained: the higher velocity part recollected from the bottom (heavier particles) and the slower velocity recollected from the top (lighter particles) [21].

1.3 Microfibers Counting

Nowadays, polymer characterization is commonly done through Raman spectroscopy, vibrational spectroscopy technique. It is based on the molecular vibrations caused by the inelastic scattering of light. A vibrational spectrum is given as a response, allowing the identification of the present compounds (fingerprint). This technique is non-intrusive, non-destructive and requires a low amount of sample [22].

2 Experimental

2.1 Synthetic Water Samples

As it was aforementioned, a high amount of microfibers is daily produced washing clothes. Synthetic water was produced adding 10 g of synthetic fibres to 10 L distilled water and stirred to homogenize the sample.

2.2 Real Water Samples

Three different glasses of water arising from WWTP were collected as well. Samples of 5 L were collected at the purification plant inlet after grinding and outlet. Moreover, water coming from the anaerobic digestion was collected too.

WWTP water contains a high amount of organic matter (OM) and sludge that can hinder macro and microplastics separation because of dragging. For this reason, it is

necessary a pre-treatment in order to remove them before the operation. Sieving pre-filtration and Fenton process were done.

2.2.1 Samples Pre-treatment

2.1.1.1 Sieving

Samples were pre-filtered with a sieving plate of 800–1000 μm of diameter in order to remove the organic matter with higher size.

2.1.1.2 Fenton

The organic matter present in the samples was degraded using the Fenton process. The development of this process included the reagents iron sulphate (II) heptahydrate (catalyst) and hydrogen peroxide (oxidant). Moreover, oxidant and catalyst concentration were optimized testing different quantities and analysing the removal efficiency. The temperature must be maintained under 40 °C. Temperatures higher than 70 °C produce the degradation of microplastics. It has to be taken into account that there is no microplastic degradation on this process.

Preliminary experiments were carried out using 100 mL of sample. Then, they were stirred on an orbital stirrer for 2 h.

2.3 Microplastics Separation

The microplastic separation was done using an elutriation column followed by a hydro cyclone and to remove inorganic matter and heavy particles from the lighter microplastic fraction Samples were filtered using a sieve of 200 μm after elutriation to remove particles with higher sizes (if any). In Fig. 1, it is shown the flow diagram of the microfibers separation process. The pre-treated sample is fed into the elutriation column on the top. The volume used was 5 L at 20 L h^{-1} and filled with tap water at a constant ascendant flux of 50 L h^{-1} from the intermediate tank. The elutriated fraction is collected at the top of the column (light fraction). Microfibers separation is carried out through centrifugal force. The aforementioned light fraction is pumped to the 15 L hydrocyclone. The separation is done in the hydrocyclone where the micro and mesofibres are separated. The lighter part is recirculated into an intermediate tank whereas the heavy fraction is settled on the bottom.

2.4 Microplastics Detection

The microplastics detection was carried out by optical and FTIR- microscope with Raman. The intermediate tank content was filtered using a glass fibre filter of 0,47 μm and dried before analysing.

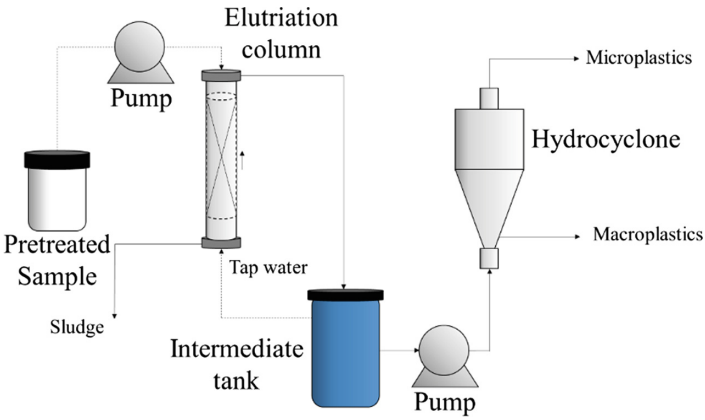

Fig. 1. Flow diagram of the whole process.

3 Results and Discussion

3.1 Fenton Pre-treatment

It was determined the good efficiency of the Fenton process in the elimination of OM and the isolation of plastic fibres. Different concentrations were tested to enhance the operability in terms of OM removal and iron sludge reduction. Optimal conditions were obtained at 2000 mg L^{-1} $FeSO_4 \cdot 7H_2O_2$ and 4000 mg L^{-1} H_2O_2 (see Fig. 2). The presence of microfibers was increased when the Fenton process was used as pre-treatment because OM sludge concentration was reduced so that less microfiber were dragged.

Fig. 2. Fenton process with different H_2O_2 concentration, from left to right: 0; 300; 4000; 7500; 15000 and 30000 mg L^{-1}

3.2 Separation Process

Visual analysis through an optical microscope (Zeiss, Axioplan 2, Spain) showed the microfiber content reduction along the time using synthetic water. However, the separation was more remarkable in the first minutes of experience. In Fig. 3, it is shown this tendency. The size of microplastics separated is between 600 and 116 μm.

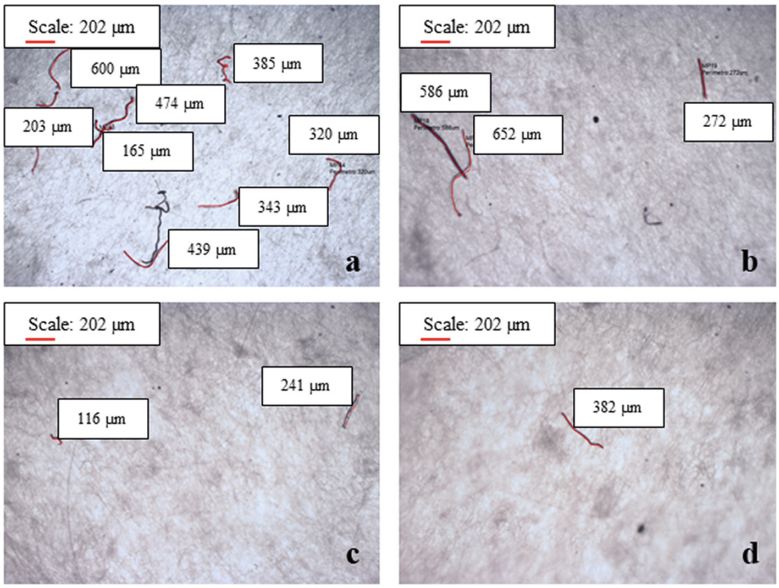

Fig. 3. Synthetic water samples at (a) 5 min; (b) 20 min; (c) 40 min; (d) 60 min

In real wastewater samples (see Fig. 4), in the visual analysis, there was a significant difference in the fibre content along the time. It was observed a higher presence of single fibres at the first minutes. As real wastewaters fibres concentration was lower than in the synthetic water, there was not fibre agglomerations as in the previous case.

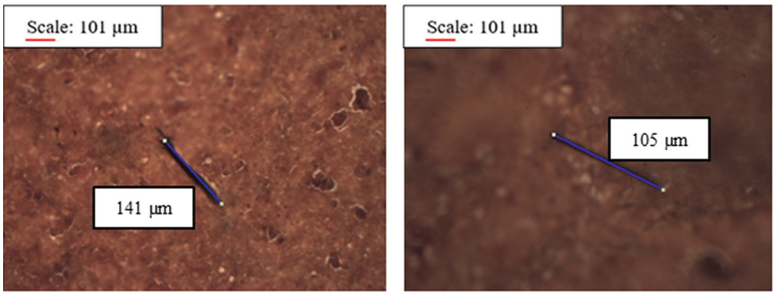

Fig. 4. Real water samples after pre-treatment and elutriation & hydrocyclone process

In real water, the presence of some microplastics was observed as microspheres, using the Kyowa stereoscope, these sphere are presumable from personal care product as exfoliants or toothpaste (see Fig. 5).

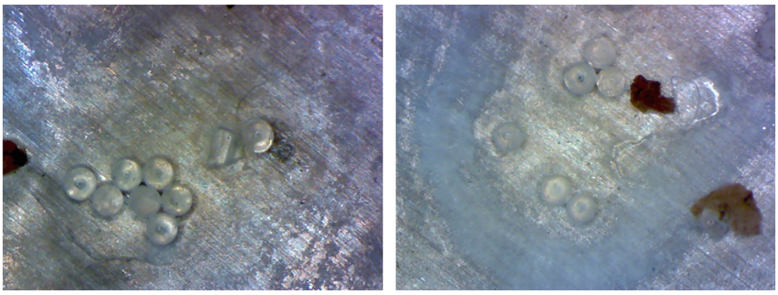

Fig. 5. Microspheres observed in real wastewater

4 Conclusions

It has been demonstrated that the optimal conditions to isolate microplastics from real wastewater were obtained at 2000 mg L^{-1} $FeSO_4 \cdot 7H_2O_2$ and 4000 mg L^{-1} H_2O_2.

The elutriation and hydrocyclone microfibres separation process shows to be a good option to remove OM from wastewater and to isolate microplastics. Qualitatively, it proves it is an inspiring treatment for future studies reaching the objective of microplastics separation. Moreover, the development of a quantitative method to recount microfibres remains.

As a novel method of microplastics extraction and purification, the optimal conditions of the whole process remains uncertain.

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Microfiber Pollution from Source to Mitigation

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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 [1]. The mechanical and chemical stresses produced on the fabrics during a wash, cause the release of microfibres 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 [2]. In the last years, an analytical protocol was developed and proved to be an 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

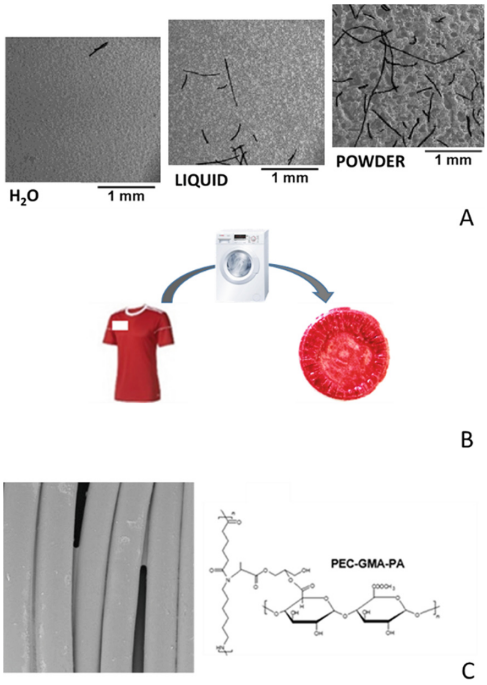

Fig. 1. Microplastic release from textiles: (a) effect of detergents on the release; (b) microfibres released in a real washing process; (c) pectin finishing treatment to mitigate the release.

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washing conditions [3]. 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 microfibres. This method allows to determine the contribution of the washing process of synthetic clothes to microplastic pollution [4, 5] (Fig. 1).

With the aim to mitigate the environmental problem caused by the microfiber pollution several mitigation strategies to reduce the release of microplastics 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 microfibres shed during a washing process and thus mitigating the environmental impact of microplastics. One of the treatment was 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 [6–8].

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Textile Fibres in Mediterranean Surface Waters: Abundance and Composition

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1 Introduction

Textile microfibres are emerging pollutants with widespread distribution in natural environments [1, 2]. They are mostly discharged into wastewater from domestic washings [3] and arrive into the environment through wastewater effluents, aerial dry or wet deposition, or through the application of contaminated sludge on agricultural soils [4]. Microfibres are commonly included by microplastic pollution studies, often accounting for 80–90% of particle counts [5], even though their synthetic nature is seldom demonstrated. Substantial concentrations have been detected in marine and freshwater ecosystems around the world [2, 6, 7]. Ingestion of fibres by marine organisms is being increasingly reported by studies worldwide [8] and adverse health effects have been demonstrated in terrestrial, marine and freshwater invertebrates [9]. In addition, a wide variety of chemicals are used during textile production including dyes, additives and flame retardants, with this raising concerns about their role as vectors of hazardous substances into the environment.

Understanding environmental concentrations of these fibres is integral to assess their environmental impact [5]. Therefore, we present here the results of a microfiber pollution survey performed in central-western Mediterranean Sea, with the main goal of providing baseline concentrations of this emerging contaminant in Mediterranean surface waters as well as an initial description of textile fibres abundance and composition in the marine environment.

2 Materials and Methods

A total of 108 seawater samples were collected at 36 different sampling stations during two research cruises (Ichnussa2017 and INFRA-Oce17) carried out in the central-western Mediterranean Sea between October and November 2017 on board the Italian research vessel “Minerva Uno”. At each station three-replicates bulk-water samples were collected using a 10-liter stainless steel bucket, triple rinsed in seawater prior to use. The bucket was lowered over the side of the ship until it reached the water. Once full, it was hauled onboard and the water poured into 10-liters pre-washed containers for subsequent filtration. All samples were collected outside of the bow wave, while the

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ship was slowly moving forward approaching a sampling station, to limit the risk of contamination from the ship itself (e.g. from wastewater outlets).

In the lab, all water samples were gravity-filtered through 20 μm mesh filters (Ø 55 mm), triple rinsed with MilliQ water prior to use. All mesh filters, lab-ware and sampling equipment were triple rinsed with filtered or underway water prior to use and samples were kept covered as much as possible during sampling and processing. Upon filtration, all samples were labeled, placed in petri dishes and stored at −5 °C in the freezer. In the laboratory, all samples were examined at the stereomicroscope and all fibres were counted by the same individual using standardized criteria [1]. Raw fiber concentrations were then computed for all samples and expressed as fibres·l^{-1} (Fig. 1).

The levels of external contamination were measured by performing two different kind of blanks during sampling operations. Aerial controls (n = 33) were made by exposing clean filters to open air both outdoor, on the ship's main deck during sampling, as well as indoor while processing the samples. Procedural blanks instead (n = 20), were made by filtering 10 L of ultrapure Milli-Q water, using the same equipment used for sampling. Aerial control samples accumulated 3.3 ± 4.5 fibres·h^{-1} (median: 0.75 fiber·h^{-1}), suggesting low airborne contamination levels during sampling (i.e. ~0.1 fibres per sample, given that processing took 5–10 min per sample and that samples were always kept covered during filtering and sorting procedures). Procedural blanks indicated a greater contamination risk (1.23 ± 1.11 fibres·l^{-1}; median: 0.85), but still significantly lower than environmental concentrations (Mann-Whitney $p < 0.0005$). Consequently, to compensate for external contamination, all samples were reduced by 1.0 fibres·l^{-1} and all negative values set to 0.

A representative subset of 336 fibres were randomly extracted for polymer identification from one of the three replicates collected at each station (i.e. ~10 fibres per sample). Individual fibres were hand-picked from the filters using ultra-fine laboratory tweezers and placed on moistened glass slides to favor adhesion on the horizontal plane. Slides were kept covered to prevent airborne contamination and left to dry in a laboratory oven for 5 h at 35 °C. All analyses were performed in the ISMAR laboratories using a LUMOS standalone FT-IR microscope (Bruker Optik GmbH), equipped with a motorized XY sample stage and operated in ATR mode (Ge crystal). Prior to each scan, fiber length and diameter were measured to the nearest 1 μm from the digital images collected by the instrument. Following background scans, ATR spectra were obtained by averaging 64 scans per item with a spectral resolution of 4 cm^{-1} (range 4000–650 cm^{-1}). After acquisition, infrared spectra were processed and analyzed using OPUS 7.5 software. CO_2 interference (adsorption at 2300–2400 cm^{-1}) was removed for clarity and polymer identification was performed by comparison with a combination of commercially available libraries and an additional custom library compiled within the framework of the JPI-OCEANS project BASEMAN [10]. To further increase identification accuracy, FTIR spectra of common fabrics, clothing and textiles were obtained and added to the spectral database according to their label information. Sample spectra were compared to the augmented database and only matches >75–80% with reference spectra were accepted as verified polymers. Fibres were then classified as synthetic (polyester, acrylic, polyamides, aramids and

polypropylene), animal (wool, silk) or cellulosic fibres consisting of both natural (cotton, linen and other plant-based fibres such as jute, kenaf, hemp, flax, sisal) and man-made cellulosics (e.g. rayon/viscose and acetate).

3 Results

Textile fibres were found in all 108 samples collected totaling 5466 fibres (mean 151.8 ± 68.9 fibres per sample). After accounting for contamination levels, an overall mean concentration of 4.1 ± 3.2 fibres·l^{-1} was found across the study area (Median: 3.2 fibres·l^{-1}). Mean fibres concentrations showed a relatively high spatial variability, but no clear trend in relation to distance with land (Fig. 1). The maximum concentration (21.5 fibres·l^{-1}) was observed in a sample collected in the Sardinian Channel,

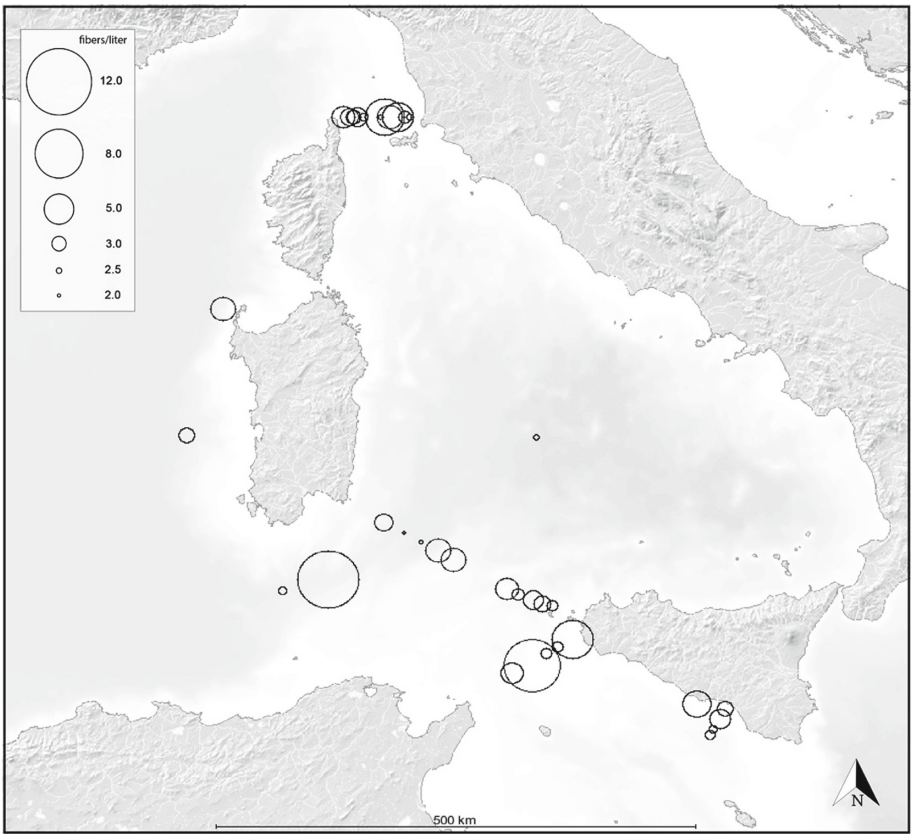

Fig. 1. Map of the study area showing the location of all sampling stations and the mean fiber concentrations (expressed in fibres·l^{-1}), obtained by averaging the values measured in the three replicates collected at each station (n = 36). The size of the circles is proportional to the concentration values on a logarithmic scale.

while the lowest concentration (0.9 fibres·l^{-1}) was found in a sample collected in the Western Tyrrhenian Sea.

Half of the collected fibres (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 (Median 1.33 mm; Range: 0.14–14.62 mm, IQR: 0.76–2.03) while mean fiber diameter was 22.6 ± 18.4 μm (Median 18.6 μm, Range: 5–200 μm, IQR: 15.0–22.9 μm).

FTIR analysis (n = 336) revealed that most fibres were made of non-synthetic materials, with the majority being made of cotton (47.3%), wool (5.4%) or other cellulosic materials (39.6%). Overall, 92.3% of all analyzed fibres were natural fibres of animal or plant origin. Only 6.9% of the characterized fibres were actually synthetic, with polyester being the most abundant polymer (4.2%), followed by nylon (0.9%), polypropylene (0.9%), aramid (0.6%) and acrylic (0.3%).

4 Discussion and Conclusions

With the exception of polypropylene, all natural and synthetic polymers found in our study have densities greater than seawater and should sink. The widespread occurrence of textile fibres in marine surface waters could thus be explained by a constant atmospheric deposition to the ocean surface [11], coupled with retention mechanisms within the sea-surface microlayer and complex turbulence and re-suspension processes [6] about which little is known.

Most fibres floating at the sea surface are not plastic, but dyed cellulose. This is in agreement with recent studies showing that cellulosic fibres account for more than 60–80% of all fibres in seafloor sediments [7], marine organisms [8], freshwater [12] and airborne fiber populations [11]. Given the dominance of synthetic fibres in current global textile production (62%, compared to 7% in our samples), the high proportion of natural fibres in the marine environment is surprising. Cellulosic and animal fibres accounted for 87% and 5% of our samples, despite comprising only 36% and 2% of global production respectively [13]. This contrasts with the pattern of macro and micro-plastic litter, where polyethylene and polypropylene are the two most common polymers both at sea [14] and produced by the plastic industry. Several factors might explain this discrepancy. Natural fabrics release more fibres than polyester during laundering [3]. Also, the historical dominance of plant and animal fiber use in textiles [13], coupled with lower-than expected degradation rates, might help to explain the current patterns. As a matter of fact, despite being considered biodegradable [15] little is known about the actual lifespan of wool and cellulosic fibres in marine environments [16]. In addition, natural yarns are often processed, finished, dyed and coated with a wide range of chemicals including resins, softeners and flame retardants, which may considerably slow their degradation. Hence all these factors together might help to explain the widespread occurrence of natural and cellulosic fibers in the marine environment.

Our results demonstrate the ubiquitous presence of textile fibres in Mediterranean surface waters. Research on the prevalence, fate and impacts of textile fibers however is

relatively young and often unbalanced in favor of plastic polymers. More information is needed on the degradation of natural fibers relative to synthetic polymers as well as a better understanding of the ecological impacts and biodegradation rates of these fibers in a range of environmental conditions.

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When Size Matters – Textile Microfibers into the Environment

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1 Introduction

Microplastics (MP from now on) are synthetic polymers (<5 mm) that have been widely found across the environment, converting these particles in an emerging and fast-growing concern. Any plastic product can contribute to the microplastic pollution, meaning that the sources are diffuse and intrinsically diverse. Within these sources, *textile microfibers* (MFs from now on) have been predominantly identified in water [1, 2], atmospheric [3, 4] and soil environments [5, 6], and also in products for human consumption [7, 8]. For this reason, MFs are considered as one of the most important *primary MP sources*, i.e., emitted to the environment in a MP size [9].

In order to evaluate MFs emissions, some estimations of their mass flow to the environment have been conducted. To achieve that, calculation approaches based on the "fibre *linear weight*" have been done. The fibre linear weight is a textile property that relates the mass and the length of the fibres. It is generally expressed in *decitex* (dtex), which corresponds to the mass in grams per 10,000 m of fibre. As far as we know, published MFs estimations have used 300 dtex in their calculations as the linear weight of the MFs. In this way, it has been estimated a global MFs' flow to the oceans between 0.2 to 0.5 E+06 tons per year [10, 11]. But, 300 dtex corresponds to an average value for *yarns instead of MFs*. In fact, *MFs must be considered as individual filaments*, which when grouped they form yarns. Therefore, their appropriate linear weight is between 1 to 5 dtex [12, 13]. In our previous work, [12] the global MF mass flow to the oceans was re-estimated by applying an appropriate linear weight (1 to 5 dtex instead of 300 dtex).

In this work, a new model for the estimation MF emissions is developed by considering the following terms: (1) MFs detachment rates per textile garment and washing cycle; (2) worldwide trends of household washing machines per world regions (type of washers and volumes of laundry water effluents); (3) municipal water treated per world regions (percentage and technologies applied); and (4) rate of synthetic materials (as polyester and acrylic) used in the manufacturing of textile garments. On

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the bases of these considerations and using the appropriate dtex value for MFs, an approximated MF flow to aquatic environments of 1.2E+06 tons per year was obtained.

2 Estimations Previously Reported

In the last years, some global estimations of the MFs' flow to the oceans have been published [10, 11]. In the most recent publication (2017), three scenarios (minimum, central and maximum) were estimated based on information obtained from previous investigations whose main objective were not to measure the textile MFs' detachment [9, 14–16]. Also, in other publications, an inappropriate linear weight for MFs has been applied [14, 15, 17]. Moreover, in our opinion, some data can be also improved by using updated published literature.

The parameters considered by Boucher & Friot can be encompassed in the Eq. (1):

$$mF_{MF} = WCP \cdot P \cdot L \cdot MF \cdot x \tag{1}$$

From this equation, a set of observations were done, which are summarized in Table 1.

Table 1. Observations onto Boucher abd Friot (2017) estimation methodology

Term	Explanation	Observation
$\boldsymbol{mF_{MF}}$	Annual mass flux of MFs, in ton MF/year	–
WCP	Average annual number of laundry cycles per capita	It does not consider the intensity of the washing procedure
P	Population	It is not directly correlated with the washing machine ownerships
L	Load per washing cycle (4 kg considered)	–
MF	MFs detached per kg of garment (mg MF/kg garment)	A linear weight of 300 dtex was used to calculate the mass of detached MFs, instead, 1 to 5 dtex should be applied. Conservative values of MFs' detachment were considered
x	Unexplained factor	Probably referring to the rate of the population owning a washing machine

If the estimation of the parameter "*MF*" is substituted in the Eq. 1 by its appropriate linear weight (3.4 dtex instead of 300 dtex), the central value for the mass flow estimation is reduced from **5E+05 tons/year** to **18E+03 tons/year**, as discussed in our previous work [12].

However, less MFs' particles are estimated by applying Boucher and Friot's method, as a length of **5 mm** was assumed in that work. Subsequent investigations

have made measurements and reported a length of **about 0.3 mm** per MF. Therefore, applying that value, the MFs' particles directly released to aquatic environments gets increased from **3.6E+15** to **1.4E+17** per year (see Fig. 1).

3 New Estimations

Our new estimation approach intends to overcome the gaps of the former proposals. Hence, in this work, it was developed an improved model including the considerations and the observations described in Table 1. In addition, other features have been introduced:

- The intensity of the washing cycle was assumed to be reflected in the volume of water consumed [18, 19].
- Municipal used-water, percentage treated in a regional and worldwide basis and retention efficiencies for the technologies applied [20–22].
- Type of washing machine used, which is an important predictor in the MFs detachment.

According to this model, the new estimation of MFs reaching aquatic environments is **1.2E+06 tons per year**, which corresponds to a MF particle flow of **9.3E+18** with an average length of 0.34 mm. In Fig. 1, it can be seen the three mentioned situations: Boucher and Friot estimations (2017), estimations of the authors (2019) obtained by applying the right values of decitex and length, [12] and current results provided by the new methodology (for the year 2020).

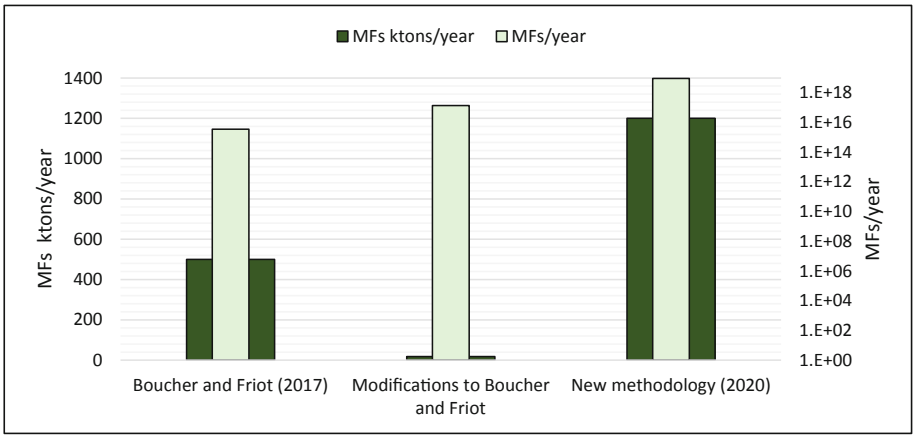

Fig. 1. Estimations MFs heading towards aquatic environments: mass (dark green, left axis) and number (light green, right axis, in a logarithmic scale)

As seen in Fig. 1, the estimated mass flow and quantity of MFs emitted to aquatic environments is significantly increased when applying the new methodology proposed.

One of the main features that promote this increment is the type of washing machine used. In fact, it has been reported that top-loading washers detach 7 times more than front ones [23]. In this way, Asian countries have a high rate of top- versus front-loading washers [18]. Hence, that region has a considerable contribution to the worldwide MF pollution.

4 Conclusions

In this work, a new estimation of the microfibers detachment and subsequently emitted to the environment is proposed. This model aims to improve our previous estimations that reported a flow of MFs to the aquatic environment of 0.5E+06 tons of MFs per year. The current estimations were obtained with an improved model which includes the following parameters: (1) intensity of the washing cycle, (2) type of washing machine, (3) type and percentage of municipal treated water. In this way, the annual mass flow emitted to aquatic environments was estimated in **1.2E+06 tons of MFs** for the year 2020. In terms of quantity, it corresponds to an annual flow of **9.3E+18 particles of MFs**.

In addition, it is important to highlight that a realistic size of the MFs should be considered when assessing its mass flow to the environment. In this way, bigger MFs, like the ones considered in the previously published estimations (5 mm), would mean a higher mass flow. However, despite the fact that measured MFs have been proved to be smaller (0.3 mm), these light-weighted particles' impacts might be worst, as they are easier to ingest for a wider range of organisms.

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Derelict Fishing Gear – Removing a Source of Microplastics from the Marine Environment

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1 Introduction

Lost fishing gear is omnipresent in the marine environment. The Mediterranean acts as a hotspot for 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 [1, 2]. In the framework of the MARELITT Baltic project, WWF Germany together with partner institutions 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 contribution, we 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. With a contribution of 30–50% of macroplastics by number being characteristically found in marine litter samples [3, 4], abandoned, lost or otherwise discarded fishing gear (ALDFG [5]) 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.

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2 The MARELITT Baltic Project

MARELITT Baltic was an EU-funded INTERREG Baltic Sea Region project carried out from 2016–2019 with partners in four countries around the Baltic Sea coast. The lead partner was the Fisheries Municipality of Simrishamn in Southern Sweden. Co-founders were WWF Poland and WWF Germany as well as the non-governmental organisations (NGOs) Keep the Estonian Seas Tidy (KEST) and Keep Sweden Tidy (KST). Additional project partners included academic institutions in Poland such as the Maritime Academy in Szczecin. A full list of project partners and associated organisations can be found on the MARELITT Baltic website https://marelittbaltic.eu. The major aim of MARELITT Baltic was to identify best-practice mitigation measures against the negative impacts of lost fishing gear on the Baltic Sea ecosystem. To this aim, three pillars were investigated, the results of which will be briefly summarised in the following sections:

1. **Search & retrieval of ALDFG**
   Development of a best-practice strategy to identify lost fishing gear on the seafloor and retrieve ALDFG in an ecologically sound way that minimises both the impact of the fishing gear as well as of the retrieval action on the marine environment.
2. **Harbour reception & waste management**
   Fishing harbours around the baltic sea were investigated for reception infrastructure and waste management options for retrieved fishing gear. A suite of processing

Fig. 1. Gillnets retrieved from the German Baltic Sea (© Andrea Stolte), showing contamination with mixed marine litter and marker buoys. Insert: Degradation of twisted net fibres into microplastics (© Wolf Wichmann).

trials was carried out to evaluate the recyclability of ALDFG and offer waste management recommendations in the form of a DFG treatment scheme.

3. **Prevention**
   Gear marking and other loss prevention and monitoring mechanisms were tested to phrase recommendations for the reduction of further losses or the facilitation of retrievals during regular fishing activities.

3 Search and Retrieval of Lost Fishing Gear (ALDFG)

In total, approximately 19 tonnes of ALDFG were retrieved with creeper, hook and diver searches in the four partner countries during MARELITT Baltic [6]. The most difficult part of ALDFG retrieval is the location of gear lost at sea. Both trawl and gillnet filaments are made from nylon (polyamide 6) or PET with a higher density than water and gillnets have attached sink lines, which cause lost gear to end up on the seafloor. Although reporting of gear loss that cannot be recovered by the fishing vessel is obligatory according to the European Fisheries Policy (Article 48 [7]), reporting does not take place regularly in the MARELITT Baltic partner countries because coverage of retrieval cost is not regulated. This leads to the situation that GPS positions of lost gear are in most cases unknown. A positive example of a functioning reporting system is Norway, where regular retrieval campaigns by the fisheries directorate and return of lost gear to the owner incentivise the reporting of loss positions. In MARELITT Baltic, different methodologies for the localisation of ALDFG were tested: (i) the search with creepers/rock hoppers, (ii) the search with small hooks or anchors, and (iii) the search with divers on wrecks where ALDFG had been reported in the past.

Results of search methodology testing: "Blind searches" with hook or creeper are not very efficient in the Baltic Sea – the 1 m width of a creeper limits the area that can be covered; the search in trawl areas did not yield lost nets even on reported underwater hook positions. The failure of blind searches in areas with active trawl fisheries can be explained by the fact that ALDFG lost earlier is recollected during regular trawling activity. For coastal gillnet fisheries, dislocation by currents is the most likely cause that very limited amounts of ALDFG were found with creepers or hooks in gillnet-intense areas.

Results of retrieval campaigns: Lifting ALDFG can be carried out with a minimum 2 tonne winch of a working vessel or a fish cutter, once netting and ropes are cut loose from obstacles and hooked by divers. On ship wrecks, professional retrieval divers are needed because of the health hazard to cut ALDFG loose, which can be lifted by lifting bags.

Recommendations: A hot spot map was developed during MARELITT Baltic where fishing intensity, seabed morphology (rocks, soft sediments), ecologically sensitive

areas and possible sea user conflict zones are displayed. This map helped to identify high-risk areas for concentrated search operations. The full report on all search & retrieval results and an environmental impact assessment for search & retrieval campaigns are available on the MARELITT Baltic website. Local knowledge by fishers and especially sport and professional divers is key to localisation of ALDFG and most of the 6 tonnes of ALDFG retrieved in Germany were based on hints by local divers.

Beyond MARELITT Baltic, WWF Germany started search operations with a dedicated 600/1200 kHz side-scan sonar. With support of the sonar expert Crayton Fenn from the Northwest Straits Foundation operating in Puget Sound, USA, who had recovered 6000 gillnets and hundreds of thousands of pots in 30 years of retrievals, the technique for sonar scanning was optimised for the shallow Southern Baltic Sea. The key is spatial resolution: a resolution of a few cm can be achieved by driving the sonar 5 m above the seafloor, with constant adjustment of the driving depth. On the scanned images, float and sink lines, floats, ropes, cables and other fishing gear attachments are resolved. As opposed to point-like searches by divers, an area of a square nautical mile (342 ha) can be scanned with a small vessel in 2–3 h. This methodology was found to be the most efficient search methodology.

4 Harbour Reception and Waste Management of ALDFG

4.1 Harbour Reception

Fifty fishing harbours were investigated for the MARELITT Baltic harbour survey (Press 2017 [8]). While 28% of fishing harbours provided regular collections for the disposal of end-of-life fishing gear, none of the fishing harbours in all four partner countries had reception facilities for ALDFG retrieved from the sea. This is particularly surprising and concerning as the European Maritime and Fisheries Fund (EMFF) provides financing for the fisheries sector for cleanup activities. Because gillnets are the most frequent type of ALDFG found in the Southern Baltic, sink lines composed of lead weights are potentially hazardous waste and shall not be mixed with commercial or household fisheries waste. Removal of sink lines from entangled trawls and gillnets implies that after retrieval, intense manual labour is required to prepare ALDFG for waste management. In all four MARELITT Baltic partner countries, this was found to be an obstacle to retrievals and proper waste management of recovered fishing gear.

Fig. 2. Left: Mix of gillnets and trawls retrieved from the German Baltic Sea with floats, swim lines and sink lines. Middle: Manual dismantling of retrieved fishing gear including metal fragments, rocks, organic matter and other marine litter. Right: Extracted metal parts from manual sorting. (© Andrea Stolte)

4.2 Waste Management of Fishing Gear Retrieved From the Sea

4.2.1 Material Recycling – A Challenge for ALDFG

Fishing gear retrieved from the sea is entangled and in most cases contains other forms of marine litter such as anchors, cables, ropes, textiles, and organic substances from dead fish, seabirds, algae, etc (Fig. 1). During MARELITT Baltic, processing trials were carried out to evaluate whether ALDFG can enter the normal waste stream or even be recycled. It turned out that pre-processing and screening, as illustrated in Fig. 2, are key before ALDFG can enter common waste streams. Several hazards were encountered and led to the following results of ALDFG processing tests [9]:

- Large metal items need to be manually and laboriously removed to avoid damage to shredding and cutting machinery (Fig. 2).
- Lead lines need to be removed to avoid contamination with hazardous heavy metals (Fig. 3), e.g. the legal threshold for lead contamination in mixed waste to be classified as non-hazardous waste is 0.25% by weight or 2.5 g/kg [10] which leads to an acceptance limit in German incineration plants of less than 0.33% by weight or 3.3 g/kg [11]. Lead lines can contribute as much as 3–30% by weight of retrieved gillnet-dominated ALDFG, which is a factor of 10–100 higher than the legal limit for non-hazardous wastes.
- Preparing materials for material recycling implies that different polymer fractions, such as floats/float lines, PET sheathing around sink lines, the nylon of the net body, need to be separated. In mixed, entangled ALDFG, this is barely possible and requires unacceptably large manual effort. As a result, material recycling of mixed ALDFG has been found unrealistic and not economically viable. In the case that individual, clean, single-material trawl fragments are recovered, material recycling remains an option, as in the case of end-of-life fishing gear.
- Fine-grained sediments and residual lead fragments are extremely difficult to remove from shredded rope & net fibres during the industrial washing process (Fig. 3, right panel). Different types of industrial fibre washers were tested and led

to the same result: Residual fine-grained sediment and, in the case of gillnets, small sub-mm size lead fragments remained present because nylon fibres tend to bend and knit together, trapping small contaminating grains.
– High residual salinity levels imply that chlorine contamination can lead to extreme corrosion and wear of involved machinery, processing and storage tanks.

These results imply that it is both technologically and economically challenging to manage fishing gear recovered from the sea to comply with the material recycling pathway.

Fig. 3. Left: Shredded gillnets with residual sink lines, the lead fragments showing at the centre of the image (© Andrea Stolte). Middle: Sink line with lead pieces embedded in PET sheathing (© Gunter Weißbach, Gilian Gerke). Right: Microscopic image of fibre mix showing twists and bents that cause contaminants to be trapped (© Gunter Weißbach, Gilian Gerke).

4.2.2 Thermal Processing of Mixed, Contaminated ALDFG

Incineration in residual waste or cement plants is the standard pathway for mixed wastes in Europe. This implies that infrastructure and facilities are available in the existing waste management system. However, with gillnet-dominated ALDFG, the legal lead/heavy metal contamination threshold for non-hazardous wastes of 0.3% by weight limits immediate processing [11]. For incineration, the following manual or technical pre-processing is required:

– removal of sink lines containing lead weights and other heavy metal items
– removal of large metal items to avoid damage to cutting/transporting machinery
– cutting into 0.5–1 m^2 maximum size fragments

This latter requirement is most crucial, as longer fragments can cause sparks to be transported from the incineration oven back to the storage bunker, which can lead to storage burn – one of the largest possible hazards for a thermal processing plant.

During MARELITT Baltic, WWF Germany tried alternative thermal processing scenarios such as pyrolysis and steam reforming (chemical conversion). Pyrolysis of nylon fibres leads to toxic emissions of hydrocyanic acid, thus requiring further alcalic gas washing or secondary burning of emission gases. In contrast to low-density polymers such as common PE and PP packaging, the return of pyrolysis condensate that might be used as engine fuel was with 2–5% too low to warrant further investigation of this technology for fishing gears. Steam reforming at >1000 °C led to a complete disintegration of organic substances, including polymers and contaminants,

and resulted in pure recondensed lead fragments among the solid residual (sediments, lead and small metal fragments). Prior to processing, gillnet-dominated ALDFG was shredded in a standard industrial rotor-shredder with reverse-antiblock system to a fibre size of 2–4 cm. The advantage of alternative thermal processing scenarios is that lead lines do not need to be manually removed, as the lead is extracted on the fly. Both pyrolysis and steam reforming systems are currently under investigation for mixed and hazardous waste fractions, as they can separate rare and common metals from electronic and medical waste and yield synthetic energy gas or condensate from contaminated polymer content. If these small-scale systems become commercially available in the near future, they can be recommended as the easiest and most efficient way to process lead-contaminated ALDFG.

Recommendations for the handling & waste management of ALDFG:

1. Pre-processing of fishing gear retrieved from the sea is the key for waste management.
2. Material recycling is only an option for clean, separable ALDFG, where end-of-life recycling facilities such as Plastix in Denmark, Antex in Spain, Nofir in Lithuania or Aquafil in Slowenia might be employed for further processing.
3. For incineration, trawl and gillnets need to be cut into 0.5–1 m^2 fragments to avoid hazards in residual waste incineration plants.
4. Lead lines need to be removed for incineration, as well as for any potential material recycling option.
5. Alternative thermal processing scenarios such as steam reforming can offer an easier solution as compared to manual labour to extract lead from sink lines and avoid dumping of mixed ALDFG in open-air, hazardous waste landfills.

5 Prevention

During MARELITT Baltic, it was found that sea user conflict and adverse weather are the predominant reasons for gear loss today. Historically, in pre-GPS times, underwater obstacles such as wrecks or rocky shores were the most common reason for gear loss esp. in the trawl fishery. For the coastal gillnet fishery, sudden ice and storm events can tear off marker buoys and cause gillnet fleets to drift from their mooring point. In Sweden, conflict between trawls and gillnets operating along the same rocky shores still leads to gear loss today. In Germany and Poland, gillnets are regularly run over by working vessels or sport boats, and cannot be recovered by the owner once flags and buoys are lost.

5.1 Gear Marking

Gillnets and trawls have to be marked with the identification of the vessel and the owner. Gillnets might not be recovered by the owner after marker buoys are accidentally or purposefully removed. In the MARELITT Baltic project area, this can lead to 500 m–2000 m long set nets to be lost on the seafloor. Gillnets can continue to catch

for several months and up to years [12]. Radio-Frequency IDentification (RFID) tags have been tested [6]. These tags are increasingly popular because they have become cheap over the past years and are readily available in sufficiently small formats to fit into small floats. However, RFID tags can only be identified as long as lost fishing gear is located on the surface. Once sunk to the seafloor, the radio transmission is reflected at the water surface and the signal becomes undetectable for nearby vessels. In addition, vessels have to be relatively near to the RFID tag for location. Other tags, such as small metal tags on each net segment, work well in the Swedish fishing fleet and help to identify the owner of lost gear and promote reporting. Passive echolocation through sound reflectors might be an ecologically friendly solution with large area coverage because all nearby echolocating vessels will capture the gear signal, which enables the identification of lost gears after marker bouys are lost. This promising technology needs further investigation [13].

5.2 Communication of and Between Sea Users

Advanced communication, e.g. through smart phone applications, might be a tool to promote the exchange between gillnets/set nets in areas where trawl fisheries are also allowed to avoid future gear conflict. In general, most fisherfolk today are aware of the high costs invoked with gear loss, both economically and ecologically by harming fishing grounds. Hence, disposal at sea has become uncommon. Involving fisherfolk in cleaning actions further nourishes the sensitivity for marine litter and for the impact of ALDFG on the seafloor and marine species. Sport boat renters and other sea users should be made aware of signals and the possible impacts of gear loss, both to the fishers and to the marine ecosystem.

Recommendations: A more densely spaced gear marking system, capturing individual gillnet segments, would help allocating ALDFG to the owner and promote gear loss reporting. An information system, e.g. a smartphone app, illustrating the location of set nets can avoid both conflict between different types of fisheries and recreational or working vessels and can also aid to inform sport boat users of the impact of fishing gear accidents. Further recommendations on prevention can be found in the MARELITT Baltic report on gear loss prevention [14].

6 Conclusions

Over centuries, every form of ALDFG from trawls to gillnets to fish aggregation devices (FADs) will disintegrate into microplastic fibres. Trawls might be treated with copper to avoid overgrowth and gillnets are weighted down with lead lines, hence adding heavy metals to the water and sediments, and fibres are prone to entering the marine food web and ultimately might end up on our plates. In addition to continued, unselective catches of fish, seabirds, turtles, and marine mammals, lost fishing gear remains a longterm source of microplastic fibres to the marine environment. The MARELITT Baltic project derived best-practice search & retrieval methodologies for the collection of ALDFG

from the sea. The EMFF provides funding support for fisherfolk and fishing associations willing to carry out cleaning actions at sea. However, for large-scale and regular retrieval operations, waste management pathways are urgently needed. MARELITT Baltic has identified a set of requirements and recommendations that would enable fishing gear retrieved from the sea to enter the existing waste management system. Alternative processing scenarios and material recycling were also investigated. The handling options, recommendations, and logistics requirements are compiled in the Treatment Scheme for Derelict Fishing Gears [15].

The full set of recommendations to policy makers and regional stakeholders, harbours and municipalities, as well as retrieval teams are summarised in the MARELITT Baltic Blueprint [16], and detailed individual reports on all three pillars investigated can be found on the MARELITT Baltic website https://marelittbaltic.eu.

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Biodegradable Plastics Do not Form Chemically Persistent Microplastics

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1 Introduction

Biodegradation of solid plastic materials is a heterogeneous reaction [1]. Only the surface is affected by biodegradation while the inner part should not be readily available for biodegradation. Current standard test methods for biodegradation are based on the exposure of a plastic sample to an environmental matrix (e.g. compost, soil, marine sediment, etc.) and on the measurement of the extra respiration possibly induced by the plastic sample in case of metabolization by microbes as an energy and carbon source [2]. Biodegradation is calculated as the mineralisation ratio i.e. the ratio between the carbon evolved as CO_2 (C-CO_2) and the total carbon present in the plastic sample (C-plastic) [3]. Under these conditions, the biodegradation rate refers to how the "C-CO_2/C-plastic" ratio changes over time. This is understood as the biodegradation rate of the polymer; however, most carbon (C-plastic) is not available but rather protected in the core of the plastic particles under testing. Thus, with the current test approach we can only determine an "apparent" biodegradation rate, most reactant being not available but nevertheless accounted in the denominator. Because of that, biodegradation of solid materials appears as a "slow" process, which suggests persistence of biodegradable plastics in the environment. In this paper, we show that the biodegradation rate happening at the surface of the plastic particles is very fast. We are currently proposing a different approach to measure the biodegradation rate of plastics, in order to determine the rate of the reaction effectively occurring at the surface of the plastics, i.e. at molecular level. To do so we suggest measuring biodegradation in samples with increasing surface areas and applying regression to determine the relevant parameters. This article is based on two previous publications where more details can be found [4, 5].

2 Experimental

2.1 Materials

Two test materials were tested: a biodegradable aliphatic polyester (polybutylene sebacate) and a commercial biodegradable plastic material based on biodegradable

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polyesters, starch, and a natural plasticizer. Both materials are produced by Novamont in the form of pellets.

Micro-crystalline cellulose in powder (Merck) was used as reference material.

2.2 Methods

2.2.1 Preparation of Samples

The plastic granules were milled by cryogenic grinding and sieved with standard sieves (Endecotts, London) of different mesh size obtaining different fractions (Fig. 1).

2.2.2 Determination of Surface Area

The surface area of the different sieved fractions was estimated with a theoretical approach by considering the particles of each fraction as spheres with a diameter equal to the median of the limits of each sieving range or using a particle sizing instrument (Mastersizer 3000 equipped with a Hydro EV manual wet dispersion unit, Malvern Instruments Limited, UK).

2.2.3 Regression Analysis

The regression analysis and data plotting were carried out using the statistical functions of Excel (Microsoft) and Statgraphics Centurion XVII.

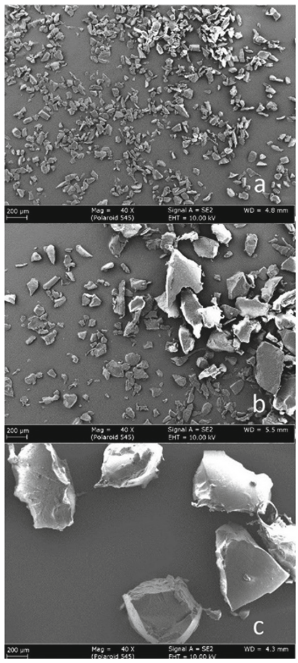

Fig. 1. Representative SEM images of three polymeric powder fractions: 50–75 μm (a), 200–355 μm (b), 500–700 μm (c).

2.2.4 Biodegradation Test

Respirometric tests were carried out in accordance with the ASTM D 5988-12 test method [6], based on the measurement of CO_2 production. 1 g of test material was mixed with 200 g of soil in a 1000 ml hermetically-sealed glass jar. The test was set up with blank jars (without material) and with reference material jars (1 g of cellulose). Two replicates were carried out for each polymer particle size, for blank and for reference, and incubated in the dark at 28 ± 2 °C.

3 Results and Discussion

3.1 Results

The different sieved fractions were tested in parallel with microcrystalline cellulose as a reference. The tested amount was normally 1 g per reactor. Some fractions were tested also at double dose (2 g per reactor). The total surface area was determined considering the specific surface area and the weight of each sample. The average biodegradation curves of the neat polymer are shown in Fig. 2. The average biodegradation curves of the plastic material are shown in Fig. 3. The curves of the plastic material are more complex because it contains several constituents. Three different kinetics were identified, possibly corresponding to (i) the biodegradation of low molecular weight constituents (first 10 days), (ii) the self-degradation of biomass formed in the first phase (from 10 to day 50), (iii) the biodegradation of the bulk polyesters.

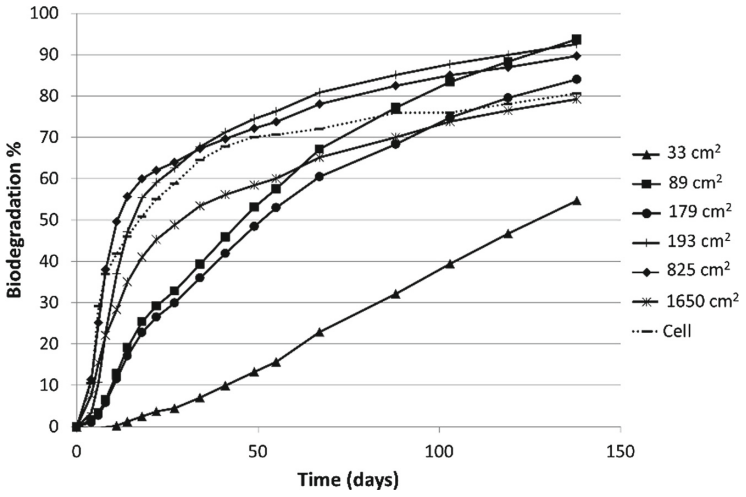

Fig. 2. Biodegradation (mineralization) curves of a polymer tested using different samples with different initial surface areas (indicated as total surface area in cm^2) and cellulose. Each curve is the mean of two replicates.

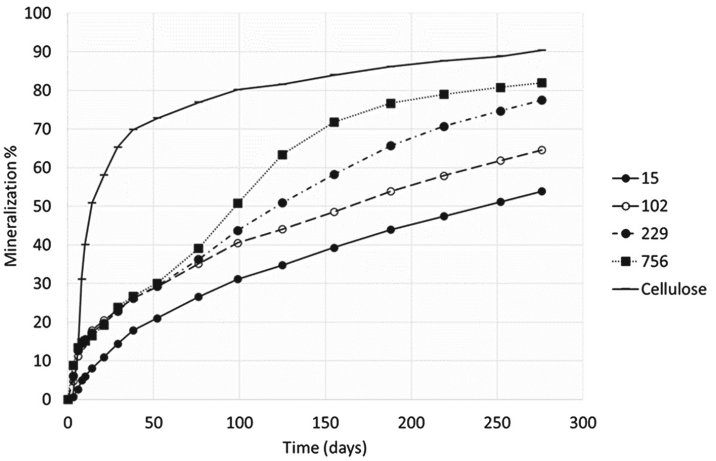

Fig. 3. Biodegradation (mineralization) curves of a multi-constituent plastic material tested using samples with different initial surface areas (indicated as total surface area in cm^2) and of reference material (cellulose). Each curve is the mean of two replicates.

The biodegradation rates have been determined on the residual carbon (i.e. the value obtained subtracting the evolved C-CO_2 from the C-plastic introduced in each reactor) by means of regression analysis. The rates are expressed as mineralised carbon per day, i.e. mg C/day and reported as positive values, for convenience, but should be considered as negative, as they are depletion rates. The relationship between the biodegradation rate and the surface area has been explored using the regression analysis (simple regression).

The double reciprocal model turned out to be the best fit for the polymer (Fig. 4 and Table 1). The double reciprocal model is also known as the Lineweaver-Burk plot, used in the Michaelis-Menten equation to describe enzyme kinetics.

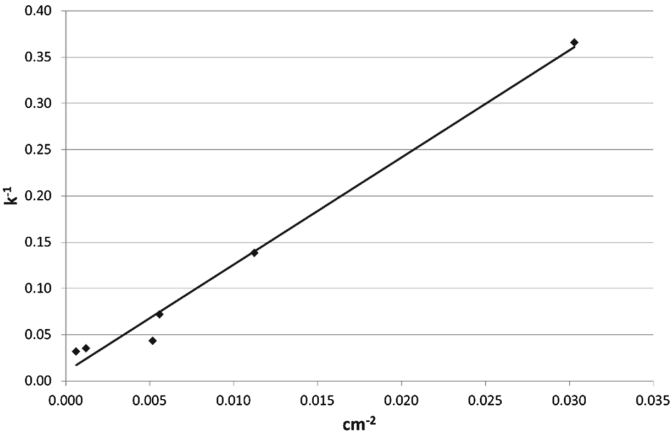

Fig. 4. The double reciprocal plot (Lineweaver-Burk plot) of reaction rate (k) and the initial total surface area of the polymer (cm^2). The continuous line is the linear regression.

The squared-Y model best fits the data of the plastic material (Fig. 5 and Table 1). The third biodegradation phase (from day 52 onwards) was considered.

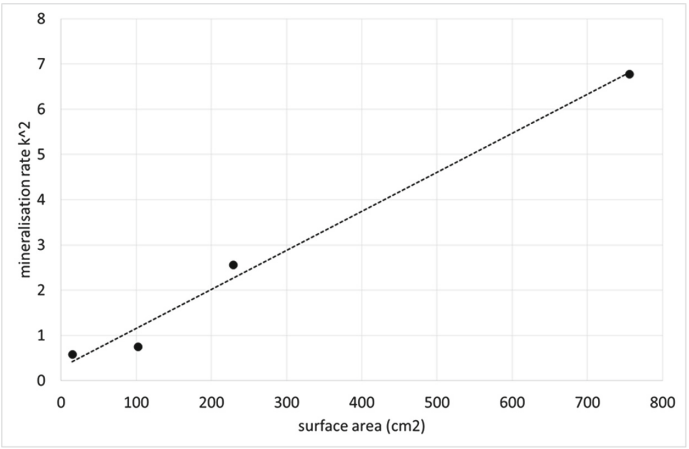

Fig. 5. The plot of reaction rates (k) squared determined from day 52 to day 125 and the respective total surface area of the sample (cm^2). The dotted line is the linear regression.

Table 1. Regression analysis of the mineralization rates and the surface areas

Sample	Applied regression model	Intercept (a)	Slope (b)	R-squared
Polymer	double reciprocal Y = 1/(a + b/X)	0.0103	11.55	98.7
Plastic material	squared-Y Y = sqrt (a + b * X)	0.008627	0.290144	98.83

3.2 Discussion

Plastics are solid materials where biodegradation happens on the surface. The idea that surface area is a factor affecting biodegradation rate is very common but few systematic studies on this factor are available. We have shown that biodegradation rate is a function of the surface area of the tested sample. The higher the surface area, the higher the biodegradation rate, all other environmental conditions being equal. The mineralization rates of polybutylene sebacate tested at different surface areas were related to the respective total available surface areas. The data are well described by a linear regression of the double reciprocal plot (the Lineweaver-Burk approach used in enzymatic kinetics) that enables the estimation of the theoretical maximum biodegradation rate (kmax = 97 mg Cpolymer day-1). The kmax can be considered as an estimation of the biodegradation rate at molecular level, when the available surface area is not limiting biodegradation. Likewise, the mineralization rates of a biodegradable multi-constituent plastic material have been determined. The relationship between

surface area and mineralization rate was determined for the third biodegradation phase using a regression analysis. The squared-Y model was the best fit in this case.

The regression models suggest that if it were technically possible to test the polymer and the plastic material in the form of nanoplastics (spheres of 100 nm diameter) it would take 15–20 days to reach full biodegradation, a time frame compatible with the OECD requirements for readily biodegradable chemicals. According to OECD, the "ready biodegradable" chemicals have to reach a 60% mineralisation in a 10-day window within a 28-day period of the test [7]. A "ready biodegradable" chemical is assumed to undergo rapid and ultimate biodegradation in the environment and no further investigation of the biodegradability of the chemical, or of the possible environmental effects of transformation products, is normally required. For example, the OECD approach is applied in Europe for detergents [8].

4 Conclusions

The data and the applied models suggest the *chemical* permanence time of the test materials is very short. On the other hand, the *physical* permanence time will depend on the surface area, i.e. on the thickness of the plastic items. This indicates 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. The biodegradable plastics sector is developing fast, and inquiries for more detailed information on environmental characteristics are also on the increase. A relevant characteristic of biodegradable plastics is the biodegradation rate, a parameter that is necessary to predict the environmental fate. We suggest a specific methodological approach to measure the biodegradation rate of plastics, in order to determine the rate of the reaction effectively occurring at the surface of the plastics based on increasing surface areas and applying regression analysis.

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Controlled Aging and Degradation of Selected Plastics in Marine Environment: 12 Months of Follow-up

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1 Introduction

The concern about plastic marine debris has steadily been rising year after year, because millions of tonnes of plastics are released in the ocean every year, causing tremendous ecological and economic repercussions. The degradation of polymers in sea water is studied under different aspects, as it could lead to (i) the fragmentation of plastic objects and the production of microplastics, (ii) leaching of environmentally harmful additives, and (iii) preclusion of the possibility of plastics recycling [1–3].

Polymer degradation generally begins at the outer surface and penetrates into the bulk material [4].

Photo-degradation, thermal degradation, biodegradation and mechanical deterioration are the main factors responsible for the decay of plastic functionality during weathering.

Chemical degradation processes often involve the interaction between free radicals and oxygen: free radicals undergo termination via recombination and/or disproportionation, thus yielding variations in molecular weight and crosslinking density. Free radicals are mainly produced when polymers are exposed to high temperatures, UV or other high energy radiation.

Abiotic and biotic degradation can accelerate the kinetics in the first steps of the damaging processes of some polymers, but also acts as main factor in the final steps after mechanical fragmentation. In particular, polymers with carbon backbone generally undergo abiotic degradation before biodegradation, whereas polymer fragments with lower molecular weight can afterwards be more efficiently biodegraded [5].

Natural weathering is responsible not only for visible deterioration of the plastic objects, but also for reduction of molecular weight, mechanical properties, thermal stability, etc. We have systematically monitored the variations of these properties by analyzing samples after various aging periods [6]. The aim of this study is to shed more light on the mechanisms and kinetics of degradation of plastic objects in marine environment, taking in the account the combined and synergistic effects of sun, sand, seawater and mechanical stresses.

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2 Experimental

Plastic samples were aged in three different environments: sandy shoreline (S), surface of seawater (MS) and 1 m underwater (MF). The experiments were carried out in Villasimius (CA), Italy, in collaboration with the staff of the Marine Protected Area of Capo Carbonara.

2.1 Materials

Selected common plastic items have been subjected to the aging procedure: thermoformed cups made of polystyrene (PS), polypropylene (PP) and polylactic acid (PLA); water bottles of poly(ethylene terephthalate) (PET); packaging films (PP and PE) and expanded polystyrene boxes (EPS). Cups, films and bottles were purchased from local markets, EPS boxes were kindly provided by operators of the Municipal Market in Cagliari, Italy.

2.2 Methods

The aging process started in November 2017, with the installation of the containers in appropriate locations, such as Sandy Shoreline (S) at Lat 39°12866′ N, and Long 9° 5062′ E; Marine Immersion (MS and MF) at Lat 39°11754′ N, and Long 9°5080′ E.

In the course of the aging process, the samples were periodically controlled by the staff of the Area Marina Protetta di Capo Carbonara. The containers consisted of a main structure made of poly(vinyl chloride) tubes, wrapped by PP fishing net. The samples were harvested after 3, 6, 9 and 12 months of the selected exposition and sent to the University of Trento for the physical-chemical characterization.

2.2.1 Preparation of Samples

As the samples aged in water exhibited biofouling proportional to the exposition period, they were cleaned with water, lightly rubbed with a soft sponge and dried at room temperature for one month before testing. The fouling of the samples aged 12 months was difficult to remove, so that another procedure was followed: the samples were dried at room temperature for 2 months, washed with a 1M HCl solution, and dried for one week at room temperature. The test specimens were cut with a die press for mechanical test, with a hollow punch for the calorimetric measurements, and with scissors for the others tests.

2.2.2 Analytical Techniques

Tensile tests were performed by means of Instron testing machine mod.4502 at 10 mm/s. The tests were carried out on dumbbell specimens 20 × 5 mm cut from original and aged samples. All data have been normalized with respect to the thickness of the original samples to take into account variability of the thickness of the samples caused by biofouling on the surface of materials.

The viscosity-average molecular weight M_v was calculated from the intrinsic viscosity of the sample $[\eta]$, determined for chloroform solutions by means of the Ubbelhode viscometry at 25.0 °C, using Mark-Houwink equation:

$$[\eta] = KM_v^{\alpha} \tag{1}$$

where $K = 5.45 \times 10^{-4}$ and $\alpha = 0.73$ are constants for PLA [7].

Plate-plate rotational viscometry, performed with Anton Paar Physica MCR301 rheometer, was used for polyolefins at 200 °C with variable shear rate from 0.01 to 100 s^{-1}. The zero-shear rate viscosity was correlated with the weight-average molecular weight calculated by the Ostwald - de Waele model:

$$\eta_0 = AM_w^n \tag{2}$$

where η_0 is the zero-shear viscosity read off as the value in the initial plateau of viscosity curves, M_W is the weight-average molecular weight (A = 5.8×10^{-14} Pa·s and n = 3.4 were used for PE, according to literature [8]). The relative ratio of melt-viscosity or Mw for the original and aged samples was exploited to evaluate the percentage of molecular weight reduction as shown in Figs. 3, 4 and 5.

Fourier Transform Infrared (FTIR) spectroscopy was carried out on polypropylene, polyethylene and polystyrene to detect the carbonyl peak, indicating the oxidation of the material. The Oxidation Index (OI) can be defined as the relative intensity ratio of the carbonyl peak to the reference peak:

$$OI[\%] = \frac{\%T_{oxy}}{\%T_{ref}} \times 100 \tag{3}$$

where $\%T_{oxy}$ is the transmittance of the carbonyl peak, whereas $\%T_{ref}$ is the transmittance of the fingerprint peak at 690 cm^{-1} for PS, and at about 2915 cm^{-1} for PE and PP.

Differential Scanning Calorimetry (DSC) was performed using Mettler Toledo DSC30 from 0 to 300 °C at flushing air at 100 ml/min to evaluate Tg, crystallinity, and melting temperature, and also to monitor the Oxidation Onset Temperature (OOT) of the polymer samples. Crystallinity of PLA was evaluated from the melting and crystallization heat, and referred to 93.6 J/g, the melting enthalpy of 100% crystalline PLLA, as described in literature [9].

The color variations of the samples were detected by testing the blank and selected aged samples with a Konica Minolta CM-2600 Spectrophotometer performing three scans for each sample. Data were analyzed by the software SpectraMagic NX, following the ASTM Standard E805.

The quantification of the color variation was carried out by measuring the distance on the CIELAB color space. The distance between two points in the CIELAB space is equal to:

$$\Delta E = \sqrt{\left((\delta L)^2 + (\delta a)^2 + (\delta b)^2\right)} \tag{4}$$

"L" is the grade variation from white to black, whereas "a" and "b" range from green to red, and from yellow to blue, respectively, as reported in the technical literature [10].

3 Results and Discussion

The selection of relevant results in this extended abstract mainly regards the properties of virgin samples and samples after 9 months of aging. Properties of polyester – polycondensation based products (PET and PLA) are compared in the initial paragraphs. The analogous data of polyaddition products, PP, PE and PS (film, cup and foamed box) are also presented.

3.1 Results

Polyethylene terephthalate PET bottles exhibited a consistent geometrical integrity along the studied period. Some small variations could be observed along with the reduction of strength in longitudinal direction, and strain at break was also reduced (Table 1).

Table 1. Strength and strain at break of the PET samples in longitudinal/transversal direction obtained for aging conditions (MF, MS and S).

PET sample	Strength [MPa]	Strain at break [%]
Original	176 ± 18 / 120 ± 4	75 ± 11 / 95 ± 24
9MF	167 ± 11 / 131 ± 7	68 ± 7 / 78 ± 17
9MS	164 ± 16 / 144 ± 3	70 ± 11 / 80 ± 2
9S	154 ± 19 / 136 ± 7	58 ± 8 / 84 ± 9

The samples aged in water appeared more resistant after 9 months than those exposed to sandy shoreline aging. However more significant changes can be expected at longer times. It appears that hydrolysis and other weathering factors have complex and hardly predictable effects on the mechanical properties of PET, that is an aromatic polyester, with a polymer chain of relatively long-time stability. Molecular weight has not been investigated.

More significant effects of aging were observed for samples of poly(lactic acid), because this aliphatic polyester is highly susceptible to hydrolytic degradation [9]. Viscometric molecular weight Mv and longitudinal mechanical properties of PLA samples are reported in Table 2.

The molecular weight of PLA goes down as the marine aging is prolonged. In the same time also mechanical properties progressively decrease, and after 9 months exhibit only 70–75% of initial strength and strain at break both in longitudinal and in transversal direction. The samples aged on water surface (MS) showed the lowest molecular weight, obviously due to the simultaneous action of solar radiation and seawater environment. As the samples aged in the sand (S) were not in direct contact

with seawater, the hydrolysis might have deteriorated the material only via the humidity absorbed from the environment. Therefore, the interaction between the solar radiation and polymer appeared as a synergistic component of the degradation process. Partial crystallization of PLA could affect the stiffening and prelude to embrittlement. Moreover the different behavior of PLA vs. PET can be also related to their glass transition temperature, about 64 °C vs. 73 °C respectively [9].

Table 2. Molecular weight, strength and strain at break in longitudinal direction, crystallinity and OOT o PLA samples aged 9 and 12 months in different conditions (MF, MS and S).

	Mv [Da]	Strength [MPa]	Strain at break [%]	Crystallinity [%]	OOT [°C]
Original	39230	175 ± 12	45 ± 9	3.2	238
9MF	29770	136 ± 17	39 ± 17	3.6	218
9MS	28750	119 ± 12	36 ± 16	10.5	226
9S	31640	130 ± 8	36 ± 18	4.1	218
12MF	28400	111	/	3.6	223
12MS	26270	101	/	5.2	224
12S	28620	118	/	25.5	220

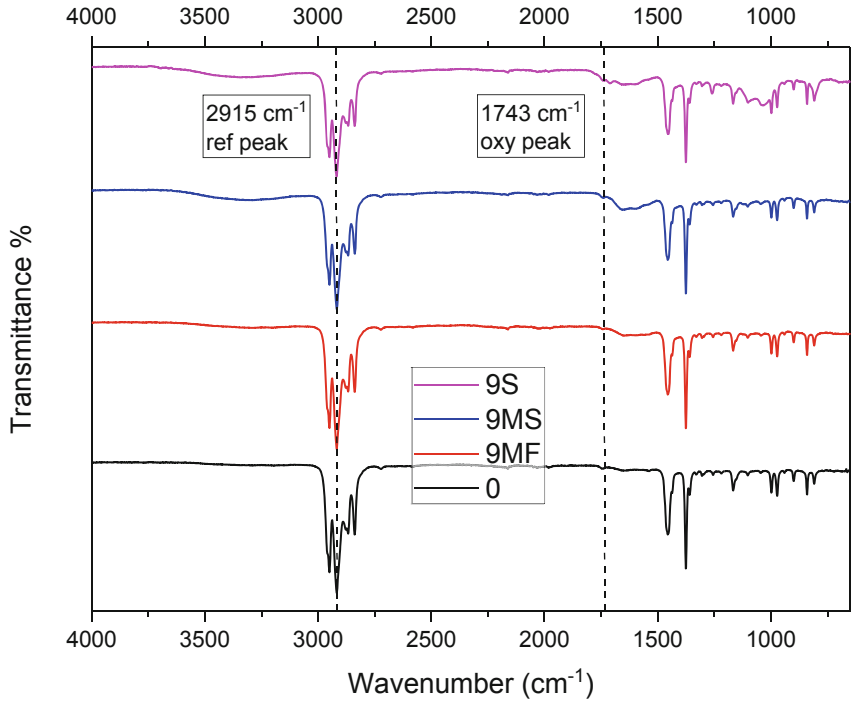

Fig. 1. FTIR spectra of polypropylene film samples: original (0) and after nine months aging under different conditions (MF, MS and S).

A dissimilar trend was observed for objects made of vinyl polymers (PE, PP and PS), with some peculiar behavior reflecting also on their geometry (film, cup, foamed box). Selected properties were compared for samples before and after 9 months of aging.

The first indication of aging is the oxidation as shown in Fig. 1 for PP film and quantified by the Oxidation Index.

The decrease in mechanical properties is accompanied by the increase in Oxidation Index (see Table 3), the reduction of Oxidation Onset Temperature OOT (Fig. 2), but also to the decrease of molecular weight and the increase of color variation (see Fig. 3).

Polypropylene films aged in water had a large portion of the surface covered by biofouling, which may have affected their real exposition to UV radiation.

Polyethylene film, similarly to PP film, shows the detrimental effect of the chemical degradation and the mechanical properties. In particular, Table 4 summarizes the data on zero-shear viscosity (η_0), calculated molecular weight, tensile strength in transversal direction and the oxidation onset temperature (OOT).

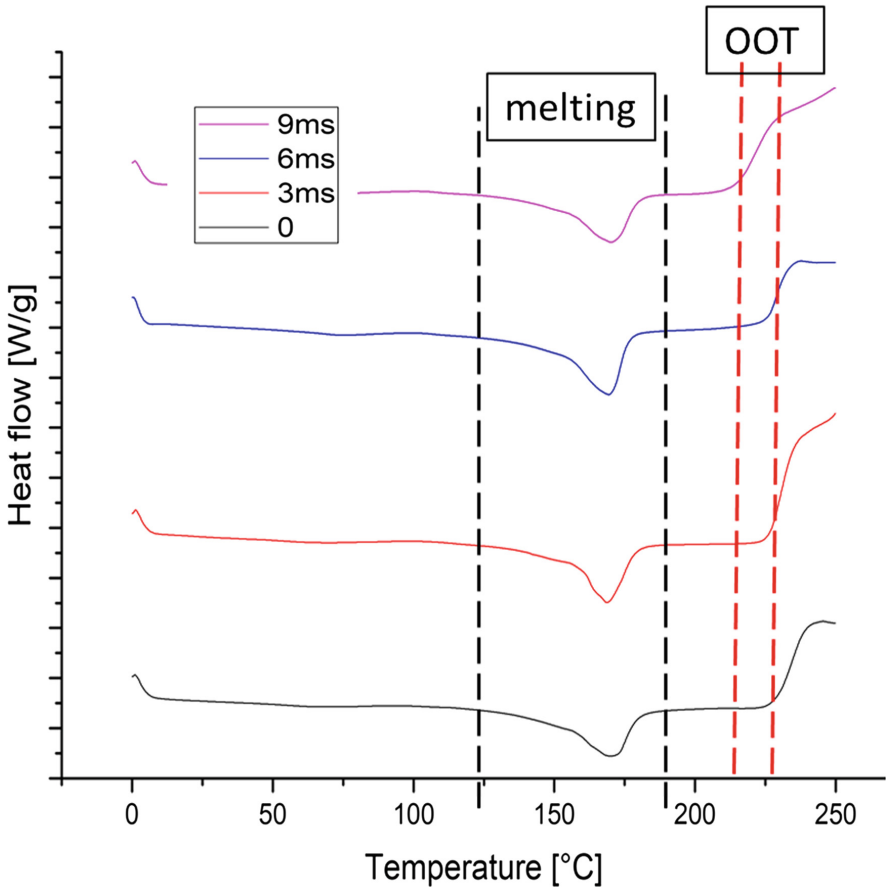

Fig. 2. DSC thermograms of polypropylene film samples: original (0) and after 3, 6 and 9 months of aging under MS conditions.

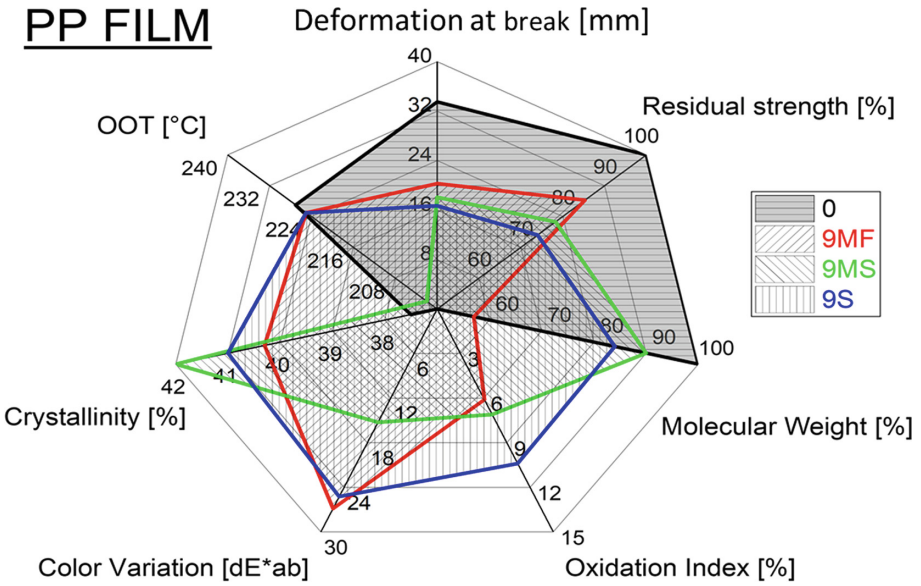

Fig. 3. Comparison of the data for original PP film (0) and for samples exposed to 9 months of aging (mechanical test were performed in longitudinal direction).

Table 3. Strength and strain at break of the PP film samples in longitudinal direction.

Sample	Strength [MPa]	Strain at break [%]	Oxidation Index [%]
Original	57.4 ± 0.8	168 ± 10	0.0
9MF	49.0 ± 3.7	101 ± 21	6.1
9MS	45.0 ± 7.3	90 ± 46	7.1
9S	42.3 ± 9.1	84 ± 47	10.4

Table 4. Rheological properties and other properties of polyethylene films.

Sample	η_0 [Pa s]	Molecular weight [dalton]	Strength [MPa]	OOT [°C]
Original	3700	90520	15.5 ± 1.4	212
9MF	3430	85270	12.7 ± 2.2	196
9MS	3020	88520	13.0 ± 1.2	200
9S	2450	80180	10.4 ± 0.5	203

The decrease in molecular weight is documented by the drop of viscosity. Simultaneous decrease of OOT and strength in transversal direction (Table 4), along with the increase of both color variation and Oxidation Index (Fig. 4) are clear indication of polymer degradation. The apparent decrease in crystallinity might be attributed to the effect of biofouling.

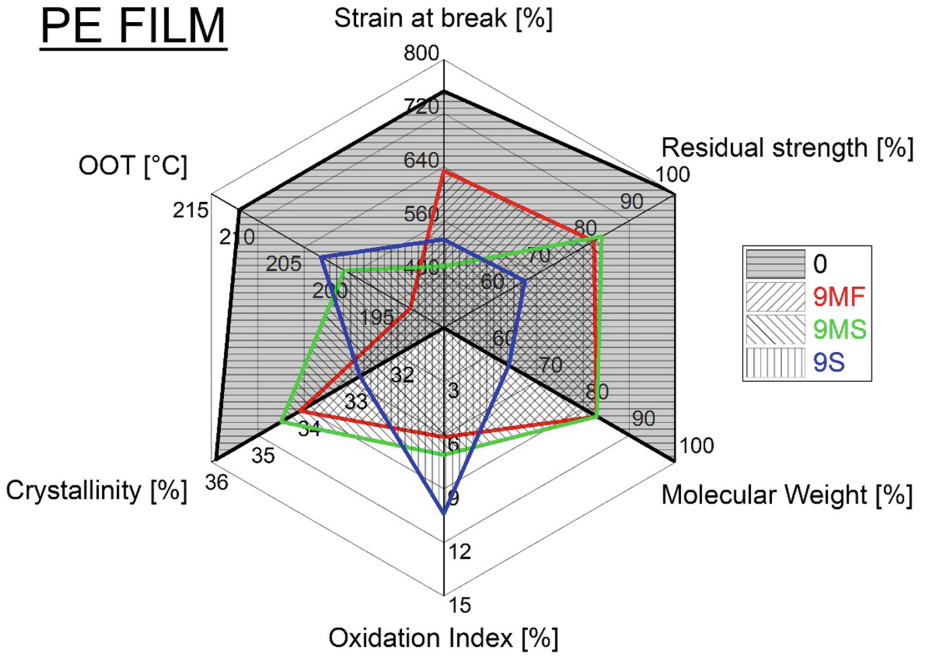

Fig. 4. Comparison of the data for original PE film and for samples after 9 months of aging (mechanical test were performed in transversal direction).

Specific behavior was observed for plastic cup obtained by thermoforming which showed different residual stresses affected by the shape factor and the production process. The results for PP and PS cup are compared in Figs. 5 and 6.

The thermal stability of the polypropylene cup samples decreased very abruptly with aging time, in particular after aging in the sand. The samples aged in water displayed monotonous decrease in thermal stability as a function of time, but slightly less steep than that of the S samples. In the same time the crystallinity evidenced a slight increase with the aging time up to 9 months, particularly 9S samples, reaching values close to 50%. Correspondingly mechanical properties, such as stress and strain at break, decreased.

The values of melting enthalpy, selected mechanical properties and OOT for polypropylene cups aged under various conditions are reported in Table 5. Significant reduction of the strain at break of 9MS sample is also worth noting.

Table 5. Values of melting enthalpy, strength and strain at break (longitudinal), and oxidation onset temperature for polypropylene cup samples.

Sample	Melting integral [J/g]	Strength [MPa]	Strain at break [%]	OOT [°C]
Original	95.9	99 ± 8	174 ± 29	231
9MF	97.3	96 ± 9	123 ± 27	217
9MS	95.6	88 ± 8	42 ± 19	212
9S	99.1	74 ± 12	143 ± 26	200
12MS	89.3	73	/	220
12S	90.6	61	/	220

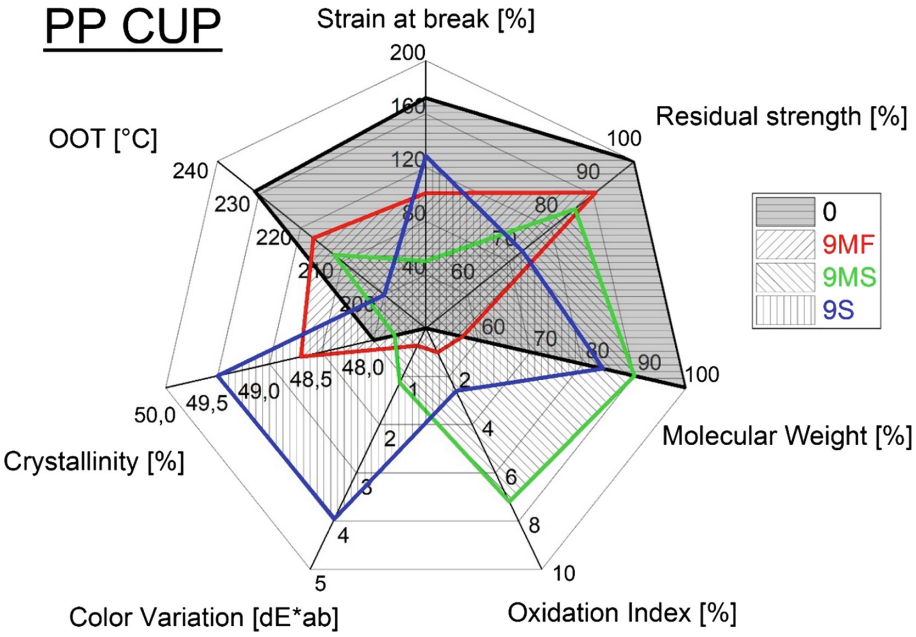

Fig. 5. Comparison of the data for original PP cup and for samples after 9 months of aging (mechanical tests were performed in longitudinal direction)

Similarly to PP cups, the effect of aging on PS cups caused progressive thermal oxidation documented by the decrease of OOT and by the increase of both Oxidation Index and color variation (see Fig. 6 and Table 6). As expected, the deterioration of mechanical properties was manifested by the significant embrittlement of PS cup, especially for samples exposed to solar light (S and MS samples). Selected results of the colorimetric and mechanical tests are reported in Table 4. Polystyrene cups aged in water showed a very small color variation, whereas the samples aged in the sand exhibited a value almost double value of the perceivable threshold (2.3 is considered the Just Noticeable Difference).

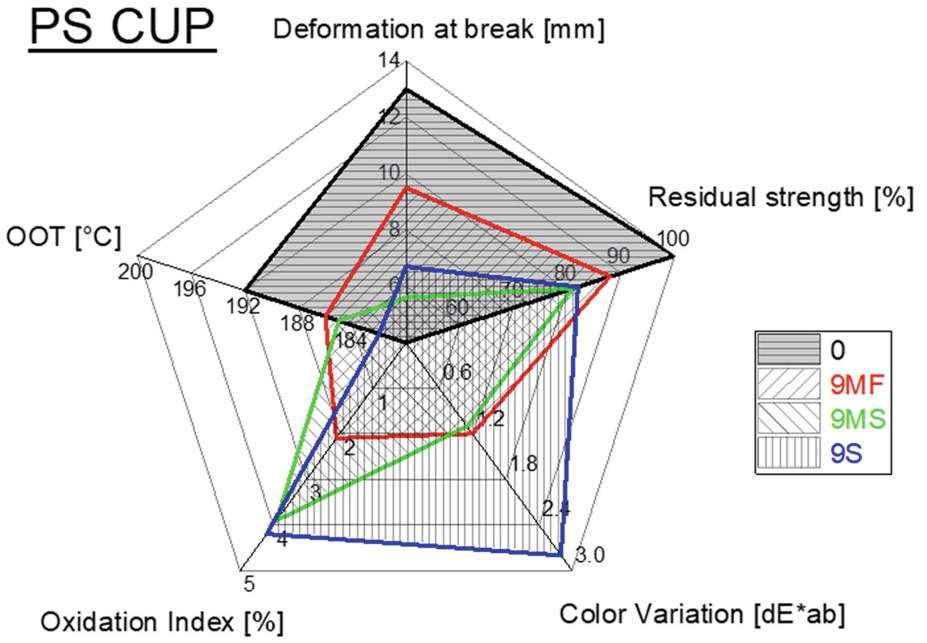

Fig. 6. Comparison of the data for original PS cup and for samples after 9 months of aging (mechanical tests were performed in longitudinal direction).

Table 6. Results of the colorimetric test and of the selected mechanical properties in longitudinal direction of PS cup samples.

Sample	δL	δa	δb	$\delta E * ab$	Strength [MPa]	Strain at break [%]
Original	0	0	0	0	42 ± 3	65 ± 12
9MF	−0.12	−0.16	0.30	0.36	37 ± 6	50 ± 10
9MS	−0.68	−0.16	0.89	1.13	34 ± 1	28 ± 13
9S	−0.47	−0.65	3.87	3.95	35 ± 2	33 ± 12

Table 7. Results of the colorimetric test for the PS foamed samples after aging in sandy shoreline.

Sample	δL	δa	δb	$\delta E * ab$
Original	0	0	0	0
6S	−0.08	−0.07	0.96	0.97
9S	−0.15	−0.6	2.9	2.97
12S	−1.0	−0.5	3.6	3.75

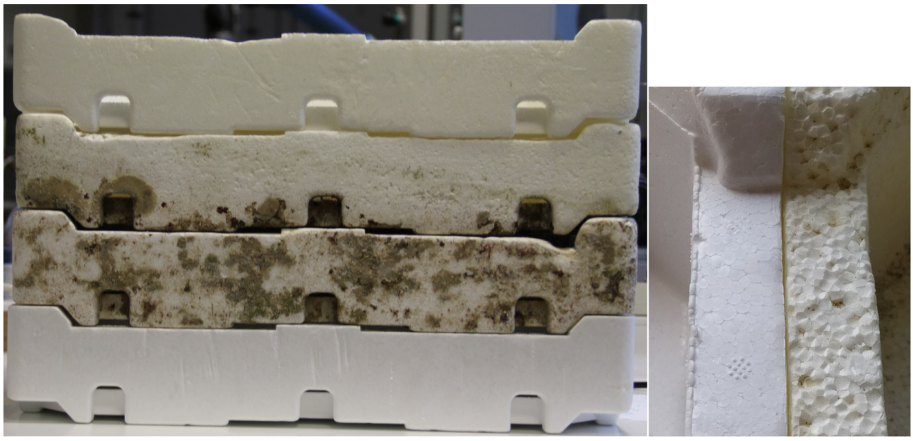

Fig. 7. Right: lateral view of the EPS samples (Blank, 9MF, 9MS and S from bottom to top). Left: upper border of the EPS box before and after 9 months of aging in sandy shoreline (corrugation effects).

Foamed polystyrene boxes after 9 months of aging maintained their general integrity (Fig. 7a), probably due to the low level of mechanical stress both in the sandy shoreline and in sea water immersion. Samples immersed in water evidenced progressive colonization and biofouling. On the other hand, a significant color variation was found after 9–12 months of direct sun exposition (S-samples), along with progressive corrugation of surface profile was detected (see Table 7 and Fig. 7b). No fragmentation has been observed after 12 months.

4 Conclusions

In previous papers, the samples aged in the sand were found as the most degraded, but our analysis shows that it may not be necessarily be quite general case. In fact, the combined effect of water and light can be in some cases more detrimental for specific polymeric samples. In particular, the samples aged underwater showed the smallest degradation effects for vinyl polymers (PE, PP, PS), whereas polyesters such as PET and PLA, showed higher sensitivity to the weathering due to hydrolysis phenomena. The most detrimental factor for vinyl polymer degradation appears to be the (UV) light.

In the same time, after the sea water immersion of the samples accounts for biofouling reaching macroscopic scale within weeks. After nine months, large species such as mussels, murices, sea urchins and sea cucumbers were attached to the samples. The presence of these species undoubtedly protected the polymers against oxidative phenomena and solar irradiation, thus making the aging process less effective. This may explain why many samples aged in water showed smaller decrease in measured properties in comparison with the original samples.

Our experimental findings lead to the hypothesis that a nonlinear relationship may exist between the time of exposition and the polymer degradation in seawater, whereby acceleration or deceleration can be observed. Moreover, it is worth underlining that the aging effects should be related not only to the period of exposition, but also to the season of the year in which the sample underwent aging and the possible surface coverage of the sample by various biological species.

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Inhalable Microplastics: A New Cause for Concern?

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1 Introduction

Microscopic plastic particles – microplastics – are a global issue for aquatic habitats. Recently, they have been reported in 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 [1].

Accurate estimation of the number of microplastic particles, films, and fragments is subject to significant methodological challenges. Correlating spectral data against plastic-only references may increase the chance of false-positive results, and visually discriminating between similar polymer spectra (such as polybutylene terephthalate and polyethylene terephthalate) could result in misclassification. Much work is still required in this area, and analytic techniques capable of detecting plastic particles at the nanoscale are needed.

Here we present data on the presence of microplastics in total atmospheric deposition sampled over one month at an urban background site in London, UK. Given the potential for microplastic misidentification due to overlapping aesthetic, morphological or fluorescent properties with non-plastic particulates, spectroscopic analysis is an analytical requirement. Raman Spectral Imaging (RSI) has been used as an alternative approach to combat operator bias and improve spectroscopic analysis of contiguous particulates.

2 Experimental

This study uses Raman spectral imaging for the identification of microplastics (≥ 2 μm) in ambient particulate matter, using different chemometric techniques. We show that Raman spectral images analysed using univariate and multi-variate statistical approaches is appropriate for the identification of both virgin and environmental microplastics ≥ 2 μm in size.

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2.1 Materials

Ethanol (EtOH) was sourced from Fisher Scientific UK (Loughborough, UK). 1, 2, and 10 μm PS microspheres were sourced from Sigma Aldrich (UK). 4.16 μm PS microspheres were sourced from Spherotech Inc. (Illinois, US). PA, PE, PET, PS, PP, and polyvinyl chloride (PVC) particles were sourced from Goodfellows Ltd (UK) (otherwise referred to as virgin plastics). These were all stored as per the supplier recommendations. Environmental plastics collected opportunistically from a European Beach (Arenal d'en Castell, Menorca) were milled to reduce their finesse.

2.2 Methods

2.2.1 Preparation of Samples

To validate the proposed method against a representative background a 24-h PM10 (Particulate matter <10 D) archived air sample collected from an urban road-side site (Marylebone Road, London, UK) was used. The sample was collected onto a Teflo filter (Polytetrafluoroethylene; PTFE) using a Partisol™ Plus 2025 Sequential Ambient Particulate Sampler (flow rate of 16.7 L/min). PM was extracted by submerging the filter in 5 mL of EtOH and agitating in a sonicating bath (5 min). The extracted PM was dried, weighed, and re-suspended in EtOH to a concentration of 312 μg/mL. Which corresponds to the mean daily sample weight collected by the desired method of sampling (MVCS) during a spring 2017 sampling campaign.

The PM was spiked with 2, 4, and 10 μm PS microspheres at a total concentration of 30,000 particles/mL (10,000 particles/mL for each size). The sample was diluted 1 in 10 and a 100 μL aliquot was dried dropwise on to an aluminium slide. A micrograph and SIs were acquired of the entire dried PM drop-cast. Due to the dried drop cast's area being relatively large and concerns over file size, the SI was acquired in 6 separate units at $\sim$2.6 μm spatial resolution. Each individual SI unit once analysed was tiled together to generate a sample wide SI.

2.2.2 Analytical Techniques

To evaluate the chemometric protocols, microplastics identified in the SI were counted and compared to the expected concentrations. Image analysis (particle counts) was completed on all analysed SI in Icy and ImageJ. Image pre-processing of the SIs included a 64-bit raw grey scale image conversion, applying a gaussian blur filter with a sigmoid radius of 1.0, the application of a watershed transform [2], and a Huang thresholder to extract the objects (areas of Raman signal) from the background using Shannon's function [3]. Microplastics in the processed SI are counted using an Undecimated Discrete Wavelet Transform (UDWT) detector, which produces a multi-resolution representation of an image [4].

3 Results and Discussion

3.1 Results

From the SIs obtained of 1, 2, 4, and 10 μm PS microspheres dispensed onto a range of substrates, aluminium slides were found to be optimum for visualising PS microspheres and supressing background fluorescence, while low-E slides performed worst due to optical contrast and substrate fluorescence (LOD: 10 μm PS microspheres at ~2.6 μm spatial resolution) (Fig. 1).

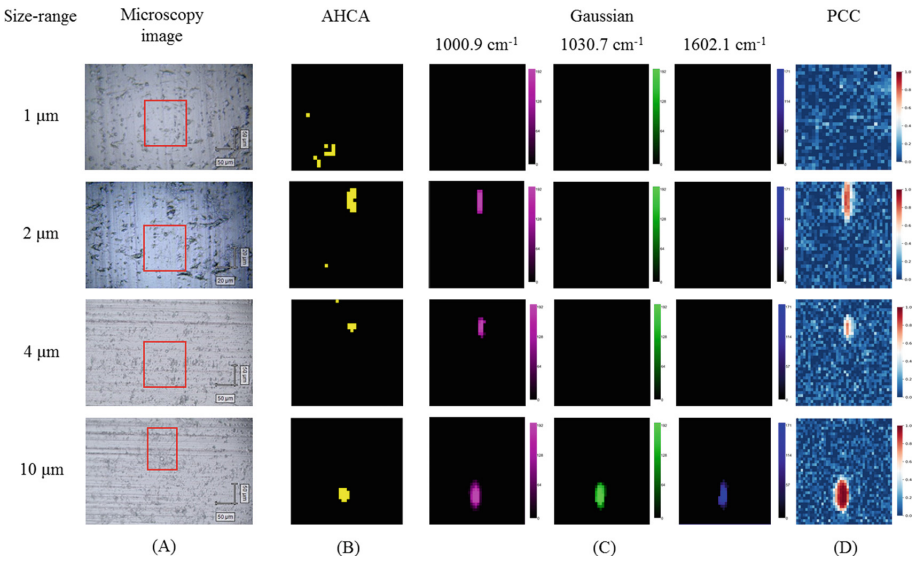

Fig. 1. Size dependent identification of polystyrene (PS) microspheres using Raman spectral imaging. Raman spectral images (SI) approximate area shown in Red and corresponding bright field micrographs (A) of PS microspheres were acquired. The SIs were analysed using AHCA (B), Gaussian (C), and PCC (D) to identify PS microspheres. 4 and 10 μm PS microspheres were obtained at 2.6 μm spatial resolution, and 1 and 2 μm PS SIs were acquired at 1.1 μm spatial resolution

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.

The proposed chemometric methods were analysed for their speed at plastic classification in an SI of PS particles deposited on an aluminium slide (7875 spectra). The chemometric techniques identified the presence of PS in 13.3 s, 105 s and 2100 s for PCC, AHCA, and Gaussian analysis, respectively.

PS microsphere counts differ between the chemometric techniques, with PCC analysis detecting the greatest number of microspheres (particle count: 212), followed

by AHCA (particle count: 169), and lastly Gaussian (particle count: 111). Analysis of the mock ambient sample for the remaining plastics in the plastic spectral library resulted in the identification of PE (n = 35), PET (n = 2) and PP (n = 5) particles. The identified airborne microplastics ranged from 4.7 to 51.8 μm in size in their longest dimension. The total concentration of airborne microplastics in the 24 h urban roadside sample was calculated to be 174.6 microplastics/m^3.

3.2 Discussion

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.

The chemometric analysis methods utilised successfully identified 2, 4, and 10 μm PS microspheres dispensed on aluminium-, CaFl-, gold-coated-, and stainless steel-Raman substrates in SIs. However, the intensity profile of Raman bands associated with PS differed among the test substrates. Aluminium-, CaFl-, gold-coated-, and stainless steel- slides permitted the identification of ≥ 2 μm PS microspheres in SIs. As CaFl-, gold-coated-, and stainless steel- substrates are relatively expensive, substrate reuse would be necessary to maximise economic viability. The low-cost of aluminium slides will permit sample archiving, future reanalysis and/or sample extraction.

RSI and the applied chemometric methods were capable of distinguishing virgin microplastics reflecting the most commonly observed plastic types in the environment, i.e. PA, PE, PET, PS, and PVC. However, in the environment, microplastics will undergo photo-, thermooxidative-, hydrolytic- and bio- degradation [5]. Depending on the plastic type, this has been found to impact or alter Raman spectra [6]. Whilst the duration and level of photodegradation is unknown, the identification of environmental microplastics in the current study (PE, CuPc, PP, and PS) indicates that this method is suitable for aged particles of a similar composition [6]. However, Lenz and colleagues [6] observed PVC's Raman spectrum is modified with UV-degradation and is completely altered after 1634 days of simulated noon-sunlight. Thus, it's important to continue to validate analytical methods on aged microplastics and recognise detection limits.

4 Conclusions

In conclusion, we have demonstrated that RSI analysed using Gaussian, AHCA, and PCC chemometric techniques can identify microplastics in the inhalable size-range. In this study, SIs were acquired at 1.1 μm and 2.6 μm spatial resolution, which enabled the direct identification of 2, 4, and 10 μm PS microspheres in ambient PM. In addition to ambient samples, the presented RSI method could be adapted to identify microplastics in marine and terrestrial samples, though adequate sample pre-processing

is recommended. Particulates shown to generate plastic signal in the SI should be further optically inspected to conduct morphological analysis (i.e. size, shape, colour).

RSI has a clear advantage of removing operator bias, while permitting the identification of particulates in the inhalable size range, enabling data on microplastic abundance, size distribution. Chemical composition and morphology to be attained. However, it suffers long acquisition and data processing times. For the field to progress towards microplastic monitoring in any environmental matrix, more streamlined instrumentation is required. Hence, RSI should be a complimentary technique alongside alternative methods, such as Py-GC-MS, to generate mass related datasets.

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Microplastics and Nanoplastics Occurrence and Composition in Drinking Water from Akureyri Urban Area, Iceland

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1 Introduction

Microplastics as a potential health and environmental problem has gained increasing attention recently. Microplastics are defined as plastic pieces smaller than 5 mm in diameter, and there are many sources of such microplastics [17]. Current literature reports that microplastics are ubiquitous worldwide. While several authors report on fragments of different polymers being observed, practically in all environmental areas of marine [3, 5, 12–14, 16, 19], freshwater [1, 20] and terrestrial ecosystems [2, 4, 6–9]; others point out that the accumulation of micro and nanometer-sized plastic particles throughout the marine and terrestrial food webs, is posing a risk to marine and terrestrial life, and ultimately to human health [18]. Despite 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 drinking water and their implications on human health. According to 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. Recent publication indicates that drinking water, uptake through seafood, and airborne exposure are the main sources of microplastics in humans [15].

The aim of the present work is:

I. to map published and available literature in the field of microplastics in drinking water,
II. to develop and optimize a standardized, fast and sensitive protocol for sampling and quantification of nano/microplastics particles in drinking water of Akureyri, Iceland,
III. to analyze and detect microplastic particles in drinking water supply systems with special focus on different polymeric composition and size fractions.

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2 Experimental

2.1 The Study Area

The study area is Akureyri, a town populated by approximately 18,000 inhabitants and its surrounding area. Figure 1 shows a map of the area with location of the sampling sites which are numbered. Akureyri is located by Eyjafjörður bay in North Iceland surrounded by mountains on two sides to the East and to the West. The drinking water supplies are two areas of underground wells in the mountains east of the town. The third water supply is a river in a nearby valley (Site no. 1 in Fig. 1) which is a mixed run of rainwater and glacial water that is filtered through the riverbed. The areas around the three water sources are scarcely populated and can be considered almost free of

Fig. 1. Overview of Akureyri and its surroundings. The dark lines show the main plumbing and the numbers indicate the eight sampling sites of drinking water.

anthropogenic impact. The mountain catchment is shown in light a grey color on the left in Fig. 1. The drinking water is of high quality and contains negligible of suspended solids. The quality of the water is controlled regularly, and the water needs no treatment at all before use (source: Norðurorka Ltd.).

2.2 Sample Collection

The sampling took place during spring and summer 2018. Samples were taken from eight different sites (Fig. 1). Samples taken from Site nr. 1 contain water from the river at Vaglir, while samples from Sites 5 and 6 contain water from the wells in the mountain called Hesjuvellir and Torfdalur respectively. The other sampling sites (2, 3, 4, 7 and 8) are within the plumbing system of the town at a different distance from the sources. The sampling sites in the town contain mixture of water from all three water sources. Three replicates were taken from each sampling site to give the total of 20–25 L of samples per site. The samples were collected into glass bottles that were previously cleaned by heating them to 520 °C for three hours to get rid of any contaminants. The bottles were then sealed with aluminium caps until they were prepared for analysis.

2.3 Preparation of Samples

All tools, GF/C fiberglass filters and glassware, were pre-heated and carefully rinsed with distilled water that had been filtered through 0.7 μm GF/C grade fiberglass filters (Whatman).

Preparation of the samples for analysis consisted of vacuum filtering each sample through two fiberglass filters of 1,2 μm and 0,7 μm in order to get the two size fractions. Trapped material on fiberglass filters was treated with hydrogen peroxide to remove interfering organic matter and finally, the filter placed on pyrolysis tin cups following the method highlighted by Gomiero et al. (2019). Procedural blanks were run in parallel to each sample to exclude plastic contamination in the analytical process. 500 mL of distilled water pre-filtered through 0,7 um GF/C filters, were put in 1 L beakers and used to evaluate possible sample contamination from atmospheric fall-out. The blanks were then treated in exactly the same way as the drinking water samples and analysed in PYR-GCMS.

2.4 Analytical Techniques

The analytical technique used in this research was developed and is described in detail by Gomiero et al. [11, 12]. Pyrolysis GCMS measurements were performed by a Shimadzu Optima 2010C GCMS controlled by GCMS solution V 4.45, equipped with a Rxi-5ms column (RESTEC, Bellefonte, PA) and coupled with Frontiers Lab's Multi-Shot Pyrolizer EGA/PY-3030D with auto-shot sampler (BioNordika, Norway). Pyrolysis was performed at 590 °C on a tin pyrolytic target cup. Thermochemolysis was performed by adding 10 mL of tetramethylammonium hydroxide (TMAH, 25% in water) in tin cups pre-loaded with samples, allowing it to dry at 40 °C on a heat plate prior to pyrolysis. Eight of the most commonly used plastic polymers of >99% purity such as polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride

(PVC), polyamide (PA6), polymethyl methacrylate (PMMA), Polycarbonate (PC) and polyethylene terephthalate (PET) were purchased from Goodfellow Ltd. (Huntingdon, England) to set up the calibration and quantification curves.

3 Results and Discussions

The samples were tested for the following polymers; polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyamide (PA66), polymethyl methacrylate (PMMA), polycarbonate (PC) and polyethylene terephthalate (PET). The detection limits of Pyrolysis GCMS was calculated 1 μg for each of them and recovery from 81–97% depending on the polymere.

Triplicate samples were analysed from each sampling site and the results are presented as a mean of the three measurements.

Only two types of polymers, PVC and PE, were detected in the samples of drinking water in Akureyri as can be seen in Table 1.

In Sites no. 6 and 7 PE was measured in the range of 2 μg/L in the size fraction >1,2 μm. However, PVC was measured in both size fractions and was present in sample Sites no. 1, 3, 5, 6, 7, 8.

Sample Site no. 7 contained the most PVC or 5,2 μg/L, while the other sample sites contained less than 1,5 μg/L of PVC. Sample Sites no. 6 and 7 were the only once containing both PVC and PE. Sampling Site no. 7 was at the end of the plumbing in an old part of the town and was the only sample that went through a plumbing of cast iron. Apart from Site no. 7 no space related trend can be detected.

Table 1. Results of measurement of microplastics in drinking water in Akureyri 2018. Sampling sites where polymers were not detected (n.d.) or detected (indicated with the sampling site number).

Polymers	Size > 1,2 μm	Size 0,7–1,3 μm
Polyethylene (PE)	6, 7	n.d.
Polypropylene (PP)	n.d.	n.d.
Polystyrene (PS)	n.d.	n.d.
Polyvinylchloride (PVC)	1, 6, 7	1, 3, 5, 8
Polyamide (PA66)	n.d.	n.d.
Polymethyl methacrylate (PMMA)	n.d.	n.d.
Polycarbonate (PC)	n.d.	n.d.
Polyethylene terphtalate (PET)	n.d.	n.d.

4 Conclusions

These results are the first obtained on microplastics in freshwater in Iceland. Limited amounts of PE and PVC was detected in the drinking water of Akureyri, but PP, PS, PA6, PMMA, PC and PET were absent. The plumbing is mostly from HDPE that may

explain the presence of it in two of the samples, but the analytical method does not distinguish between high, medium or low-density PE. The presence of PVC has no obvious explanation, other than the fact that PVC is a frequently used polymer. About 10% of plastic demand in Europe is PVC. However, PE (LD, MD and HD) is the far most common polymer at 30% of the total demand in Europe [17]. The results are in line with the current literature reporting that microplastics are ubiquitous worldwide. Recently attention has been given to possible atmospheric fallout of microplastics, even in remote mountain catchments [2, 8, 9].

The presence of plastics in the size range 0.7–1.2 μm raises concerns since the so called nanoplastics (plastics < 1 μm) can enter the cell walls and interfere directly with the cell metabolism [18].

There is a need to examine further the drinking water in Iceland with more samples and larger volume samples.

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Association of Potential Human Pathogens with Microplastics in Freshwater Systems

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1 Introduction

Microplastics (MPs) have become the main form of pollution in the world's oceans (80% of marine litter consists of plastic) because of their slow degradability leading to their accumulation in the environment [1]. MPs have become a global hazardous pollutant [2, 3]. MP particles result from the degradation of large plastic materials in the environment via physical, chemical or biological processes. Potential risks of plastic particles in ecosystems have been reported through both laboratory and field investigations [4]. These include potential endocrine disruption in vertebrates and some invertebrate species, physical injuries of gastrointestinal and digestive tracts of animals, and tissue damage [5–7]. Over the past decade, numerous studies have demonstrated that MPs can serve as vectors for the dispersal of toxic substances such as heavy metals, persistent organic pollutants (POPs), and pathogens, increasing their dispersal opportunities in marine and freshwater systems [4, 8, 9]. Concerns have been raised regarding the potential for MPs to represent new substrates for microorganisms, mainly harmful and pathogenic ones [10]. Recently, MPs have been observed in drinking water sources, which has triggered discussions on their possible implications for human health [11].

The aim of this study is to look at potential human pathogens that can be found on the surfaces of MP particles after being released into the freshwater environment. The current study enhances our knowledge of freshwater microbial assemblages on MPs, while focusing on pathogenic bacteria, and assessing the potential risks that their presence in freshwater has for humans.

2 Experiment Design and Set up

Polyethylene (PE) particles (0.96 g/cm^3, 1000 μm diameter) were treated with chicken egg white lysozyme used to burst the bacteria by degrading the polysaccharide chain found in bacterial cell walls. 100% ethanol was then used to sterilise MP particles prior to the experiment. MP particles were then incubated for 14 days in freshly sampled river water taken from the River Barrow, Ireland (52°49′37.9″N 6°56′17.9″W). A total

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of 18 MP samples (1.0 g each) were used in this study, where nine MP samples were directly immersed in the River Barrow (RB) (52°49′37.9″N 6°56′17.9″W) and nine were placed in water samples collected from the River Barrow (RB) and incubated at room temperature in the laboratory (RBL). Both sets of microplastics were left immersed for 14 days.

The hypotheses are that environmental factors play a major role in bacterial colonisation of MPs surfaces. Specifically, we wanted to test the hypothesis (1) that microplastics act as a reservoir for a diverse microbial community, some of which could be potential human pathogens; and (2) that environmental conditions in the river (specifically water temperature) will have an impact of the composition of these microbiomes with potential impacts on the risk of these MP in acting as reservoirs and vectors for human pathogens.

2.1 Materials

Total microbial genomic DNA was extracted from microplastic particles using E.Z.N.A Bacterial DNA Kit, according to the manufacturer's protocol. DNA concentration and quality were evaluated by optimal density using Denovix DS-11 spectrophotometer at wavelengths of 220 and 340 nm. DNA purity was then evaluated using 1.5% agarose gels. Water samples were collected from River Barrow (52°49′37.9″N 6°56′17.9″W). A two-hundred- μm pore sized net was used to hold in place MP particles for exposure purpose in River Barrow (in situ).

2.2 Methods

MP Samples Incubation

The nets were sterilised using 100% ethanol to eliminate bacteria prior to the experiment, and approximately 0.1 g of MP particles were placed inside the nets. Nine nets containing MPs (n = 9) were exposed to river water by placing them directly in the river (52°49′37.9″N 6°56′17.9″W). (in-river), and the other MP samples (n = 9) were exposed to river water samples taken from the River Barrow and held at room temperature in the laboratory (in-laboratory) for 14 days.

DNA Extraction from MP Samples

Total microbial genomic DNA was extracted from MP particles using E.Z.N.A Bacterial DNA Kit, according to the manufacturer's protocol. DNA concentration and quality were evaluated by optimal density using DeNovix DS-11 spectrophotometer at wavelengths of 220 and 340 nm. DNA purity was then evaluated using 1.5% agarose gels. The DNA samples were stored at −80 °C prior to sequencing. DNA of sufficient quality and quantity was extracted from 11 out of the 18 MP samples and these 11 MP samples were used for microbiome analysis, where six (n = 6) were selected from the in-river samples and five (n = 5) were selected from in-laboratory samples.

2.2.1 Library Preparation and Sequencing

PCR was carried out on the DNA samples to amplify the V3-V4 region of the bacterial 16S gene. Sequencing libraries were produced using NEBNext® Ultra™ DNA Library Prep Kit for Illumina, according to the manufacturer's recommendations, and index

codes were added. The generated libraries were quantified via Qubit and qPCR. The library was sequenced on Illumina platform, and 250 bp paired-end reads were generated. The sequencing was conducted by the Beijing Novogene Corporation (Beijing).

2.2.2 Data Analysis

The paired-end reads from the raw DNA fragments were merged using FLASH software, a very fast and accurate analysis tool, which was designed to merge paired-end reads when at least some of the reads overlap the generated reads from the opposite end of the same DNA fragment, and the splicing sequences were called raw tags. Quality filtering on the raw tags were performed under specific filtering conditions to obtain the high-quality clean tags according to the Qiime quality controlled process (V1.7.0, http://qiime.org/scripts/split_libraries_fastq.html). The chimera sequences were removed using UCHIME Algorithm. Representative sequence for each operational taxonomic unit (OTU) was screened for further annotation. For each representative sequence, Mothur software was performed against the small subunit ribosomal ribonucleic acid (SSUrRNA) database of SILVA Database. The effective tags were then clustered into OTUs at $\geq 97\%$ sequence similarity, in order to analyse the species diversity in each sample. Heatmaps were generated using the FactoMineR package and ggplot2 package in R software (Version 2.15.3) with taxonomic data.

3 Results and Discussion

Six samples (n = 6) of microplastics that were left in situ in the River Barrow for 14 days (referred to hereafter as RB) and five samples (n = 5) of the same microplastics exposed to river water from the River Barrow under laboratory conditions for a period of 14 days (referred to as RBL) were analysed for the microbiome attached to their surface. Samples were used to generate V4 16S rDNA gene profiles. A total of 1,135,496 clean reads were obtained in the analysis, with an average of 83,845 ± 16,035 reads for the RB samples and 126,485 ± 16,363 reads for the RBL samples. The higher number of reads obtained in the laboratory incubated samples (RBL) are likely due to the rapid replication of mesotrophic bacteria in these laboratory incubated samples. The average read length was 422 bp, with an average GC content of 54%. Based on the rarefaction curves (data not shown), OTU saturation was obtained at about 30,000 reads per sample.

The top 10 most abundant phyla and genera were identified (Fig. 1A and B) in all the MP samples. At the phylum level, proteobacteria was the most predominant group, comprising of between 30−40% of the microbiome in RB samples and 80−90% in RBL samples. The data shows that the in-river incubated MP samples (RB) had a much greater number of OTUs associated with them (on average 2596 ± 258 OTUs) compared to the ex-situ laboratory incubated (RBL) samples, which had on average just 865 ± 258 OTUs associated with them. This represented a 66.6% reduction in the microbial diversity of laboratory incubated samples (RBL) compared to in-river incubated samples (RB) and this proved to be statistically significant using both t-tests and Wilcox tests ($P < 0.05$).

At the genus level, there was a high diversity of bacteria within the in-situ MPs (RB), and a much lower diversity was observed in those MP samples exposed to river water under laboratory conditions (RBL). Interestingly, OTUs representing the *Escherichia-Shigella* genera were identified in all eleven MP samples. These microbes are coliforms, which are indicators of faecal contamination within the river and which can be human pathogens. In the microbiomes of the river incubated samples (RB) *Escherichia-Shigella* OTUs represented on average 2.5 ± 1.1% (1.1–4.5%) of the total genera

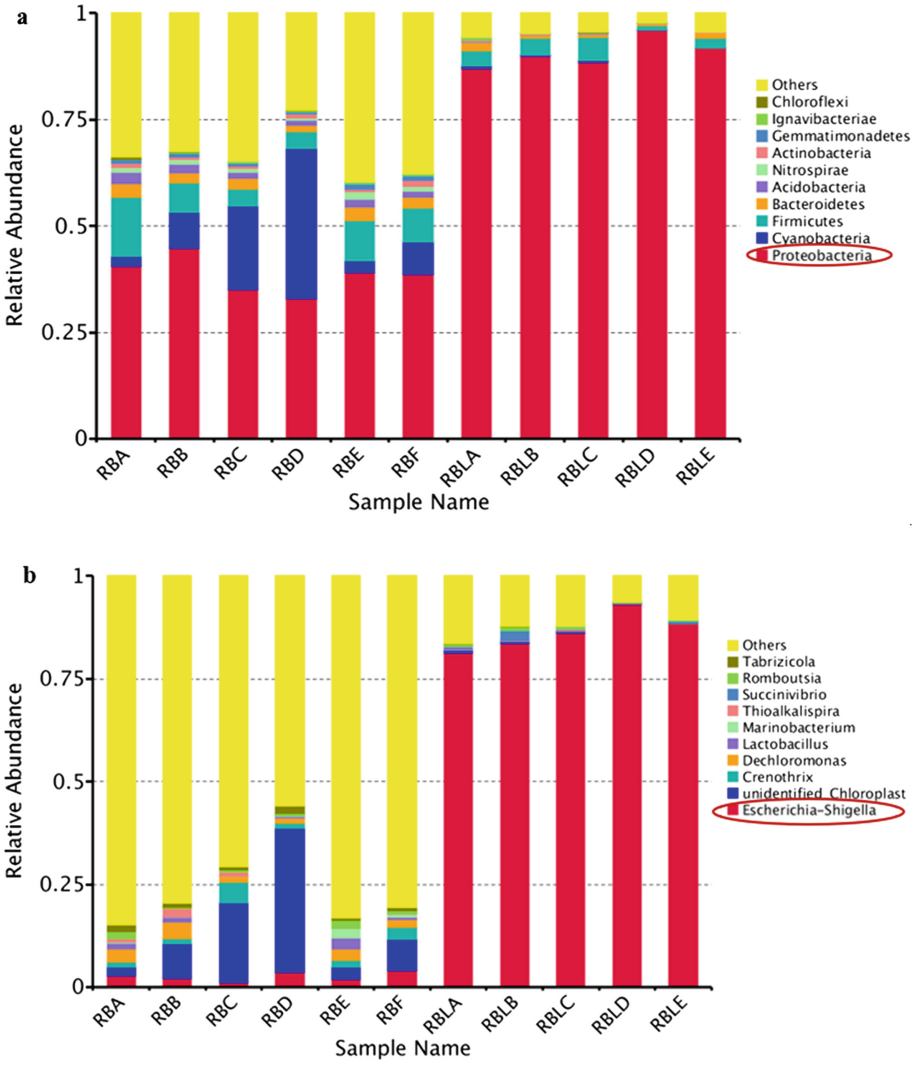

Fig. 1. **(A)** Phylum level bacterial community composition in in-situ and laboratory exposed MP samples and **(B)** Genus level composition. RB (River Barrow) refers to the MP samples incubated in the river for 14 days and RBL (River Barrow laboratory) refers to the MP samples incubated in river water samples held at room temperature for 14 days in the laboratory.

identified, whereas in the laboratory incubated samples the *Escherichia-Shigella* OTUs represented 81–93% (mean 86.5 ± 4.5%) of the genera identified.

The alpha diversity of the bacterial communities was determined using Chao1, Simpson, ACE, Goods coverage and Shannon's diversity metrics (Table 1). Both Observed OTU numbers and Chao1 showed a high level of species richness in the in-river incubated microplastic microbiomes and a low level of species richness in the laboratory incubated samples. Likewise, both Shannon and Simpson indices show a high level of species richness and evenness in the in-river incubated microplastics samples and a low level of species richness and evenness in the laboratory incubated samples.

Table 1. Alpha diversity indices for the microbiomes of river incubated (RB) and laboratory incubated (RBL) micro-plastic samples. The data shows the averages and standard deviations.

Sample name	Observed number of species	Shannon	Simpson	Chao1	ACE	Goods coverage
RB (n = 6)	2596 ± 258	8.42 ± 0.89	0.97 ± 0.04	3233 ± 648	3165 ± 424	0.985 ± 0.0040
RBL (n = 5)	865 ± 316	1.60 ± 0.51	0.25 ± 0.08	1244 ± 452	1332 ± 498	0.992 ± 0.0029

Analysis of the phylogenetic composition of MPs bacterial community between the six replicates of RB samples indicated no significant difference in microbial diversity within the community. Similarly, there was no significant difference in the microbial communities across the five replicates of the RBL samples. However, between the in-river incubated samples and the in-laboratory incubated samples there was a significant difference observed in all the indices used (Table 1). Alpha diversity comparison based on Shannon's diversity index can be seen in Fig. 2A and beta diversity can be seen in Fig. 2B. Beta diversity indices also confirmed a significant difference between the in river incubated and in laboratory incubated samples, and again showed that between replicates there was very little difference in the bacterial communities.

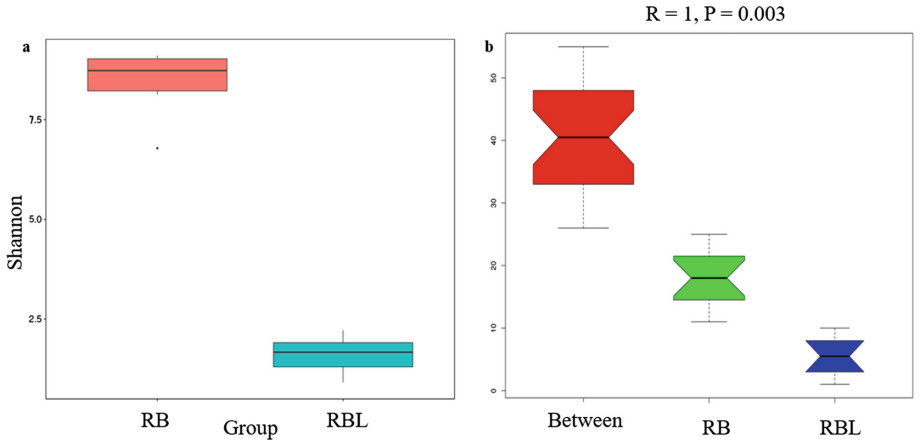

Fig. 2. Alpha diversity **(A)** and beta diversity **(B)** comparison of the microbiomes of RB and RBL samples. RB (River Barrow) refers to the MP samples incubated in the river for 14 days and RBL (River Barrow laboratory) refers to the MP samples incubated in river water samples held at room temperature for 14 days in the laboratory.

Examination of the OTU table generated in the analysis shows the presence of potentially pathogenic groups such as *Faecalibacterium*, *Enterococcus, Enterobacter*, *Campylobacteraceae*, *Rumunicoccus*, *Helicobacter*, *Clostridia*, *Romboutsia*, *Burkholderia* and *Escherichia-Shigella* on the surfaces of MPs after 14 days in the river. At the species level potential pathogens such as *Escherichia coli*, *Enterococcus faecalis*, *Helicobacter rodentium* and *Clostridium perfringens* were found on the microplastic surfaces.

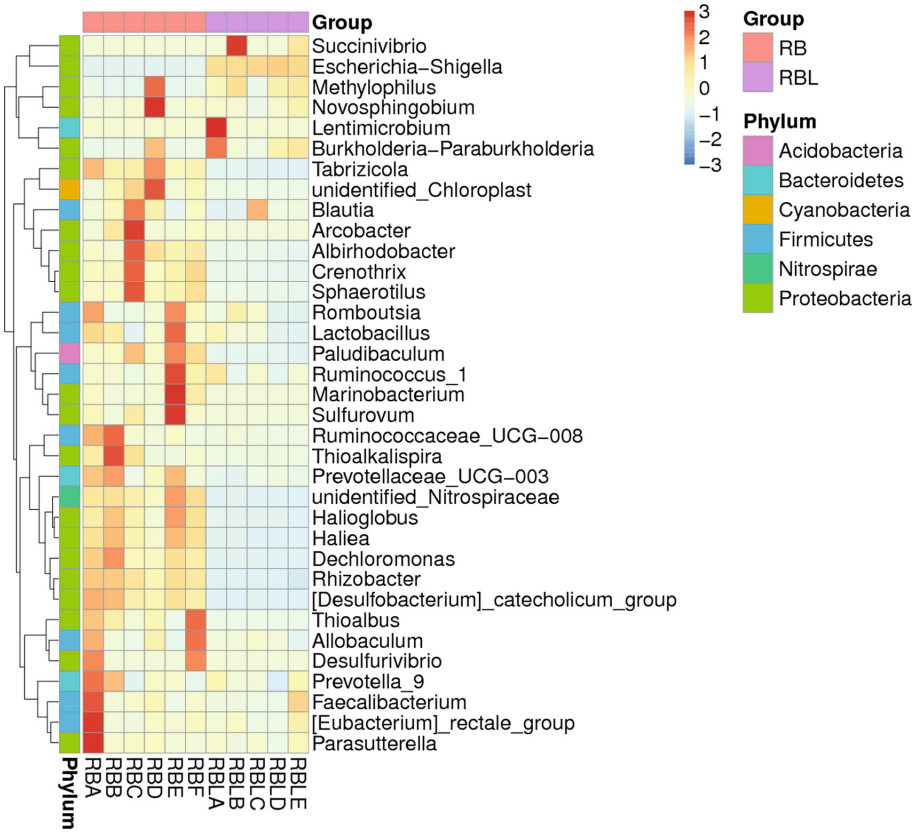

Fig. 3. Heatmap showing the 35 genera with significance differences of relative abundances among the environmental and laboratory samples. The colour-coded indicates the heatmap scale from −3 to 3.

The heat map shown in Fig. 3 shows that there is no obvious correlation of any particular genera with the in river incubated samples. With the exception of one or two cases these groups appear to be evenly associated with the six replicates of MP beads that were incubated in the river. Similarly, in the laboratory incubated microplastic samples there seem to be a relatively even distribution of microbial groups across the five replicates. However, in these samples there does appear to be a marked reduction

in the abundance of groups such as Tabrizicola, Paludibaculum, Nitrospiraceae, Halioglobus, Haliea, Dechloromonas, Rhizobacter and Desulfurivibrio while at the same time a significant enrichment in the abundance of the *Escherishia-Shigella* genera. The depletion in the abundance of these microbial groups on the microplastic surfaces may reflect a lack of adaptation ability of these microbes to increased water temperatures. Typical room temperatures in the laboratory ranged from 20–25 °C, while typical temperatures in the River Barrow during the same time period range from 13–16 °C. Another possibility is that these depleted genera lack niche competiveness compared with the *Escherishia-Shigella* genera, or perhaps even the production of growth inhibiting substances by the *Escherishia-Shigella* strains.

3.1 Discussion

Microplastics offer a stable surface onto which complex microbial biofilms can develop. In natural water systems there is the potential for pathogenic microbes to colonise microplastic surfaces. The subsequent ingestion of these plastics by aquatic fauna such as fish and shellfish, or the direct consumption of microplastic particles through drinking water/or leisure related activities such as swimming, are potential pathways for the entry of these pathogenic microbes into the human food chain. Our study investigated the potential of microplastic beads to pick up potential human pathogens from natural river water. Our analysis found that the Proteobacteria were among the most abundant group of microbes present on the microplastics. There are numerous Proteobacteria that have negative impacts on human health, such as *Escherichia coli* and *Shigella* which cause diarrhoea and fever [12] when ingested. We observed the occurrence of potentially pathogenic *Faecalibacterium*, *Enterococcus*, *Enterobacter*, *Campylobacteraceae*, *Rumunicoccus*, *Romboutsia*, *Burkholderia*, *Escherichia* and *Shigella* on the surfaces of MPs after 14 days in the river. This is an indication of possible human or animal fecal contamination entering the river water. Among the listed genera, some of them are known to cause diseases. For example, *Faecalibacterium* is linked with inflammatory bowel disease and colorectal cancer. *Ruminococcus* is a microbe associated with the gut microbiome and again is a strong indicator of faecal contamination. It is also linked to individuals suffering from irritable bowel syndrome. *Romboutsia* is linked with inflammatory bowel disease, *Burkholderia* is linked with opportunistic pathogenesis in individuals with cystic fibrosis, and *Escherichia Shigella* is linked with serious, life-threating gastrointestinal illnesses.

This study demonstrated that microplastics have the potential to act as reservoirs and vectors of potential human pathogens in freshwater ecosystems. Previous studies on potential associated pathogens have identified the genus *Arcobacter* and *vibrio* in a higher abundance on the surfaces of MPs [13, 14]. This study revealed that *Escherichia-Shigella* was in a higher abundance in all the samples compared to other genera. Although our study shows the presence of these potential pathogens, it is likely that these are not pathogenic members of these groups. More work needs to be carried out to determine if these strains are indeed pathogens using either molecular methods (e.g. PCR) to identify pathogenic traits or through whole genome sequencing.

Environmental conditions in surface waters such as nutrient levels (nitrogen, phosphorus, dissolved and particulate organic matter), dissolved oxygen and

temperature are likely to play a significant role in the development of the microbial communities on the surfaces of microplastics. We observed that increased water temperature significantly altered the community richness and evenness of the microbiomes of microplastic beads. Specifically, the increase in temperature significantly increased the abundance of *Escherichia coli*. While the experimental increase in water temperature was quite extreme ($\sim$10 °C increase), climate change models are predicting more extreme weather events; increased average temperatures and more extreme weather events such as prolonged periods of heatwaves and droughts. Over the last decade Ireland has experienced its warmest average summer temperatures on record. Therefore, these changes in environmental conditions may lead to similar shifts in the bacteria community structure of microplastics present in natural water ecosystems, increasing the risk of these plastic particles acting as a reservoir for human pathogens.

4 Conclusions

In conclusion, microbial community characteristics of 11 MP samples in freshwater (River Barrow) were studied using 16S rDNA amplicon sequencing. Proteobacteria was the predominant phyla across all the samples, particularly in the laboratory samples (RBL) when compared to the environmental samples (RB). The results indicated the presence of potential human pathogens in all the samples, confirming our hypothesis that MP particles act as a reservoir for pathogens. We also showed that environmental conditions (water temperature) have a profound effect on the microbiome composition on the surface of MPs. Increased water temperature favoured the development of bacterial communities with high proportions of potential human pathogens. The results give a better understanding of MP particles in freshwater ecology. Such information can be used as a scientific tool to help understanding the ecological and human health consequences of MP particles in the environment. MPs are ingested by seafood, for example fish, shrimps, crab and other animals, which could be contaminated with polluted MPs in the aquatic systems and potentially enter the human food chain. Therefore, limiting the emissions of plastics into the environment should be a top priority, in order to reduce the risk of these particles, and the potential pathogenic bacteria that they carry, entering the human food chain.

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Sample Preparation and Analysis Methods of Microplastics

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1 Introduction

This research study made in collaboration between Aquafil Spa and CNR STIIMA (Biella Department) is aimed at the creation of a standard method applicable to the determination of microplastics (MP) in different matrices present in the textile field such as waste water from clothes washings, water effluents or solids from industrial processes, air. A very recent dossier [1] compiled by ECHA (European Chemicals Agency) for EU commission defines "microplastic" as a material consisting of solid polymer-containing particles, to which additives or other substances may have been added, and where $\geq 1\%$ w/w of particles have all dimensions 1 nm $\leq$ x $\leq$ 5 mm, or, for fibres, a length of 3 nm $\leq$ x $\leq$ 15 mm and length to diameter ratio of >3. The sources of microplastics are numerous, from tires, to plastics abandoned in the environment (terrestrial and/or marine, to paints, textiles, etc.). Variations are also due to their shape and size. In the case of those released by textiles the typical morphology it is the fibrous one and their diameter and length can vary depending on the construction parameters of yarns and fabrics or on washing conditions.

Because of the environmental problem represented by microplastics, their number, shape and size are relevant parameters for assessing their impact and consequently the development of a counting technique is the only logical approach. Moreover, many of the microparticles analyzed are not of synthetic origin and therefore there is a need to identify and distinguish them from microplastics.

The method is designed to provide the nature, numerical concentration, surface area (estimated) of microplastics in an aqueous or aeriform matrix.

Depending on the matrix, it may be necessary to pre-treat the sample to concentrate the microplastics and eliminate inorganic and organic contaminants (e.g. biological) that could interfere with their identification. The method involves a preliminary observation of the sample under an optical microscope and subsequently identification of microplastics with molecular spectroscopy. The method foresees the possibility of using two different molecular spectroscopy techniques, Micro-FTIR (Fourier

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Transform InfraRed Spectroscopy coupled with optical microscopy) and Micro-Raman (Raman Spectroscopy coupled with optical microscopy) to identify and count plastic particles up to a submicronic dimension. The parameters determined could be useful also for subsequent ecotoxicological studies. Moreover, the analysis of the MP will be suitable for specific textile production, textile products of the market (allowing the evaluation of MP production during the life of garments) or any other process/semi-finished/manufactured item.

The standard method describes the analysis method for a single filter. However, the errors on the qualitative and quantitative determination of the microplastics that can derive from presence of contaminants or variability between different filters imply the need to perform replications to establish accuracy and precision.

2 Experimental

2.1 Pre-screening

In case of samples of unknown origin a series of pre-screening tests are provided for the possible presence of salts or organic matter. For aqueous samples standard tests such as conductivity [2], chemical oxygen demand (COD) [3], total suspended solids (SST) [4], optical microscopy (OM) for fibre identification and evaluation of image quality [5, 6] are recommended. For what concern airborne microplastics the reference methods are those used for dust and particles emission in the work environment and in atmosphere [7].

2.2 Materials

Laboratory glassware is washed with demineralized water filtered on nitrate, acetate, mixed esters filters (0.45 μm porosity). Moreover, anodisc composed of a high purity alumina (0.02−0.2 μm porosity), silicon (5 μm) and gold polycarbonate membrane (0.8 μm) for μ-FTIR and Raman analysis are used.

To avoid contaminations only glass caps are used. Furthermore, operator should preferably wear clothes made of natural fibres. In any case, contaminations can be taken into account performing blank tests. All reagents for preparation of the sample and cleaning of filters were RPE- for analysis.

2.3 Methods

2.3.1 Preparation of Samples

Preparation of samples depends also by the pre-screening tests. Pre-treatments can be necessary before or after filtration of the washing effluent with a 15% H_2O_2 solution for a duration between 7–30 days with the aim of eliminating any trace of organic matter without damage MP including Fig. 1 shows some examples of hydrogen peroxide pre-treatments carried out on nitrocellulose filters.

Always depending on the pre-screening in OM it will be possible to decide to perform a pre-dilution with water from the starting sample in order to produce a

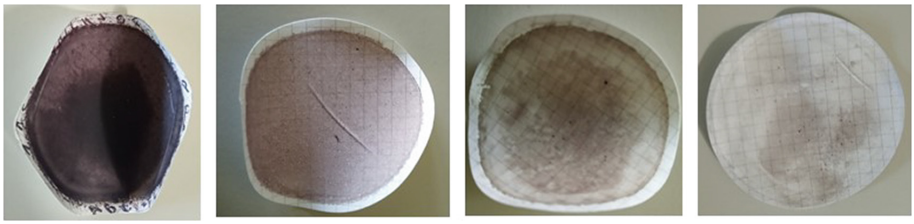


Fig. 1. Examples of nitrate/acetate filters after different pretreatments

sub-sample to be tested with micro-FTIR/Raman spectroscopy for the identification and counting of the MP.

To obtain a homogeneous distribution of MP on the filter the water sample is first homogenized it is placed in vigorous stirring (or ultrasonication) at 30–50 °C for at least one night in order to improve the hydration of any MP present. Reducing the wall effect and the possible flotation due to the surface tensions and the hydrophobic nature of the MPs. The sub-sample aliquot will then be diluted to a factor that allows an adequate particle count in the final filter.

The dilution is carried out with pre-filtered demineralised water and by washing (with recovery solution) of the equipment (pipettes, cylinders, etc.) used for the collection and dosing of the primary sample.

2.3.2 Filtration

If the sample has a high presence of MP and suspended solids, even after the pretreatment, the filtration will take place in two separate steps: filtration of the decanted supernatant, filtration of the final deposit (precipitate) with recovery by final washing operation. The subsequent analysis will be conducted on the two filters produced and the final report must consist of the sum of the results obtained. In order to be able to determine the MP in a matrix, a significant aliquot must first be transferred to a suitable filter (made with a vacuum system) to allow the subsequent quali-quantitative analysis. Consequently three different approaches must be followed depending on the physical state of the starting matrix: a powdery solid or a compound of several solid materials, an aqueous solution (liquid), an aeriform.

In the case of solid samples, if powdery type, a preliminary dispersion in a known volume of demineralised water or in a dispersing solution consisting of a surfactant in demineralised and filtered water will be carried out. If it is a non-powdered solid sample, it is first necessary to proceed with a suitable disintegration treatment (for example ultrasound and/or acid/basic/oxidant digestion). The filtration of a volume of the suspension will be carried out on a filter of suitable material, porosity and shape (as a function of the spectroscopic technique used). For the analysis of aqueous liquids proceed with filtration and related purification, washing and recovery procedures on 0.45 micron filters of cellulose esters.

In the case of aeriform samples, air will be sampled following the specific reference standards for the collection of dust in the air (work environment and/or emissions) using suitable filters for the subsequent analysis.

2.3.3 MPs Characterization

The microplastics are preventively observed and measured (length and diameter) with an optical microscope in reflected light at 50X and 100X magnifications on a filter that allows to detect the position of the particles for subsequent analyses and to exclude from the count any fibers (e.g. natural), contaminating particles and purification level of organic substances. Figure 2 shows some examples of microplastics in fibrous form, but it is possible find also particles or other forms.

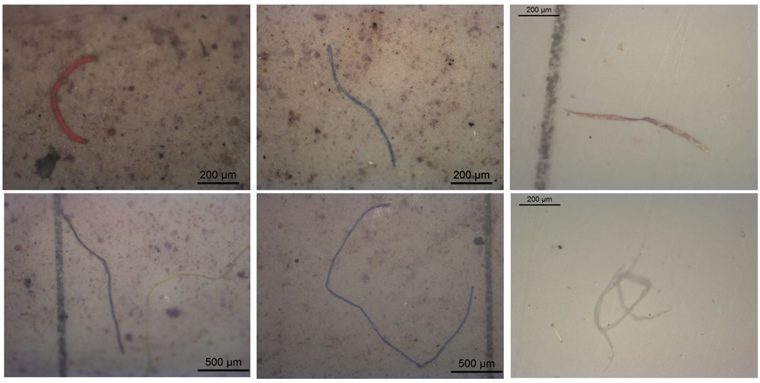


Fig. 2. Examples of microplastics observed in OM

Then a known volume of the previously treated aqueous dispersion will be filtered on a suitable filter (e.g. silicon filter) for identification and counting of microplastics present with spectroscopic techniques. For the identification and quantification of microplastics two molecular spectroscopic techniques can be used: micro-FTIR (for microplastics up to the size of 5–10 µm) or micro-Raman (for microplastics up to the size of 0.2–0.5 µm). In the case of micro-FTIR, to identify materials (microplastics) as small as 10 microns speed, resolution, and analytical power are increased using a liquid nitrogen cooled MCT detector. The image analysis software measures particles size, sets best fit aperture, collects spectra and background and compare spectrum with libraries. Particle analysis is simplified obtaining material identification, size, percentage of distribution and chemical image of particles within an area. Depending on the analysis performed, the most suitable filters can be selected. For transmission mode alumina or silicon filters are employed (Fig. 3), while in reflection mode gold coated filters can be used (Fig. 4). For attenuated total reflection (ATR) mode cellulose nitrate/acetate filters (porosity not higher then 5 µm). One of the advantages of µ-FTIR and/or Raman identification of microplastics, is to avoid over estimations due to the possible presence of non-plastic material in the sample, such as cellulose fibres.

The image analysis aims to acquire maps and mosaics on specific areas of the sample, identify the particles to be analyzed by size and shape and to determine other characteristics such as area and length. It can be both manual or automatic (Fig. 5). In this last one, the visual image consists of more than 200 video captures combined into a

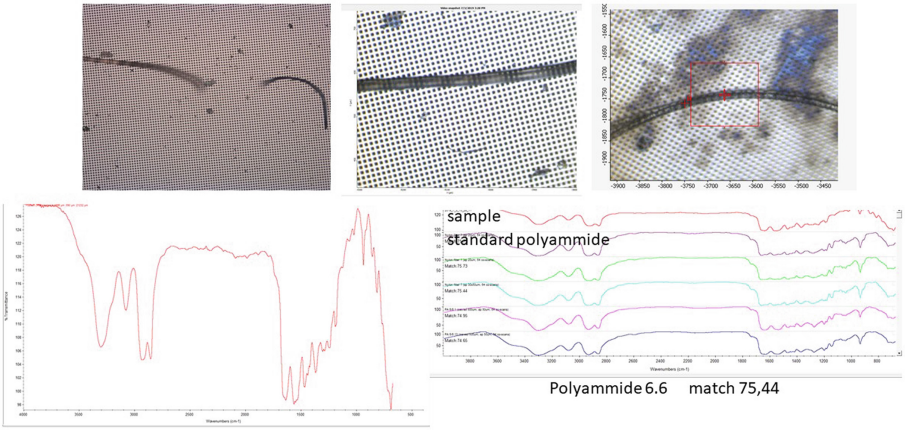


Fig. 3. Examples of microplastics and spectrum on silicon filter in transmission mode

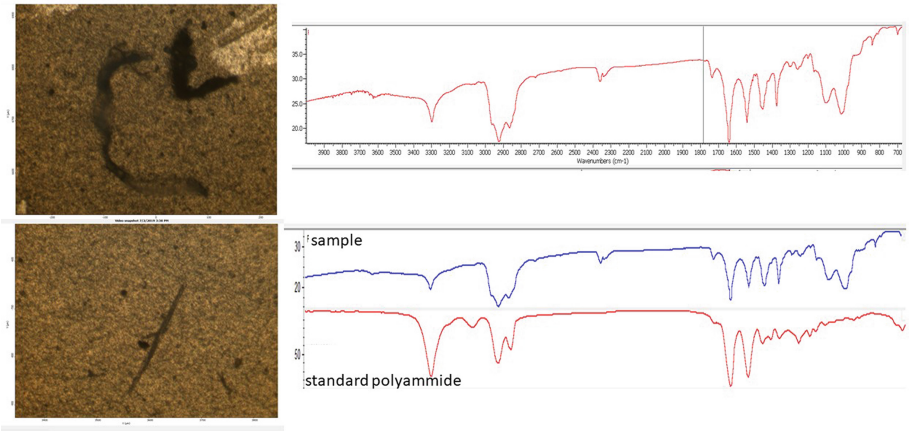


Fig. 4. Examples of microplastics and spectrum on polycarbonate filter in reflection mode

mosaic covering approximately 1 cm^2. After a region from the video image is selected, the software identifies the target particles and proceeds to produce spectra for each particle/microfiber. These spectra are then searched against a spectral library, and a report catalogs the number of particles/fibers in the inspection area and classified according to their morphology and size, providing additional information on the sample such as: percentage distribution, morphology, number and size, mapping, section area estimation of microparticles and microfibres.

The other analytical technique used is micro-Raman spectroscopy, that exploiting sub-micron wavelength lasers as its light source is capable of resolving particles down to 1 μ and less. The other advantages compared to micro-FTIR are the ease of sampling, the use of white-light microscopes that facilitate easy viewing of particles and the

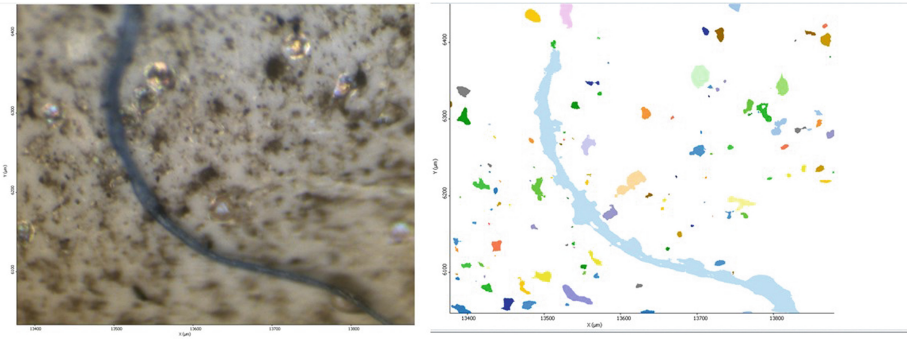


Fig. 5. Examples of automatic image analysis

non sensitivity to the presence of water. The major drawbacks are possible fluorescence in the samples (intrinsic or caused to impurities) that can disturb the acquisition of Raman spectra and the long time of acquisition due to the low Raman energy emission. However, the interference of fluorescence can be circumscribed with the chose of appropriate purification step before analysis and selection of acquisition parameters such as laser wavelength, laser power, photo bleaching, increasing exposition time in order to degrade the contaminants at the focal point. Moreover, the time consuming can be reduced with an automated procedure for the recognition of particles and automated measurements.

No limitations on the material of filters are reported. In general, depending on the different kind of spectroscopy used, the dimension of filters can vary in terms of shape (circular, square) and dimension (13, 25, 47 mm of diameter or 10 mm side) [8].

2.3.4 Optical, Micro-FTIR and/or Raman Test Report

The test report will consists of a summary table indicating: morphological and dimensional classes of microplastics, types of polymers found, estimated area (expressed in mm^2/unit of sample analyzed) per each polymer type, estimated weight (expressed in mg/unit of sample analyzed) per each polymer type. Eventually test report can include also photographs of the samples, optical images, particle mapping images, spectra, summary charts of the different classes identified (morphological, dimensional, polymer types) (Fig. 6). Moreover, the classification of MP analyses into size classes was carried out following recommendation of TR21960 ISO This classification is based on a numerical model and the "historic definition" of MP. The following size classes are proposed: 5,000–1,000 μm, <1,000–500 μm, <500–100 μm, <100–50 μm, <50–10 μm, <10–5 μm, <5–1 μm. The maximum dimension of a particle or film fragment or the length of a fibre defines the size.

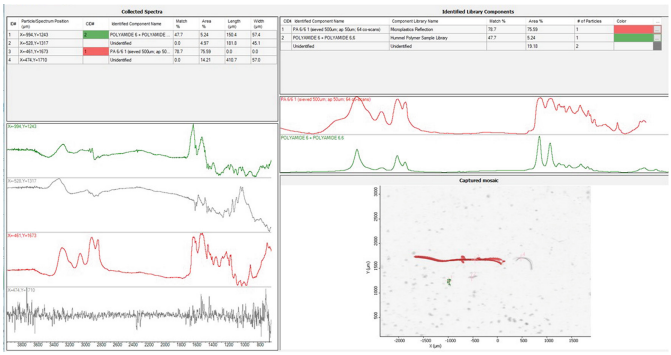
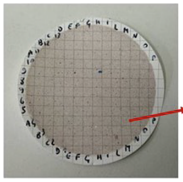


Fig. 6. Examples of one report from IR software

Figure 7 shows an example of classification of MP of water effluent textile industrial carried out by optical microscopy. In this case the length of microfibres is between 1000−100 μm.



filter 1		
Coordinates	lenghth [μm]	diameters [μm]
H 7	563,64	116,15
P 6	656,3	17,39
O 6	1068,27	26,73
L-M 6-5	754,66	37,79
N-O 5	886,72	15,6
M 4	467,11	29,48
M 4	291,82	52,87
i 3-2	328,82	42,47
D-E 3	432,31	15,04
F 2	576,77	14,42
H 2	166,66	46,56
F 13-14	560,57	14,3
D-E10-11	380,51	24,58
i-H 10-11	214,7	10,3
G 10	538,13	9,48
D 10	616,9	13,16
C 9	848,38	14,68
E 8-9	935,73	7,31

	length of the microplastics [μm]			
filters	1000-500	<500-100	<100-50	<50-10
1	11	6	1	0
2	12	25	0	0
3	24	15	0	0
4	11	7	0	0
5	25	38	3	0
6	24	39	0	0

Fig. 7. Example of classification of MPs (length and diameter) carried out by optical microscopy

3 Conclusions

In this work, made in collaboration between Aquafil Spa and CNR STIIMA, a draft of a standard method to identify and quantify microplastics deriving from waste water from clothes washings, water effluents or solids from industrial processes and air is described. Different starting matrices and sample pre-treatments are considered and optical microscopy and molecular spectroscopy (micro-FTIR and micro-Raman) are used in combination as analytical methods. The work is in progress and a first version has already been presented to UNI/CT 046/GL 12 “Sustainability” and it will be soon evaluated by a CEN (European Committee for Standardization). Once set up, it can be

foreseen that this method could be expanded and made suitable for the analysis of microplastics of different origins.

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INTO THE MED: Searching for Microplastics from Space to Deep-Sea

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1 Introduction

Microplastics (MP) are defined as "any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1 μm to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water" [1]. Being mostly composed by plastic-fibres (i.e. microfibres) [2], research findings from the past decade reveal that MP are ubiquitously present in marine and freshwater-ecosystems [3]. While there has been a growing interest in studying plastic pollution by linking its sources, pathways, sinks, and effects [4, 5], knowledge about the precise locations of accumulation, the behaviour and/or the fate of MP within the marine-realm remains

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poorly understood [6]. Additionally, these measurements tend to be restricted to particular areas (e.g. subtropical-gyres/coastal-areas) where more directed MP-sampling take place. Most studies focus on surface and/or near-surface water-column estimations, which can lead to underestimation of the global plastic marine-debris budget and hinder realistic model-calibration [7, 8]. Numerical models that study pelagic-plastics attempt to simulate different abundance scenarios in the marine environment [9, 10]. However, in order to calibrate models, they need to be "fed" authentic and validated MP-measurements [8, 11]. Therefore, it is fundamental that further measurements on the MP-distribution takes place throughout the water-column (i.e. from surface to deep-sea) and in the open-ocean. In this context, an oceanographic cruise was conducted in 2018 from Terceira, Azores (open-ocean) to Sicily, Italy (Mediterranean). Water samples were collected at discrete depths using real-time CTD-profiles to characterize the vertical-distribution, composition and concentration of MP in these waters, and to infer if their main patterns were shaped by specific physical/biological-mechanisms.

2 Experimental

Water-sampling took place aboard the *R/V-Pelagia* (PE442 "Microplastics-Transit-Cruise") from 27th July to 7thAugust, 2018. A total of 12-hydrocasts (C01–C12; Fig. 1), encompassing 146 water-samples (2L each), were performed along a longitudinal-transect, using a clean-multivalve-carrousel (24 bottles, 12L-each) coupled with a CTD-system (Seabird-SBE21). At each station two 1L seawater-replicates were collected at selected depths: 1 m, 5 m, 25 m, Deep-Chlorophyll-Maximum (DCM) intervals (i.e. DCM^{-}, DCM and DCM^{+}), 500 m, Mediterranean outflow (MOW), and 1500 m. Extra surface (1 m-depth) samples were collected via a flow-through-seawater-system directly on deck. An adaptation of the Hidalgo-Ruz *et al*. [12] and Barrows *et al*. [3] methodologies were followed.

2.1 Materials and Methods

Seawater was collected in 1L glass-jars with aluminium-foil lined lids, which were rinsed with tap water three times (3x) before sampling, instantly capped, and then rinsed 3x immediately before sampling. Hands/forearms were constantly rinsed with tap-water (3x) before all the preceding steps. One blank-sample per hydrocast (i.e. 1L glass-jar filled with tap-water) undertook the same cleaning-procedure/methodology-steps as the other seawater-samples. Possible contamination from tap-water, airborne, and the equipment cleaning-process was assessed.

2.1.1 Laboratory-Processing

Before sampling all laboratory surfaces/equipment was cleaned with a bright-coloured cellulose-sponge. The entire filtration-process occurred inside a still-hood to minimize potential airborne microfibre-contamination. Prior to opening any sample, hands/forearms, glass-petri-dishes, tools (metallic tweezers), and equipment (glass-filtration-apparatus, vacuum-pump, tubes), were thoroughly rinsed 3x under high-

pressure tap-water. Jar-lids were carefully opened (facedown, not touching any surface), and the sample was directly poured into the glass Buchner-funnel, while pumped through a 0.8 μm filter (Whatman mixed-cellulose-ester, 47 mm). Simultaneously, a clean filter was placed in a glass-petri-dish (which underwent the same cleaning-procedure) and exposed to air to control for airborne-microfiber-contamination. The latter was only uncovered during processing of samples from the same hydrocast. Filters were dried at room temperature, covered with aluminium-foil and stored.

2.1.2 Laboratory-Analysis

A MP-analysis laboratory was set-up: ventilation-vents were closed and/or isolated, doors permanently-shut, surfaces covered with glass, and cotton coats were worn. Samples were assessed via a stereo-microscope (under a glass-still-hood). The petri-dish cover was removed, and the whole sample was manually-scanned at 35x. Simultaneously a dry-filter (previously inspected) was air-exposed during all filter-readings.

The visual distinction of potential plastic-particles *versus* natural-fibres followed guidelines of other known observations of synthetic-fibres [2, 3, 12]. The morphological characteristics (i.e. shape/colour) considered in this study included: absence of cellular and/or organic structures, striations, and even width along the particle-length. Additionally, MP-particles were categorized by colour (transparent/blue/black/red/other), shape (fibre/angular/other), and size (<0.51 mm; 0.60–1.53 mm; 1.60–3.10 mm; >3.20 mm).

No chemical inspection that positively confirms polymers was performed at this stage. However, this study enforced a very conservative approach regarding MP-identification. The calculation of potential MP-presence in the water-column was reported as: number of MP-particles-per-liter at a certain depth ($MP.L^{-1}$).

Vertical CTD-profiles were performed along with oxygen-concentration, surface-irradiance, photosynthetic available radiation, turbidity, and fluorescence measurements.

Data Interpolating Variational Analysis of the 1 m-bin averaged data was conducted through a spatial (vertical/horizontal) gridded interpolation with the Ocean Data View software package. This considered the error in the observations and the typical spatial scale of the underlying field into account.

MODIS/Aqua near-surface chlorophyll-*a* (Chl*a*, $mg.m^{-3}$) and skin Sea Surface Temperature (SST, °C) Level-2 imagery data were obtained daily from the NASA Ocean Color web-resource with 1 km spatial resolution. Daily-imagery were further processed using the CANOPUS satellite-processing-system (developed by the University of the Azores). Monthly-averages for both months of the PE442-cruise were calculated.

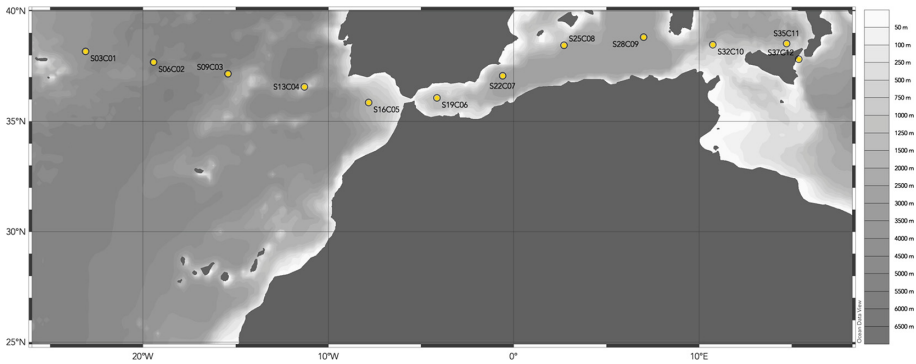

Fig. 1. Bathymetry map with the 12-hydrocasts (C01–C12) performed during the PE442-cruise.

3 Results and Discussion

A total of 192L of seawater was sampled, processed and examined for MP-particles. Microplastics were detected in all 12 hydrocasts, at all depths and in both the Atlantic-Ocean and Mediterranean-Sea (Tables 1, 2 and 3). A total of 263 MPs were identified from all filters (including blanks/air contamination); fibres were the dominant particle-type (257-fibres, 98%). These were predominantly transparent (75%) or blue (16%) and within the 0.51 mm size-classification (length). The mean concentration of MP for all samples was calculated as 1.302 ± 3.439 MP.L^{-1}. Two singular samples contained higher MP-concentrations (C02: 26 MP.L^{-1} at 1500 m; and C07: 40 MP L^{-1} at 45 m). For a total of 204 filters (including blanks/air-contamination), 83 filters were clean (no MP counted). Blanks were contaminated with plastic fibres at five hydrocasts (C05/C06/C08/C10/C11) with 1–3 MP, whereas the remaining six blanks were clean. A slight increase of contamination was recorded in the exposed air-filters: at seven hydrocasts (with exception of C01-C04 and C10) the air-blanks contained plastic-fibres, ranging from 1–4 MP. With this in mind, two conservative scenarios for interpretation purposes were applied. In the first, the exact MP-number (i.e. same shape/colour/size) found in the blanks was subtracted (245 MP remained, 7% cut-off). In the second scenario both blanks and air-contamination particles where subtracted (225 MP remained, 14% cut-off).

Looking at the MP-presence *versus* water-column variability, some interrelation-ships could be found.

The results show a clear separation between the less saline/colder Atlantic-Water (AW) (C01-C05) and the saltier/warmer Mediterranean sub-basin-water (WMed) (C06–C12), visible in the monthly-derived MODIS/Aqua-imagery (Figs. 2, 3 and 4). Time variability is observable with surface waters heating from July (23.5 °C) to August (24.2 °C), while mean Chl*a*-concentrations decrease from 0.15 mg.m^{-3} to 0.14 mg.m^{-3}, respectively. These are mainly shaped by well-identified mesoscale-structures (i.e. eddies/fronts/filaments), that characterize the mesoscale-eddy field in the region. Interestingly, the Strait-of-Gibraltar (SoG) is mostly occupied by an anticyclonic-vortex (lower/higher Chl*a*-concentrations in the centre/periphery of the

eddie, respectively) in the WMed region (e.g. C06) (Fig. 2c/d). Simultaneously, a cyclonic-eddy, coincident with a region of sharp sub-surface isopycnals uplift and shoaling (suggesting upwelling-mechanisms and frontal-signatures), was observed (Fig. 4). Here (C07), where concentration of Chl*a*/turbidity/dissolved-oxygen increased (~40–50 m, results not shown), the highest MP pieces-per-litre were detected (Table 1).

Table 1. Total MP-pieces-per-liter (mean ± standard deviation, SD) with coefficient-of-variation (CV), per sample-station stations.

Station-Number	Mean ± SD	CV(%)
S03C01	1.083 ± 0.996	92%
S06C02	3.000 ± 6.229	208%
S09C03	0.750 ± 0.683	91%
S13C04	0.786 ± 0.893	114%
S16C05	0.667 ± 0.686	103%
S19C06	0.929 ± 1.141	123%
S22C07	3.571 ± 10.545	295%
S25C08	1.143 ± 1.406	123%
S28C09	1.375 ± 1.668	121%
S32C10	1.375 ± 0.893	114%
S35C11	0.857 ± 1.099	128%
S37C12	1.143 ± 1.406	123%

Table 2. Total MP-pieces-per-liter (mean ± standard deviation, SD) with coefficient of variation (CV), ordered by depth, per sample-station.

Depth	Mean ± SD	CV(%)
Surface	1.278 ± 1.364	107%
5 m	1.125 ± 1.035	92%
25 m	0.818 ± 1.053	129%
DCM^{-}	2.542 ± 8.022	316%
DCM	0.792 ± 1.444	182%
DCM^{+}	0.792 ± 1.062	134%
500 m	1.130 ± 1.140	101%
MW	0.875 ± 1.000	114%
1500 m	3.700 ± 7.903	214%

Vertical spatial analysis denotes that surface/upper-waters in the AW-region (SW) are characteristically well oxygenated (>220 $mol.kg^{-1}$) and warm (>16 °C) (Fig. 4). Below these, two main layers are detectable: Eastern-North-Atlantic-Central-Water (ENACW; salinity: 35.45–35.75; temperature: 10.5–12.0 °C; depth: 100–

Table 3. Total MP-pieces-per-liter (mean ± standard deviation, SD) with coefficient-of-variation (CV), ordered by depth/geographic location (Atlantic-Ocean, C01–C05; Mediterranean-Sea, C06–C012), per sample-station. Mediterranean-outflow water (MW) is not found in the Mediterranean-Sea.

	Atlantic-Ocean		Mediterranean-Sea	
Depth	Mean ± SD	CV(%)	Mean ± SD	CV(%)
Surface	1.000 ± 0.816	82%	1.357 ± 1.499	110%
5 m	1.500 ± 1.179	79%	0.857 ± 0.864	101%
25 m	1.000 ± 0.926	93%	0.714 ± 1.139	159%
DCM^{-}	0.800 ± 0.789	99%	3.786 ± 10.460	276%
DCM	0.400 ± 0.516	129%	1.071 ± 1.817	170%
DCM^{+}	0.700 ± 0.675	96%	0.857 ± 1.292	151%
500 m	1.000 ± 1.000	100%	1.214 ± 1.251	103%
MW	0.875 ± 0.991	113%	–	–
1500 m	4.375 ± 8.789	201%	4.375 ± 8.798	141%

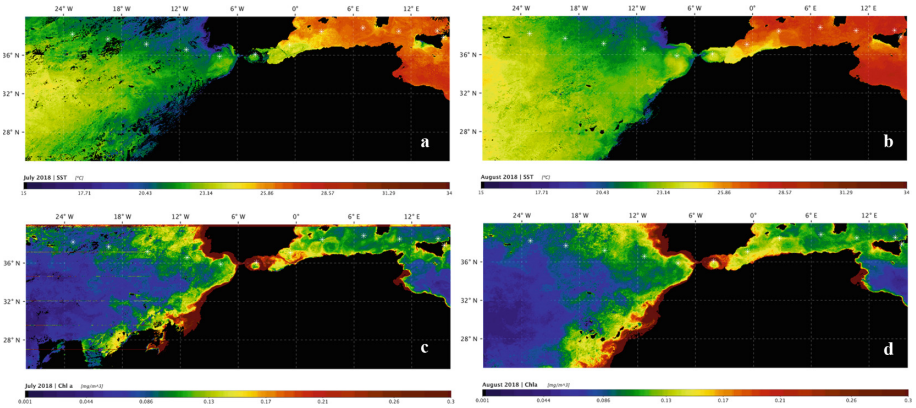

Fig. 2. MODIS/Aqua-1 km resolution monthly composites of sea-surface-temperature (SST, °C) and chlorophyll-*a* (Chl*a*, mg.m^{-3}) for the PE442-cruise. **A** and **b** SST for July and August-2018, respectively; **c** and **d** Chl*a* for July and August, respectively.

600 m) as a regular strait-line with diapycnal-mixing, and Subarctic-Intermediate-Water (SAIW; salinity: 34.9–35.1; temperature: ~4.0–7.0 °C; depth: 450–700 m) (Fig. 4: $26.800 < \sigma_\theta < 27.200$; $27.200 < \sigma_\theta < 27.922$, respectively). Furthermore, signals of Labrador-Sea-Water (LSW; salinity: <34.9; temperature: ~3.0–4.0 °C; depth: 800–2000 m) and MOW (salinity: >35.7; temperature: ~7.0–10.0 °C; depth: 600–1000 m) are also noticed. The latter is detectable in the TS-diagram (Fig. 3), and in C03/C04 shaped as a sub-surface anticyclonic-vortex ("Meddie") between 800–1200 m, with increased salinity (36.2 in the core), downward tilt of the 11 °C isotherm, decreased oxygen, and interestingly, no MP-detection (Fig. 4). Contrastingly, C02

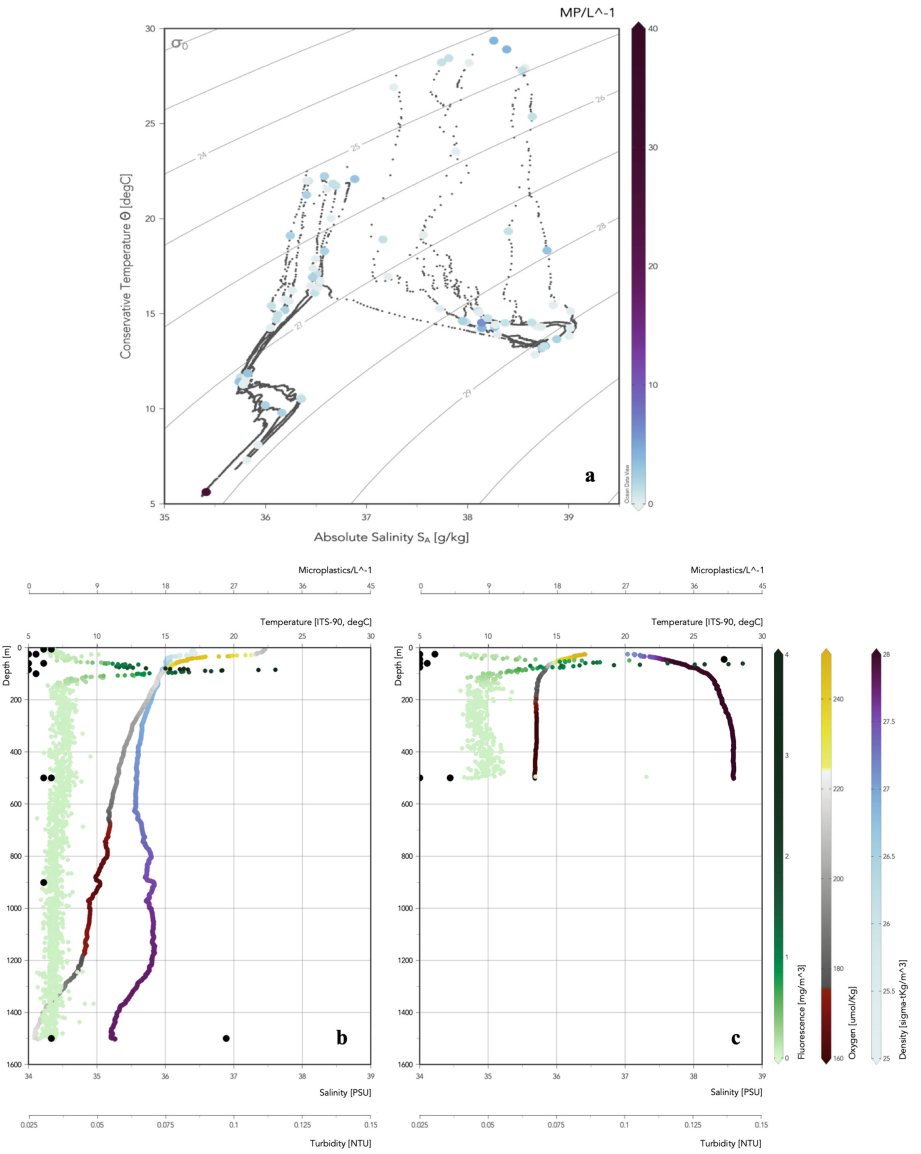

Fig. 3. Parameters measured during the PE442-cruise: **a** Temperature-Salinity-diagram (Atlantic-Ocean: light-grey; Mediterranean-Sea: dark-grey) for all stations overlaid with MP-concentrations (black dots); CTD vertical-profiles at casts S06C02 **b** and S22C07 **c**.

(at 1500 m) showed the second largest number of MP (Table 1, Figs. 3 and 4). In this region, there is an increase in dissolved oxygen (>220 mol.kg^{-1}), a sharp gradient of the isotherms/isohalines, indicating a frontal region of colder/less-saline LSW-waters, typical of these intermediate-waters [13].

In the WMed-region, during summer, surface-waters (up to 100 m, seasonal thermocline) are well stratified (Fig. 4). The surface layer is dominated by the inflow of relatively low salinity/modified AW (MAW) through the SoG (C06-C09: salinity-minimum at ~100 m; Figs. 3 and 4). As described in Hainbucher *et al*. [14], the MAW (50–200 m-thickness; salinity: 36.2 near SoG, 38.6 near Levantine-basin) is continuously modified by atmosphere/older-surface-waters/underneath-water mixing-interactions, which translates in a complex and mostly-cyclonic circulation. Furthermore, Levantine Intermediate-Water (LIW) (salinity: 38.4–39.1; temperature: 13–15.5 °C; depth: 200–800 m), can be traced in C10 (salinity: >38.4 at 300–350 m). Additionally, hook-like TS-structures found at mid-depths to bottom (colder/more-saline waters) typify Western-Mediterranean-Deep-Water (WMDW) (salinity: 38.4; temperature: 12.7 °C), a mixture of water masses that result from deep convection in the Gulf-of-Lion [15] (Figs. 3 and 4).

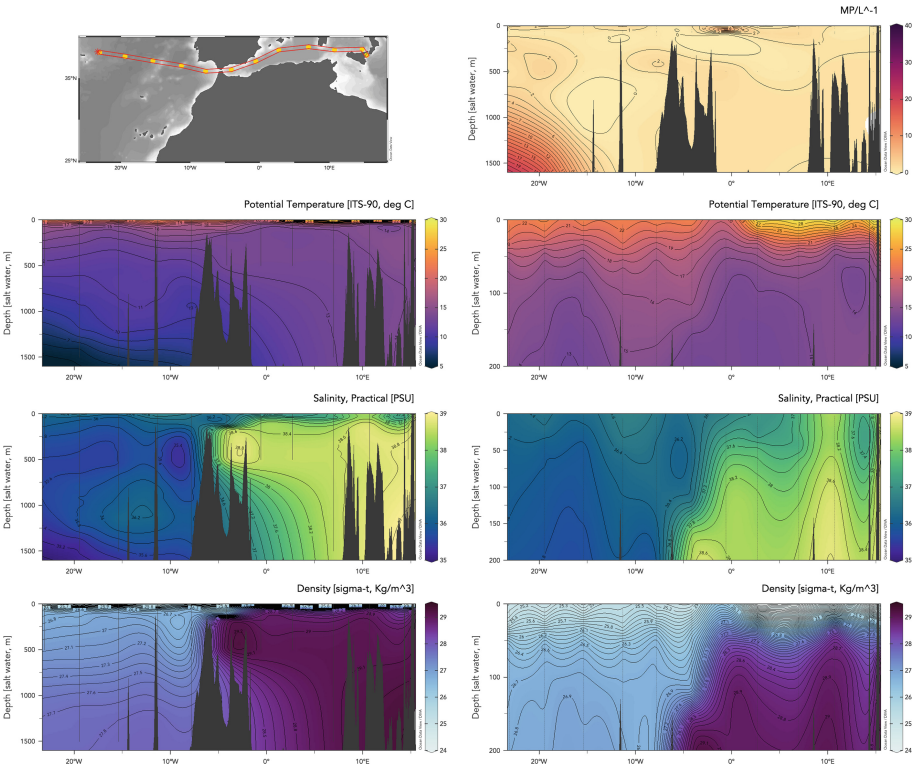

Fig. 4. ODV-plots based on the longitudinal PE442-cruise section (top, left), for MP-concentrations. The potential-temperature, salinity and density are plotted down to 1500 m (left figures, respectively) and down to 200 m (right figures, respectively), overlaid with isolines.

4 Conclusions

Our results support the main theory that MP move through several pathways/mechanisms that can lead to accumulation in the marine environment. Furthermore, this variability is revealed in two sampling points located in regions characterized by enhanced horizontal gradients (i.e. physical/chemical/biological properties): one deep-station (C02) in the Atlantic and one at relatively shallow depth (C07) in the Mediterranean. This further supports that MP can be either mixed downwards with other particles (e.g. biological) or be fragmented, altering their size and/or floating/sinking behaviour in the water-column.

We propose that local passive accumulation, enhancement and trapping areas of MP are related with zones of physical convergence and with strong frontal transitions.

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Analysis of Marine Microplastics in the Water Column Sampled up to 300 M Depth

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1 Introduction

The presence of microplastic at open ocean is a well documented fact. Microplastic is considered as plastic fragments below 5 mm length. These microplastics can proceed from macroplastic fragmentation (secondary microplastic) or from virgin pellets (primary microplastic).

Plastic concentration on the ocean has increased in last years [1, 2], but estimations has been done based on macroplastic values, but it is necessary to do more studies of microplastic concentration. Moreover, microplastics have been determined basically in the first 5 m of the water column or near the seabed, not at different depths on the water column.

The buoyancy plastic is predicted to find it on seawater surface, but due to the small size and biofouling process, density of the plastic can vary and vertical transport of these small fragments can take them below surface [3, 4]. Then these microplastics is sinking gradually on the ocean in function of ocean dynamic and turbulence [5, 6].

Some studies have reported the accumulation of microplastic at subtropical gyres as North Atlantic Subtropical gyre. In this areas the microplastic concentration can be up to 9 times bigger than in other regions [7].

Most of studies based on experimental data have sampled microplastics between 0 and 20 m depth [6, 8], but recent studies have reported the presence and accumulation of microplastic between 200 and 600 m depth [9].

In this work it has been determined the concentration of microplastics at open ocean between 0 and 300 m, the plastic compositions of some of the fragments found and the persistant organic pollutant (POPs) concentration adsorbed over this microplastic.

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2 Experimental

Based on this idea, specific sampling of microplastics at the water column in the North Atlantic Subtropical gyre were done. Stations were located near Canary Islands, but at oceanic areas, at the European Station for Timeseries in the Ocean Canary Islands (ESTOC), far enough for avoiding island influence.

72 L of seawater were filtered per sample with a mesh size of 100 μm. Sampling was done between 0 and 300 m 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 (Fig. 1).

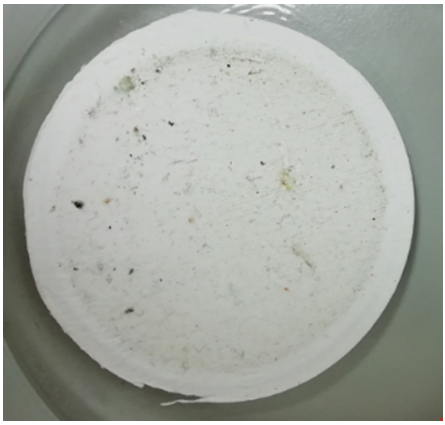

Fig. 1. Whatmann glass filter (47 mm) example (sampled between 0 and 300 m).

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 m depth.

2.1 Material

2.1.1 Reagents

The compounds analysed were 15 organochlorinated pesticides (OCPs), 8 polychlorinated biphenyls (PCBs) and 6 polycyclic aromatic hydrocarbons (PAHs), listed in Table 1.

Table 1. Compounds list analized by GC-MS, classified as PCBs (polychlorinated biphenyls), OCP (organochlorine pesticides) or PAH (Polycyclic aromatic hydrocarbons). Retention time (min) and SIM conditions for each compound.

SIM Groups	Analyzed Compounds			Retention Time (min)	Quantita -tion Ion	Qualifer Ion		Dwell Time (ms)
	Nº	Groups	Compound Name					
1 (10.00-12.35min)	1	OCPs	AlphaBCH	11,465	219	181		50
	2	OCPs	Atrazine	11,911	215	217	220	50
	3	OCPs	BetaBCH	12,067	219	183		50
	4	OCPs	GammaBCH	12,242	219	181		50
2 (12.35-13.20min)	5	PAHs	Anthracene	12,630	178	176	179	60
	6	OCPs	DeltaBCH	12,815	219	181		60
3 (13.20-14.36min)	7	PCB	PCB28	13,680	258	186		75
	8	OCPs	Heptachlor	14,128	272	100		75
4 (14.36-16.00 min)	9	PCBs	PCB52	14,635	292	220		75
	10	OCPs	Aldrin	15,113	263	293		75
5 (16.00-16.87 min)	11	PAH	Fluoranthene	16,437	202	200	201	100
6 (16.87-17.84min)	12	PCBs	PCB101	17,280	326	256		75
	13	OCPs	Endosulfan I	17,431	241	195		75
7 (17.84-18.62min)	14	OCPs	4,4-DDE	18,197	246	318		75
	15	OCPs	Dieldrin	18,254	263	79		75
8 (18.62-20.31min)	16	OCPs	Endrin	18,956	263	81		30
	17	PCBs	PCB118	19,289	326	254		30
	18	OCPs	4,4-DDD	19,564	235	165		30
	19	OCPs	Endrin Aldehyde	19,877	345	250		30
	20	PCBs	PCB153	20,024	360	290		30
9 (20.31-21.60min)	21	OCPs	Endosulfan Sulfate	20,661	272	387		50
	22	OCPs	4,4-DDT	20,794	235	165		50
	23	PCBs	PCB138	20,946	360	290		50
10 (21.60-24.50min)	24	PCBs	PCB180	23,184	396	324		150
11 (24.50-28.90min)	25	PCBs	PCB194	25,751	430	358		60
	26	PAHs	Benzo(b)fluoranthene	26,181	252	248	250	60
	27	PAHs	Benzo(a)pyrene	27,040	252	248	250	60
12 (28.90-32.00min)	28	PAHs	Indene(1,2,3-cd)- pyrene	29,997	276	275	274	100
	29	PAHs	Benzo(g,h,i)perylene	30,741				

The patterns of reference of the POPs analysed were obtained from a an EPA pesticide mix (Supelco®), a mix of PCB 32 (Sigma Aldrich®) and an assortment of PAHs (Supelco®).

The reagents used to determine the POPs were: methanol (Merck®), n-hexane SupraSolv (Merck®) and Milli-Q water (Millipore®). The internal standards (IS) used

were: Chrysene D12, Acenapthene D10, Penanthrene D10, Perylene D12 (Supelco®), at a concentration of 2.5 ng·mL^{-1} and Telodrin for analysing OCPS from Dr. Ehrenstorfer GmbH®, at a concentration of 25 ng·mL^{-1}.

2.1.2 Equipment

- Stereo-microscope: VWR®, model SZB250 - for visually identifying the microplastic samples.
- Ultrasonic bath: Cole-Parmer® model 08895-22.
- Vortex shaker: VWR®
- Rosette with 24 Niskin bottles (12 L each), for collecting deep-water samples (0–300 m depth). Seawater sample is filtered with a pore size of 100 μm. The samples kept at a 47 mm Whatmann GF/F filters.
- GC-MS for POP determination: Agilent Technologies® gas chromatography with mass spectrometry (GC-MS) equipment, model 7820A and 5977B MSD, with an HP-5MS Ultra Inert 19091S-433UI column of the same brand, following the Agilent Technologies® multi-residual analysis methodology [10].
- Analytical pyrolysis (Py-GC-MS) was performed in a double-shot pyrolyzer instrument (Model 2020i, Frontier Laboratories) coupled to a gas chromatograph (Agilent 6890 N) and a selective mass detector (Agilent 5973 N). The pyrolysis temperature was 400 °C for 1 min [11]. The pyrolysis products were identified and characterised using the Wiley and Nist computer libraries and by comparison with published polymer mass spectra [12].

2.2 Methods

Samples was taken at 150, 100, 50 m depth and surface (vary between 3 and 10 m).

72 L of seawater was filtered and kept in a Whatmann filter. Each sample were kept at −80 °C for later visual assessment and analysis.

3 Results and Discussion

Sampling at ESTOC was repeat during three different oceanographic cruises, between April 2017, September 2017 and March 2018.

Samples were taken at the ESTOC time series oceanographic station located at 29º10′N and 15º30′W, to the north of the Canary Islands.

Microplastics appear in all the samples analysed, with an especially predominant presence of fibres [13], but small fragments can also be found down to a depth of 150 m. Fibres that could be similar in appearance and number to those identified in the blank run on board were discarded (Fig. 2).

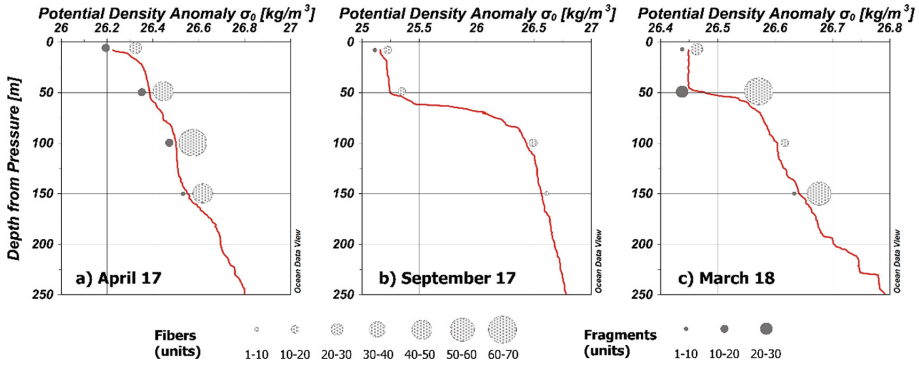

Fig. 2. Vertical density profiles of sea water (red line) measured in the campaigns conducted at the ESTOC in April 2017 (a), September 2017 (b) and March 2018 (c). The units of fibres and fragments of microplastics counted in 72 L of sea water filtered per sample were assessed for the surface, 50, 100 and 150 m.

4 Conclusions

Some 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.

This fact can indicate that there is an underestimate amount of plastic below surface that are not being studied yet, and it is highly necessary to increase studies of microplastic presence and concentrations between 0 and (at least) 600 m depth.

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Macro and Microplastics in Stormwater and Combined Sewer Overflows in Paris Megacity

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1 Introduction

Plastic waste is currently an important environmental issue. Due to its noteworthy mechanical and chemical properties (*e.g.*, lightness, waterproofing, non-biodegradability and low reactivity), plastic production has known an exponential increase, from 1.5 million tons in 1950 to 350 million tons in 2017 [1]. Plastics often release into environment, particularly into freshwaters, subsequently into the marine environment. The release of plastics from rivers to ocean was estimated to be 1–2.5 million tons per year [2]. However, most plastics are characterized as non-biodegradable, thus easily accumulate in all environmental compartments, which may be harmful to ecosystem. Plastic wastes can have sanitary risks due to their accidental ingestion. Moreover, there are evidences reporting that micropollutants (e.g., PAHs, PCBs, BPA) can be adsorbed on plastic waste, which further increases its environmental risks, even though it is still debated [3].

Since 2009, plastic waste has been categorized as macroplastic (>5 mm) and microplastic (<5 mm) based on its size distribution [4]. Microplastics can be further classified as primary and secondary microplastics. Primary microplastics are designed as plastic particles smaller than 5 mm, whereas secondary microplastics are originated from the degradation of bigger plastic waste. Previous works have reported that plastic waste found in ocean were mainly coming from freshwater environments [2, 5, 6]. Moreover, it has been demonstrated that the discharge of plastic waste is generally linked to two factors: (i) the population density and (ii) the efficiency of waste management [5]. In the case of Paris megacity, a metropolis of more than 10 million inhabitants, the urban metabolism of plastic waste (*i.e.* the whole entering/outgoing of plastic fluxes in the urban area) is not precisely understood. Previous works have clarified the questions concerning the estimation of floating debris in Seine River using floating booms [7]. Preliminary results were provided for microplastics in runoff and

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combined sewer overflows (CSOs). However, only few numbers of rain events were studied, and the sample volumes were relatively low, which led to high uncertainties [8]. The objective of this study was to obtain mass concentrations (kg of plastic/m^3) and annual fluxes of macroplastics in CSOs and stormwater runoff. Moreover, we also focused on the concentrations of microplastics during several rain events in those urban water compartments. The results from this work will help to compare the inputs of plastic waste from various sewer systems and to clarify the urban metabolism of plastic waste.

2 Experimental Section

2.1 Sampling Sites

Two sampling sites were selected. For stormwater, Sucy-en-Brie catchment (situated in the south east of Paris metropolis) was selected in collaboration with the Direction of the sanitation and environmental services of Val-de-Marne (DSEA 94). This area has been chosen because it is particularly well documented and well-instrumented to study rain events. The characteristics of the Sucy-en-Brie catchment is summarized in Table 1.

Table 1. Characteristics of Sucy-en-Brie catchment [9]

Catchment	Surface (ha)	Runoff Coefficient (%)	Land use
Sucy-en-Brie	228	21	Mainly a residential area with large number of multi-occupancy family houses, limited commercial activities

A stormwater reservoir is located at the catchment outflow. This reservoir is equipped of a basin and a lamellar clarifier which is used to remove particles. A 60 mm grid (written GR1, for Grid Refusal 1), is situated at the stormwater reservoir entrance to prevent the arrival of debris in the basin. The stormwater of the basin is pumped to the lamellar clarifier for decantation. A 10 mm grid (GR2), located just before the lamellar clarifier, is devoted to protecting it from debris bigger than 10 mm. The accumulated debris on the two grids are used to study the macroplastic concentrations.

The CSO Clichy outfall (in the north east of Paris), which is one of the most important outfall in Paris megacity and managed by the Paris Public Sanitation (SIAAP), was also selected as sampling site. For example, in 2015, 5.6 Mm^3 of those effluents discharged in the Seine, those volumes make it an important sampling site [10]. Concentrations of plastic waste in the CSO of Clichy will be studied in the outlet channel.

2.2 Methods

2.2.1 Stormwater of Sucy-en-Brie

To study macroplastics, debris were collected from the two grid refusals (between 30 and 60 kg per grid). They were weighted and pretreated. The grid refusal debris were collected once per month for one year (from February 2018 to March 2019) to follow the concentration variations of plastic waste. 80 to 100 L of water from the inlet canal (upstream of the grid refusals) were collected during four rain events for microplastic studies. This water was then filtered through 80 μm net. For a given event, between 3 and 5 samples were collected for each rain event. The characteristic of rain events (e.g., precipitations, flowrates) was provided by DSEA. It is important to note that macroplastics were studied monthly whereas microplastics were investigated only during rain events.

2.2.2 CSO of Clichy

Debris of Clichy outfall were collected using a net with a mesh size > 5 mm and with an exposure time of 10 min during an overflow discharge. Microplastics were collected from the outfall canal during a rain event using two different nets: a 300 μm net with 30 s to 1 min exposure time and an 80 μm net, using a 10 s exposure time to prevent clogging.

2.3 Analytical Procedure

2.3.1 Macroplastics

A subsample of the debris collected at different sampling sites (from 3 to 6 kg) was randomly selected, weighted and dried in an oven at 40 °C for at least 10 days. They were then weighted and visually sorted. Plastics and other anthropogenic debris (e.g., aluminum cans, health-care waste…) bigger than 5 mm were classified.

2.3.2 Microplastics

Microplastic extraction was performed in 3 main steps: (i) the samples were sieved using a 5 mm and a 1 mm sieve, (ii) then separated with NaI density separation method (d = 1600 kg/m^3), (iii) and finally digested with H_2O_2 solution (30%) to remove the organic matter. The samples were then filtered and microplastics were counted with a stereomicroscope (Leica MZ12) coupled with an image analysis software (Histolab). The fragments and a part of the fibers (10−30% of the total fibers) were analyzed with FTIR spectroscopy (ThermoScientific Nicolet iN10 MX).

3 Results and Discussion

Results from Sucy-en-Brie catchment were presented here as an example.

3.1 Macroplastics in Grid Refusals of Sucy-en-Brie

The different characteristics of some of the collected macroplastic samples were summarized in Table 2 for GR1 (60 mm) and GR2 (10 mm).

Table 2. Characteristics of the grid refusal samples and plastic waste mass percentages

Sample	Sampling Date	Wet mass (kg)	Dry mass (kg)	Water mass percentage (%)	Number of plastic debris found	Plastic mass (kg)	Plastic mass percentage (%)
GR1	04/12/2018	5.74	1.44	74.9	71	0.20	13.9
	05/22/2018	3.46	1.28	63.0	81	0.48	37.5
GR2	03/02/2018	16.89	4.20	75.2	405	0.19	4.5
	04/12/2018	7.17	1.44	79.9	312	0.12	8.0
	05/22/2018	5.00	1.34	73.2	140	0.02	1.5

Those samples were characterized by high water proportion (a mean value of 70.3%). The dry masses were all approximately the same (except for 03/02/2018 sample). We observe a high difference between GR1 and GR2 in terms of number of plastic objects found. On average, we observed 76 objects for GR1 and 286 objects for GR2, which were accumulated for the same time intervals. The plastic mass fraction was more important for GR1 than GR2, which reached 37.5% as the highest percentage. The mass percentage of plastic in GR2 was in the range of 1.5 to 8%. As illustrated in Fig. 1, eight different types of macroplastics were found in the grid refusals: fragments of plastic bags and/or films, packaging food (e.g., candies, biscuits), bottles, solid fragments with unknown origin (written FWUO), cigarette filters, garbage bags, polystyrene fragments, and plastic cups.

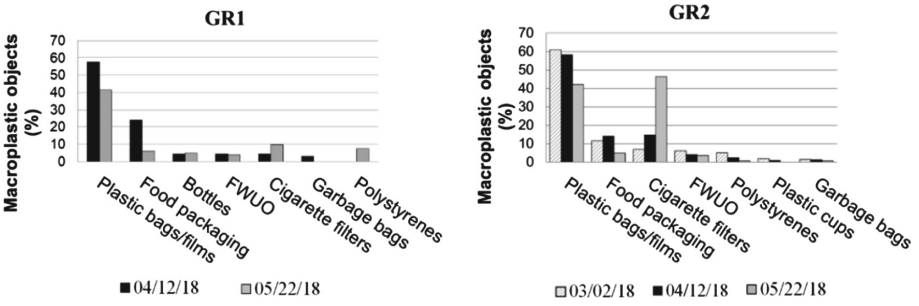

Fig. 1. Proportions of the main macroplastic objects found for GR1 and GR2

For all the samples, films, plastic bags, and food packaging represented the majority of the macroplastic debris, except for 05/22/18 GR2, for which the cigarette filters were found in high proportion (46.4%). Plastic bottles were exclusively found in GR1, due to the large mesh size (60 mm). It should be noted that the waste collected at GR1 came directly from the stormwater (direct precipitation), whereas GR2 debris were from stormwater, which was pumped in the reservoir, then brought to the lamellar clarifier. Thus, the pumping process might have an influence on the macroplastic repartition in GR2. For both GR1 and GR2, we observed the presence of fragments from bigger debris with unknown origin (FWUO). Preliminary assessment of the macroplastic mass concentration in stormwater was proposed. The runoff volume at the outflow of Sucy-en-Brie catchment was approximately 54,000 m^3 during March-April 2018. About 0.32 kg of macroplastics were collected for GR1 and GR2 subsamples for this period. If we extrapolate this value to the entire debris accumulated in the grid refusals, these debris were estimated to contain approximately 1.82 kg of macroplastics. Thus, the macroplastic mass concentration in stormwater of March-April 2018 was about 3.4×10^{-5} kg/m^3. For the sampling period of April-May 2018 we found a macroplastic mass concentration quite similar to the March/April period: 4.3×10^{-5} kg/m^3.

Future studies will focus on the results from other samples to investigate the variation of macroplastic mass percentages with sampling points and periods.

3.2 Microplastics in Stormwater of Sucy-en-Brie

First results of microplastic on 06/11/18 rain event were illustrated in Fig. 2, presenting the concentration of microplastic fragments (1–5 mm) in fragments/L, in relation to time and flowrate of the inlet canal of Sucy-en-Brie.

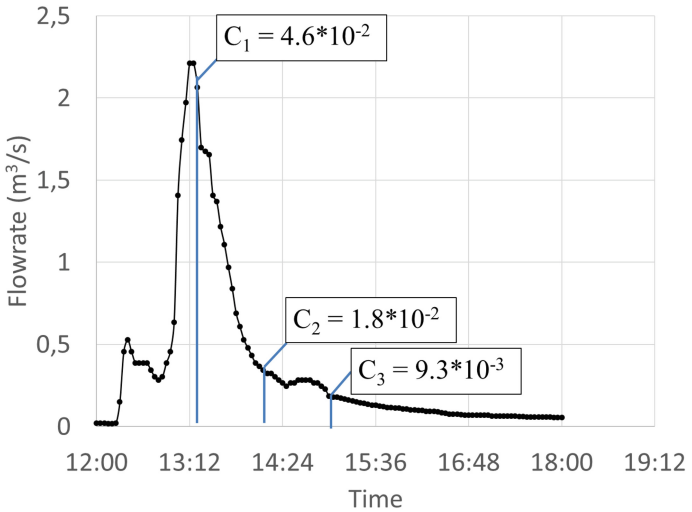

Fig. 2. 1–5 mm microplastic concentrations (fragments/L) as a function of time and flowrate in the inlet canal of Sucy-en-Brie watershed for the rain event of 06/11/18 (data from DSEA 94)

Results indicated that the concentration of microplastics increased with flowrate, but reduced with time. Previous works have reported 2–29 fragments/L of microplastics from the same catchment [8]. Lower values found in this study could be explained by the fact that we did not analyze the smaller microplastics (1 µm to 1 mm). Infrared spectroscopy confirmed the presence of polyamide and polyethylene. 6 fragments found during this rain event were not analyzed due to their high degradation. An infrared microspectroscope will be used to analyze smaller fraction. Analyses on the other rain event samples are in progress.

4 Conclusions

The macroplastic mass concentrations in stormwater of Sucy-en-Brie catchment for March/April and April/May 2018 turned out to be similar, 3.4×10^{-5} kg/m3 and 4.3×10^{-5} kg/m^3 respectively. The six main plastic objects found are: plastic bags/films, food packaging, bottles, fragments with unknown origins (FWUO), cigarette filters and garbage bags. Microplastics with size of 1–5 mm during first rain event were analysed. For this size range, results indicated that microplastic concentrations were in the range of 4.6×10^{-2} and 9.3×10^{-3} fragments/L. The smaller fraction (<1 mm) will be soon analysed. The study of macro and microplastics in stormwater and CSOs is challenging, especially during rain events, but it could help for a better estimation of plastic waste fluxes in the Paris megacity. The results and various analytical protocols applied in this study can contribute to the establishment of monitoring system of plastic waste repartition and can be applied to other agglomerations for better understanding the source of plastic wastes. Moreover, it could serve to prioritize the actions for the reduction of plastic inputs into urban areas and environment.

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The Effect of Drinking Water Ozonation on Different Types of Submicron Plastic Particles

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1 Introduction

Plastic pollution has increased in line with the increased plastic production over the last 60 years, having reached all environments globally and potentially affecting drinking water supplies [1–3]. For example, microplastics have recently been detected in both potable water sources and bottled water for consumption [4], leading researchers to question the efficacy of current water treatment practices. While the magnitude of particulate plastic concentrations and compositions varied by water source and location (1×10^{-2} to 10^{8} particles/m^3 in rivers, lakes, groundwater, tap water and bottled water; [3]), it is hypothesized that all waterways will continue to experience an influx of particulate plastic (including down to the nanometer scale) as degradation of larger plastic items continues. However, detecting these smallest size particles is analytically challenging, wherefore, assessing the plastic concentration, and consequently removal during water treatment, has been limited to date.

Besides sand and activated carbon filtration, ozonation is a key treatment step in modern drinking water treatment plants (DWTPs) due to its excellent disinfection and oxidation properties. Chemical oxidation using ozone has been proven to be an effective treatment process for a wide spectrum of organic pollutants during bench-, pilot- and full-scale experiments in drinking water [5]. Yet studies addressing the impact of ozonation on plastic particles are still lacking. Moreover, to what extent plastic particles are removed or altered by processes applied in the conventional DWTPs has not been addressed yet.

Here, 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. In order to follow a realistic scenario, the three plastic particles were treated with ozone at different concentrations in a lake water (used as a source of drinking water for the city of Zurich, Switzerland). Depending on how particles transform and behave in this system may have implications on their

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further removal efficiency throughout the entire drinking water treatment process. In an ongoing study, we explore the efficiency of particulate plastic retention through column experiments representing conventional sand filtration processes to assess if current practices and technologies are capable of providing a drinking water without plastic particles.

2 Experimental

2.1 Materials

To overcome analytical challenges to detect and quantify particulate plastic in complex media, metal doped submicron plastic particles had previously been synthesized and assessed in proof-of-concept studies using activated sludge as a complex matrix [6]. Briefly, following a two-step *in situ* polymerization process, two different plastic particles were synthesized in house including a smooth polyacrylonitirle particle (PAN) and a core/shell plastic with the same PAN core covered by a polystyrene (PS) shell. While the overall shape of the particles remained spherical, the surface morphology of the core/shell particle had an irregular structure characterized by round protrusions from the surface. The Pd content, which was bound inside of the particles, was approximately 0.5% by weight. The addition of Pd did not significantly change the density of the material nor did the presence of Pd change the outward chemistry of the plastic. Additionally, commercially available nominal 100 nm polystyrene (PS) spheres (3100A, ThermoFisher Scientific, Inc.) with a smooth surface were used throughout all tests.

2.2 Methods

PAN, PAN/PS and PS plastic particles were suspended in lake water (used as a source of drinking water for the city of Zurich, Switzerland; LZW, hereafter) at a concentration of 10^7–10^8 particles/mL and were reacted with selected ozone doses (0.5, 1 and 5 mg/L), ranging from concentrations which represent realistic ozone exposures in a conventional DWTP to far in excess. The hydrodynamic diameter and ζ-potential of the particles in suspension before and after the ozone treatment were measured by dynamic light scattering (DLS) and electrophoretic light scattering, respectively, using a Zetasizer Nano ZS (Malvern Instruments Ltd. United Kingdom). The decrease of ozone over time was monitored by measuring the UV absorption 260 nm (maximum absorbance of ozone, $\varepsilon = 3200\ M^{-1}\ cm^{-1}$ [7]) using a UV-Vis spectrometer (Cary 100, Varian). As PAN and PAN/PS have a metal incorporated in their cores, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to assess the total and free Pd concentration of these samples before and after ozonation to test for any potential release of Pd after the ozone treatment. Before the ICP-MS measurements, particle suspensions were digested (7.6% HNO_3, 1.3% H_2O_2 and 2.5% H_2SO_4) using an ultraCLAVE4 (Milestone Srl, Sorisole, Italy). To quantify the free Pd in solution, the plastic particles were separated from the suspensions using centrifugal filtration

membranes (Vivaspin 20 mL) with a 50 kDa MWCO cutoff (Sartorius AG, Goettingen, Germany) and the permeates were analyzed by ICP-MS. The particle nominal diameter and morphology was assessed using a scanning electron microscope (Nova NanoSEM 230, FEI, USA) and a scanning transmission electron (STEM) microscope (HD 2700 CS, Hitachi, Japan), equipped with a secondary electron (SE) detector. The STEM was operated at an acceleration voltage of 200 kV and the secondary electron signal was used for image formation. To suppress charging of the particles under the electron beam, samples were coated with a thin layer (nominal thickness 0.1 nm) of Tungsten.

2.3 Statistical Analysis

The mean and the standard deviation of the hydrodynamic diameter, surface charge and total and free Pd were calculated for each treatment from three independent replicate experiments. Statistical analyses were performed by using the R software 3.0.2 (The R Foundation for Statistical Computing©). One-way ANOVA coupled with Tukey's HSD (honestly significant difference) *post hoc* test was performed for comparison of means. Statistically significant differences were considered to exist when $p < 0.05$.

3 Results and Discussion

The pristine PAN/PS, PAN and PS had a nominal diameter of 190 nm, 140 nm and 100 nm and a hydrodynamic diameter of 215 nm, 150 nm and 105 nm, respectively (Table 1), derived from SEM and DLS measurements. Moreover, all samples showed a very narrow particle size distribution reflected by the polydispersity indices of 0.075, 0.043 and 0.021 for PAN/PS, PAN and PS, respectively. The particles had a negative surface charge (ζ-potential) in deionized H_2O (3.34 μS/cm) with values ranging from −8.6 mV to −41.1 mV. Once submicron plastic particles were suspended in the LZW, the hydrodynamic diameter of PAN/PS, PAN and PS increased slightly to 251 nm, 172 nm and 117 nm, respectively. Although the surface charge of all the submicron plastics remained negative once suspended in the experimental media (315 μS/cm), the ζ-potential changed to −11.1 mV for PAN/PS and to −12.4 mV for PAN. PS remained constant with a negative surface charge around 9 mV when suspended in LZW.

The impact of ozone treatment on the physicochemical properties of three types of submicron plastic particles was studied. There were no statistically significance differences in the hydrodynamic diameter between the untreated and the ozone treated submicron plastic particles, irrespective of the applied ozone dose. However, the surface charge of the particles significantly decreased after the ozone treatment, though this variance was different depending on the particle and ozone concentration. The ζ-potential of PAN/PS decreased from −11 mV to −14, −15 and −17 mV after 0.5, 1 and 5 mg O_3/L, respectively. Moreover, PAN and PS plastic particles also decreased their surface charge, but statistically significant differences were only found after applying the highest ozone doses (5 mg O_3/L).

Table 1. Physicochemical properties of the tested plastic particles in either DI H_2O and LZW.

			DI H_2O			Lake Zurich water (LZW)		
			Hydrodynamic diameter		Surface charge	Hydrodynamic diameter		Surface charge
Abbreviation	Description	Nominal diameter (nm)	Z-Average (d, nm)	PDI	ζ-potential (mV)	Z-Average (d, nm)	PDI	ζ-potential (mV)
PAN/PS	Raspberry PAN-Pd-PS	190	214.7 ± 1.4	0.075	−41.1 ± 0.3	251.4 ± 5.9	0.168	−11.1 ± 0.3
PAN	Smooth PAN-Pd	140	150.3 ± 0.3	0.043	−16.8 ± 1.7	172.3 ± 7.2	0.114	−12.4 ± 0.9
PS	Standard 100 nm PS	100	105.1 ± 7.5	0.021	−8.6 ± 0.7	117.1 ± 0.1	0.132	−9.5 ± 0.3

To assess if the ozone treatment altered the surface of the plastic particles, TEM images were obtained for samples exposed to 5 mg O_3/L and compared to images of the pristine particles. TEM images revealed a very comparable size and surface structure for pristine and treated particles. This suggests that ozone does not result in any visible alterations of the particles such as the formation of cracks or breaks on the particle surface. Furthermore, no differences were found for the ozone decrease in absence and presence of plastic particles for an ozone dose of 5 mg O_3/L (data not shown).

The PAN/PS and PAN plastic particles used in this study are intended to be used in a larger, pilot -scale study addressing the retention of plastic particles during conventional treatment in DWTPs. Therefore, a potential leaching of Pd incorporated into the core of the metal-doped particles during ozonation had to be taken into account as this metal will be used as a proxy for the concentration of plastic particles. Ozonation did not induce any leaching of the Pd incorporated at any ozone dose (free Pd concentration approximately 0.6% for both treated and untreated samples), demonstrating their suitability for further experiments using Pd as a conservative tracer after ozone exposure of the particles.

3.1 Discussion

Our common understanding of plastic pollution is rapidly increasing after the recent efforts to unravel the contribution of the freshwater system to the global plastic pollution problem. Recent studies point out that freshwater environments are not just mere canals to transport plastics to the ocean, but also play a key role in the distribution, fate and effects of plastics in the aquatic ecosystem [8]. Although more occurrence and monitoring studies are needed, the ubiquitous presence of plastics in lakes, rivers, streams, etc., may pose a potential risk for the human health as freshwater are usually the main source for generating potable water. Until now, there are no studies which have addressed the retention of plastic particles during different drinking water treatment processes (coagulation/flocculation, sand filtration, granulated activated carbon filtration, etc.) although microplastics have been already found in potable water [4]. Thus, the impact of ozonation on plastic particles has not been assessed yet. It is also

worth noting that the information regarding submicron sized plastic particles in experimental conditions is quite limited. Our results suggest that submicron plastic particles, based on polyacrylonitrile or polystyrene polymers, suspended in the raw water of a DWTP, may remain mostly unaltered after the ozone treatment as neither the size of the three different plastic particles or their aggregation state was altered during ozonation. Nevertheless, it is noteworthy that the particle surface charge of PAN/PS was statistically significantly decreased using relevant ozone concentrations as low as 0.5 mg ozone/L. This effect was only observed at this ozone dose for this type of particle and might be related with the higher volume to surface area derived of having a rougher morphology in comparison with the other two smoother particles. Though whether this alteration may increase or decrease the attachment of plastic particles to the sand or activated carbon in subsequent treatment steps is still unknown. Further work regarding the reason behind this effect and potential implications in a general level is currently under investigation.

Two studies have previously exposed PS surfaces to ozone for different purposes [9, 10]. The authors of both studies found that ozone effectively induced several alterations on PS material. However, the way of ozone application to plastic (direct gas), the extremely high concentrations (up to 120 mg ozone/L) and long contact times (up to 3 h) make their results incomparable to what we have obtained here, as we have approximated real operational conditions. This includes ozone exposure to the plastic in solution, concentrations typically utilized in DWTPs (approximately 1 mg/L), and contact times of 45 min. As in real systems, the ozone was in competition with natural organic matter in the LZW in addition to the spiked plastic, leading to ozone decay over the course of the experiment.

4 Conclusions

The presence of plastic particles has been confirmed in source waters of several DWTPs, raising a concern whether current conventional treatment technologies can satisfactorily remove plastic particles or whether new technologies need to be applied to appropriately remove the material. Here, we have evaluated the impact of ozonation as a key treatment process in DWTPs on different PS and PAN particles with varying size and surface morphologies. Based on the results of this study, ozonation does not appear to either fragment plastic particles or change its aggregation state. However, alterations on the surface charge were observed for ozone doses as low as 0.5 mg/L for PAN/PS particles. Further studies with both pristine particles and those which have undergone an ozonation treatment will be conducted using sand and activated carbon filtration to assess if existing methods are effective in removing this new type of pollutant.

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Microplastic in Coastal Areas - Impact of Waves, Sediments and Saltwater on the Degradation Behaviour

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1 Introduction

Around 80% of plastic found in the marine environment is discharged via rivers [1]. In many cases, these plastics are not (yet) present in the microplastic size class (smaller than 5 mm [2]). In estuaries, the flow velocity decreases and particles with a higher density than water begin to settle, while lighter particles float on the surface, although other influences like coagulation can have an effect as well [3]. A part of these particles accumulates near the coast due to various processes such as wind, waves and tides, while plastic which has already been floating in the ocean can be transported to the beaches via ocean currents [4, 5]. In addition, there are industrial discharges and pollution from nearby cities and tourism [6]. Through the effects of UV radiation, high temperatures, waves, sediment, and salt water, the beach environment acts like a mill by degrading and fragmenting the plastic [7]. However, these parameters have a varying influence on the degradation behaviour. UV-radiation combined with mechanical stress is causing the main part of the degradation, whereas the temperature can theoretically have an influence, but is kept constant as long as the plastic is surrounded by water, resulting in a reduced warming [4, 8–10]. Less known, however, is how large the effect of waves on the degradation behaviour is and which of the parameters, UV-degradation or mechanical stress, triggers the process of degradation, which in turn intensifies the other influences [4, 7, 8, 11, 12]. Both sequences appear logical and cannot be rejected on theoretical grounds, which means that long-term tests with corresponding alternating loads have to be carried out [13]. Since mechanical stress is expected to cause small cracks on the surface up to decomposition (in the case of EPS) [14], the size of the surface increases, whereby the increased amount of UV radiation per particle can penetrate into deeper layers [4]. The speed with which the plastics are crushed and the proportion of the various impacts are not precisely known [11]. The smaller a particle, the more surface it has compared to the volume, which increases its interaction with the environment. The exact consequences of microplastics in the marine environment are currently unknown, but negative consequences are to be expected, such as the transition of toxic additives from plastics into water [15]. Considering this, it is imperative that the fragmentation and degradation rate is investigated in depth for further research [16].

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Various influences such as mechanical stress and UV radiation are a normal part of standardised tests in the plastics industry [17]. In these investigations defined tests are carried out which consider individual effects like impact strength, tensile strength, and UV-radiation. But, there is no interaction tested between the various influences of the beach environment which act simultaneously and continuously on a test specimen. No documents were found about other experiments by the manufacturing industry that are comparable to the coastal environment as well. In the research question on the (mechanical) degradation behaviour of (micro)plastics on beaches, various influences have already been simulated and compared with microplastics collected on beaches [14, 18–20]. The considered influences are mainly located in the field of UV radiation and sediment [10, 21, 22]. Often, however, (sea) water was not used in these experiments and, above all, the wave influence was not simulated [19, 20, 23–26]. Only Efimova et al. used a modified concrete mixer, gravel and water to mimic the effects of storm events on mechanical degradation [14]. No documents about long-term experiments with shallow or average wave heights, sea water, and sediments have been found.

2 Experimental

The experiments investigate and quantify the continuous influence of sediments, salt water and wave action on the degradation and fragmentation behaviour of microplastics. The tests run long time (≥ 30 days) to ensure a continuous interaction of the microplastics with the sediment and the milling like processes induced through wave action.

2.1 Materials

Sediment. For the experiments the grain size distributions of a typical sand beach of the Baltic Sea are used. The grain classes are examined individually in the experiments in order to investigate the influence of different grain sizes on abrasiveness as well. Before starting the experiments, quartz sand was washed, dried and sieved into the grain classes 0.25–0.5 mm, 0.5–1 mm and 1–2 mm [27]. Any organic material has already been removed by the manufacturer.

Water. The artificial sea water is produced by means of a sea salt mixture from the zoological area. For this purpose, distilled water is mixed with the minerals according to the manufacturer's specifications resulting in a salinity of 3.5% ($\sim$19,700 mg/l Cl; $\sim$11,000 mg/l Na; $\sim$2,200 mg/l SO_4; $\sim$1,200 mg/l Mg; $\sim$420 mg/l Ca; $\sim$350 mg/l K; $\sim$180 mg/l HCO_3; $\sim$16 mg/l Sr).

Plastics. The most abundant plastics found on beaches are PE (LD+HD) and PP [28, 29]. In addition, based on its presumably fast degradation rate through mechanical weathering, EPS is tested as well [14, 19]. PE-HD was supplied by the company *MULTIPET-Kunststoffe*. It has a density of 936 kg/m^3. The particles are grey and lenticular with a mean diameter of 5 mm and a mean height of 1.5 mm.

The EPS spheres were obtained from a defective beanbag. They have a mean diameter of 4 mm. The density is around 18 kg/m^3 (data from manufacturer).

Furthermore, PP particles with a diameter of 3 mm of the company *Kugel-Winnie* are used. These have a density of 897 kg/m^3.

Test Rig. The test apparatus is a "Slosh-Box", which consists of the essential elements drive, rack, boxes and control as shown in Fig. 1. The drive is a frequency adjustable electric motor with 250 W power. The power is transferred to the frame and causes an oscillating movement. Through the oscillation agitation of the water and thus waves are generated. The frequency can be adjusted by a control unit to set the velocity of the apparatus to a desired value. The frame is made of aluminium square profiles and is

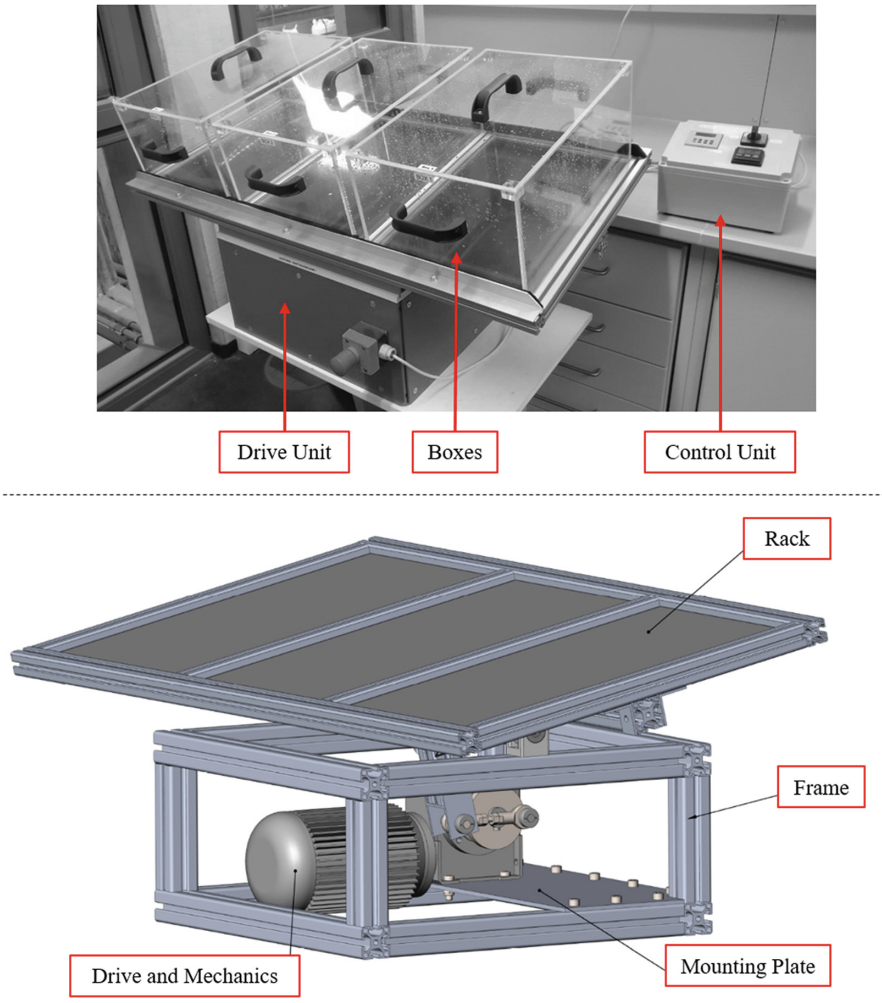

Fig. 1. Top: Slosh-Box with acryl-boxes, control and drive unit; Bottom: Conceptual sketch of the Slosh Box including drive and mechanics, frame, rack and mounting plate

1,080 mm long and 535 mm wide. In addition, there are clamping devices in which the test boxes can be fixed.

Boxes. The tests are carried out in 457 × 305 × 200 mm^3 acryl glass boxes with a wall thickness of 6.5 mm (see Fig. 1). The dimensions are limited by the test apparatus. Although they are made out of acryl glass, possible interferences from this are avoided through the analytical techniques (see Sect. 2.2.2).

2.2 Methods

The apparatus is significantly limited by the maximum box weight of approx. 30 kg (total), as from this mass an overheating of the drive would occur during long-term tests. For this reason, the layer thickness of the sediment could only be varied up to a maximum of 23 mm. In preliminary tests, a layer thickness of 15 mm proved to be useful, since at this height, together with 1.5 L of water, sediment-water interaction took place, while a constant minimum level of sand at the box's bottom was obtained, waves occurred, and the weight kept as low as possible. For the amount of plastics, the ratio of 1/200 mass MP to mass sediment [14] is used first, but is gradually increased to up to 1/10 (ratio differs for EPS). The Slosh-Box is running on a frequency of 31.4 Hz resulting in 26 agitating movements per Minute for at least 30 days of operation. The wave height due to the movement is approximately 85 mm.

2.2.1 Preparation of Samples

For sample preparation, the particles are first measured. The MP is then cleaned with distilled water to remove production residues and dried at air temperature isolated from UV light. Subsequently, the microplastic is weighed individually and an average weight is calculated. Until usage they are kept inside a desiccator covered by petri dishes while being screened from light.

2.2.2 Analytical Techniques

For the analyses following the experiments, a gravimetric evaluation will be carried out. All plastic particles will be removed from the boxes, cleaned with distilled water and then dried for 24 h under exclusion of light at air temperature (approx. 21 °C). This will be followed by weight determination and determination of mass loss using a precision balance (d = 10^{-4} g). The particles will then be reintroduced into the boxes to continue the experiments. Furthermore, scanning electron microscopy (SEM) images are planned in order to examine the surface more closely and thus determine the effects of waves, sediment and salt water on the degradation behaviour of MP particles.

3 Conclusions

Since the experiments are still running while submitting the extended abstract, no results/conclusion can be presented at this point.

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The Role of Humic Acids on the Effects of Nanoplastics in Fish

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1 Introduction

Plastics materials rarely biodegrade, instead, through different biotic and abiotic processes, they fragment into smaller microplastics (<5 mm, MPs), which have already been reported as ubiquitous pollutants in marine environments worldwide, as well as in a wide range of marine organisms [1]. Nanoplastics (NPs), the smaller than 100/1000 nm fraction of plastic fragments, are currently under focus of intense study, from multiple different perspectives. The present understanding of their occurrence, appropriate sampling techniques, physicochemical characteristics, and effects on biota is still considered scarce. In 2017, the estimated total amount plastic produced in the world was approximately 8300 million tonnes [2], from which between 1.15–2.41 million tonnes are estimated to reach the oceans as plastic waste, each year [3]. If we were to estimate de amount of MPs and NPs that might we produced as a consequence of the degradation of these average 2 million tons, the numbers of plastic particles rise exponentially.

The formation of nanometric size plastic particles during the fragmentation process of daily-use plastic objects, such as takeaway coffee cup lids or expanded polystyrene foam, has been already demonstrated. These findings show that the concentration of nanoplastics formed in the degradation process of bulk materials increases over time and that the obtained nanoparticles may present different surface characteristics [4]. Furthermore, Gigault et al. [5] collected MPs directly from the North Atlantic Gyre and documented their degradation to NPs, in environmentally realistic conditions. Although data on the occurrence of NPs in the environment is scarce due to analytical difficulty and environmental quantifications are not yet available, Ter Halle et al. [6] reported the isolation several populations of highly polydisperse particles on the nanoscale (1 to 1000 nm), from the colloidal fraction of a water sample obtained from the North

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Atlantic Gyre. In their study, the authors observed that the small microplastic sample contained a larger variety of polymers than the larger microplastic samples, documenting the presence of polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC), and Polyethylene terephthalate (PET) polymers in the nanometric plastic fraction.

In the environment, NPs can interact with other dissolved matter, such as persistent organic pollutants, other natural macromolecules and microorganisms. 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 with refractory properties and high molecular weight, make up between 60 and 80% of the DOC. It has been previously stated that dissolved organic carbon levels play a relevant role in the fate of organic chemicals, influencing their bioavailability and toxicity to aquatic organisms [7]. The effects and the potential synergism and antagonisms of mixtures of micro- and nanoplastics and organic compounds on biota are currently not well understood but instead are a cause for general concern.

In the last years, the effects of NPs' exposure on biota are being intensely investigated and hazardous effects have been found in several species, including fish. Moreover, nanoplastic particles have been identified in the brain of fish after waterborne or food-mediated exposure, indicating that they are capable of crossing the blood-brain barrier, a highly selective permeability barrier essential in the protection of the brain from systemic toxins.

Chemical contaminants can be relevant stressors to teleost fish, leading to a stress situation that comprises a wide range of responses, from molecular to physiological and behavioral. After perception of stress, the hypothalamus–pituitary–interrenal (HPI) axis is trigged, activating the steroidogenic pathways which culminate in the release of cortisol by the head kidney. The head kidney is complex organ of particular importance in fish, due to the multiple functions associated with it, including stress-related hormone secretion, immune response and hematopoiesis. It has been previously demonstrated that several chemical stressors induce changes in cortisol levels of fish, thus interfering with the endocrine function. The increase in plasma cortisol as a consequence of exposure to contaminant stressors can be part of the normal stress response to restore homeostasis, but under certain circumstances homeostasis cannot be restored and endocrine disruptive effects can occur. Plasma cortisol is broadly used as the main biomarker of physiological stress in fish, including in animals exposed to environmental contaminants. Moreover, stress episodes in fish can also lead to alterations in the immune response, due to interactions of the neuro-endocrine function with the immune system in fish. In the present study, plasma cortisol and glucose levels were measured as current indicators of the physiological stress status.

The available information on the effects of nanoplastics focusing on fish used for human consumption is practically inexistent/very scarce. For this reason, in the present research work we used the marine fish species *Dicentrarchus labrax* (European seabass) as a model. This teleost is a common and valuable product in Mediterranean area, both for the fisheries and the aquaculture sectors. Furthermore, D. labrax is a top predator and may therefore be exposed to small sized plastics both via water and the food web representing a potential risk to human health.

Based on the principle that changes at a molecular level occur prior to manifestations at higher levels of biological organization, molecular endpoints are considered sensitive and efficient tools to detect effects of xenobiotics. Furthermore, they have been appointed as a useful in a first approach to rank the toxic effects of different micro- and nanoplastics.

The purpose of this study was to evaluate if NPs could be recognised as a stressor by the HPI axis of *D. labrax* after waterborne NPs exposure, as well as to assess the role of HA in the potential effects caused by NPs. For this reason, changes in molecular biomarkers were evaluated in the head kidney of fish. Expression of target genes involved in several stress-related key functions, i.e. immune response (interleukin 1 beta - il1b, interleukin 6 - il6, tumour necrosis factor alpha - tnfa, interleukin 8 - il8, interleukin 10 - il10, transforming growth factor beta - tgfb) cellular stress (heat shock protein 70 - hsp70) and stress-related hormone secretion (melanocortin 2 receptor - mc2r, glucocoticoid receptor - gr, steroidogenic acute regulatory protein - star) was assessed in head kidney.

2 Experimental

2.1 Organisms

Juvenile sea bass (length 14.6 ± 2.4 cm; weight 21 ± 4.3 g) were acquired from an aquaculture facility (Spain) and transferred to a 1000-L aquaria containing aerated and filtered artificial seawater (Ocean Fish, Prodac). Artificial saltwater was prepared by dissolving the salt in reverse osmosis water until reaching a salinity of 34. Fish were kept in this tank for acclimation during 4 weeks, at 19 °C water temperature and natural photoperiod. During this period, the fish were fed daily with commercial fish food.

2.2 Exposure Protocol and Sampling

Procedures adopted in this experiment generally followed OECD guideline 203 (1992) for fish acute bioassays. Ethical animal care guidelines were strictly followed (EU 2010/63) and procedures adopted in the assay were previously authorized by the Portuguese legal authority (N421/2013). Six fish were randomly distributed into duplicate 20 L experimental tanks (3 fish per tank) containing 15 L test solution, maintaining the rest of the physicochemical as described for the acclimation period. Fish were exposed to 0 mg/L (control), 0.02 and 20 mg/L of PS NPs alone or in the presence of 1 mg/L of HA, for 96 h. In order to prevent significant nanoparticles deposition and to reduce the build-up of metabolic residues, 75% of the medium was renewed every 24 h. After the 96 h exposure, all fish were euthanized by over-anesthetizing them in a tricaine methanesulfate (MS222) bath. Skin mucus was immediately collected carefully rasping the dorso-lateral surface of the fish, following methodology described by Guardiola et al. [8]. Blood was promptly collected from the posterior cardinal vein with heparinized syringes, kept on ice, and posteriorly centrifuged for plasma isolation.

Head kidney was dissected and instantly frozen in liquid nitrogen, for posterior transcriptional analysis. All samples were stored at −80 °C until processed.

2.3 Quantification of Cortisol and Glucose in Fish Plasma

Plasma and mucus cortisol levels were determined by double-antibody radioimmunoassays (RIA). In brief, Cortisol RIA used Cortisol I125 (Cortisol-3-O-CMO-Histamine), specific activity 10 μCi as tracer (MP-Biomédicals-Germany); synthetic cortisol (Sigma, Barcelona, Spain) as the standard and an antibody raised in rabbits against Cortisol-3-O-Carboxymethyloxime-BSA (MP-Biomédicals-Germany), the complex was precipitate with goat antibody against rabbit IgG (Sigma, Barcelona, Spain). Dilution of samples showed good parallelism with the standard curve and recovery of spiking samples was around 100%. All samples to be statistically compared were run in the same assay to avoid inter-assay variability. The intra-assay coefficient of variation was less than 12%. The sensitivity for mucus cortisol was 39 pg/ml and for plasma cortisol was 0.5 ng/ml.

2.4 RNA Isolation, cDNA and Real-Time Quantitative PCR

The head kidney of fish was processed to extract total RNA, using Tri Reagent® (Sigma-Aldrich T9424). One microgram of RNA was retro-transcribed using the High capacity cDNA reverse transcription kit (Applied Biosystems, Thermo Fisher Scientific, USA) following the manufacturer's instructions. A Bio-Rad CFX384 Real-Time PCR Detection System (Bio-Rad Laboratories, USA) was employed to run the RT-qPCR, using the iTaqTM Universal SYBR® Green Supermix (Bio-Rad Laboratories, USA). The expression data obtained from three independent biological replicates were used to calculate the threshold cycle (Ct) value. The RT-qPCR analysis of the individual samples was determined following the same protocol described above.

In order to determine the most appropriate housekeeping gene among three (elongation factor-1α - ef1α, glyceraldehyde 3-phosphate dehydrogenase – gapdh and cDNA similar to 60S Ribosomal Protein L13α – l13α), NormFinder plug-in [9] was used. Expression of target genes was normalized with the selected housekeeping genes and relative gene expression calculated with the ΔΔCt method [10]. Genes involved in the immune response (il1b, il6, tnfα, il8, il10, tgfb), general cell-stress (hsp70) and the stress-related steroidogenic pathways (mc2r, gr star) were assessed in this study.

2.5 Statistics

A One-Way ANOVA was performed to test for significant differences between experimental groups in the measured endpoints. This analysis was followed by the Tuckey test to signal significant differences among/between all groups. GraphPad Prism 7 software pack was used to perform this analysis. Results are expressed as mean ± standard error (SE, n = 6).

3 Results and Discussion

Concerning expression of target genes related to the immune function, transcriptional levels of interleukines il1b and il6 were unaltered in relation to control group after all exposure conditions. Expression of the tnfα gene presented upregulated transcription in the 0.02 mg/L PS exposure condition, when compared to control. Transcripts of il8 were unaltered in relation to control group after all exposure conditions. The mRNA levels of il10 presented a significant increase with respect to control after exposure to 1 mg/L HA and to 0.02 mg/L PS + 1 mg/L HA. Regarding tgfb, all exposure conditions showed significantly upregulated transcriptional levels compared to the control group. Cell-tissue repair related gene hsp70, presented a significant increase in mRNA abundance in seabass exposed to 1 mg/L HA in comparison to the control group. Moreover, exposure to 0.02 PS + HA exhibited significantly lower mRNA levels than 1 mg/L HA exposure. Regarding the gene expression of steroidogenic pathway genes, a significant increase in mRNA abundance of mc2r in comparison to control was found after 96 h exposure to 1 mg/L HA and to 0.02 mg/L + HA. The exposure to 0.02 mg/L + HA showed significantly higher mRNA levels than the 0.02 mg/L PS and 20 mg/L PS + HA exposure. Transcriptional levels of gr appeared upregulated when compared to control in all exposure conditions. Finally, concerning the expression of star gene, exposure to 0.02 mg/L + HA showed significantly higher mRNA levels than the 0.02 mg/L PS and 20 mg/L PS + HA groups (Fig. 1).

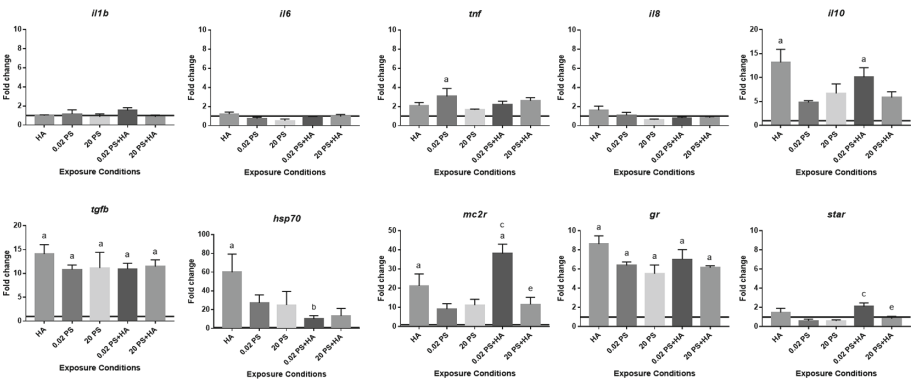

Fig. 1. Target genes mRNA levels determined in the head kidney of Dicentrarchus labrax, after 96 h of exposure to polystyrene nanoplastics (PS), alone or in the presence of humic acids (HA). Values represent the means ± SE (n = 6). Differences were determined by one-way ANOVA followed by Tukey's test. Statistically significant differences (p = 0.05) are marked as follows: a vs. control, b vs. humic acids, c vs. 0.02 PS, e vs. 0.02 PS + HA.

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Preliminary Data on the Polymer Type Identification from Estuarine Environmental Samples

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1 Introduction

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, coastal oceanic areas and 4 estuarine regions: ria de Aveiro, Mondego, ria Formosa and Mira. In this context, we report the first results in surface waters in one of the estuarine study areas (Mondego, Portugal). Water samples are taken with a manta tow net with 200 cm length and 300 µm mesh size [1] and sediments with a Petite Ponar grab [2]. 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 [3]. 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 fibres (cellulose, hemicellulose and lignin). Distinguish MPs 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. MPs data from surface water and sediments will be crossed to study the distribution of MPs. Preliminary results from water samples revealed a density of 0.81 particle per m^3 and that low-density polyethylene is the most dominant plastic polymer type (about 90 %).

2 Experimental

2.1 Materials and Methods

Surface water samples were collected with a manta trawl net while Petite Ponar grabs were used for sediments. Sample preparation includes sieving, digestion and drying

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methods. Remained particles were identified using both ATR-FTIR and micro ATR-FTIR analytical techniques.

2.2 Sampling Method

Sampling was conducted in 4 estuarine regions of Portugal: ria de Aveiro, Mondego, ria Formosa and Mira. In total, 41 samples were collected. A sediment sample was taken from all stations while a single sample of surface water (up to 15 cm depth) was taken from ria de Aveiro and Mondego. Surface water samples were collected using a manta trawl with a rectangular opening (30 cm high by 15 cm wide) and a 200 cm long, 300 μm mesh size net. The sampled transects were not equidistant, but sampling periods were about 20 min long each. Sediment samples were collected by Petite Ponar grabs.

2.3 Preparation of Samples

In the laboratory, the water sample was separated into two size ranges with 63 μm and 2 mm mesh size sieves. Both fractions ($f_1 > 2$ mm and 63 μm $< f_2 < 2$ mm) were transferred onto 500 mL beakers using ultrapure water. To remove the organic matter from the subsamples a H_2O_2 pre-treatment was applied. The subsamples were immersed in 150 mL of 30 % H_2O_2 (Merck, Germany) solution [4] with continuous stirring at room temperature for 4 days. The resulting solution was filtrated with Sartorius® nitrocellulose membrane filters with 47 mm of diameter and 0.45 μm of pore size. The beakers were rinsed with ultrapure water to ensure no visible particles were left within the beakers. Membrane filters were oven-dried at 60 °C in sealed Petri dishes [5] for 48 h. Membrane filters were stored until analysis. Blanks were made to check for any source of contamination and ensure the quality control of the sample preparation procedure (solvent, H_2O_2, filtration kit, and sieve blanks).

2.4 Analytical Techniques

Preliminary isolation of MPs in the filtered residues was conducted by visual inspection. Particles with a considerable size were analysed by ATR-FTIR (Spectrum Two) while smaller ones were identified using micro ATR-FTIR technology (Spotlight 200i). The resulting spectra were compared with the available spectrum library to identify the polymer type associated with the analysed MPs.

3 Results and Discussion

We present and discuss the provisional results of the surface water sample collected from the Mondego estuary. Other issues related to the obtained results are also discussed: the analysis of the spectra of some microparticles proved to be a challenge due to the presence of natural cellulosic fibres (cellulose, hemicellulose and lignin).

3.1 Results

MPs were detected in the surface water of the Mondego estuary sample (0.81 particles per m^3). There were two type of MPs: plastic fragments and plastic films. Fragments dominated over the other type of MPs. In total, 96.4 % of the MPs found were plastic fragments. MPs were divided into different colour categories: blue, black, brown, green, yellow, transparent and red. Grey particles were counted in the black category. The transparent category consisted of colourless particles. The red group includes pink and purple particles. In total, more than half of the MPs found were transparent (64.3 %), followed by black (17.9 %), red (10.7 %) and blue (7.1 %) MPs. Regarding particle size [6], half of them (50 %) fall into the large microplastics (LMP: 1–5 mm) category. Contributing with 25 % each are the small microplastics (SMP: <1 mm) and meso-plastics (>5 mm) categories. Although not all particles have been identified yet, polyethylene (PE) polymer type has a total contribution of 92.9 %. In addition to PE, only polypropylene (PP) was identified. Other particles remains unidentified because despite there are evidences of being polymer-based particles those are still not enough to properly identify them. Figure 1 shows two micro ATR-FTIR spectra from the same PE particle acquired in different positions of the particle.

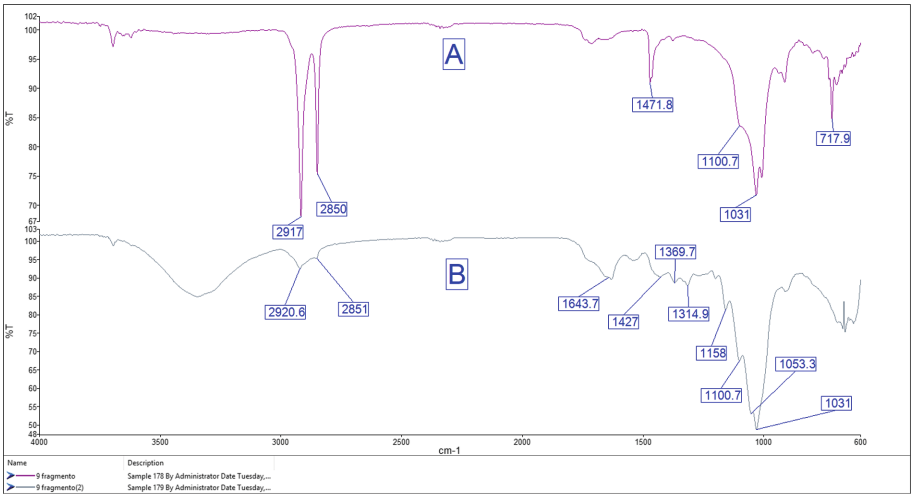

Fig. 1. Two micro ATR-FTIR spectra acquired in different spots from the same microparticle. **A**: polyethylene; **B**: cellulose.

The different spots of the particle are shown in Fig. 2. Spot A is the surface of the polymer while the spot B area is covered with cellulose.

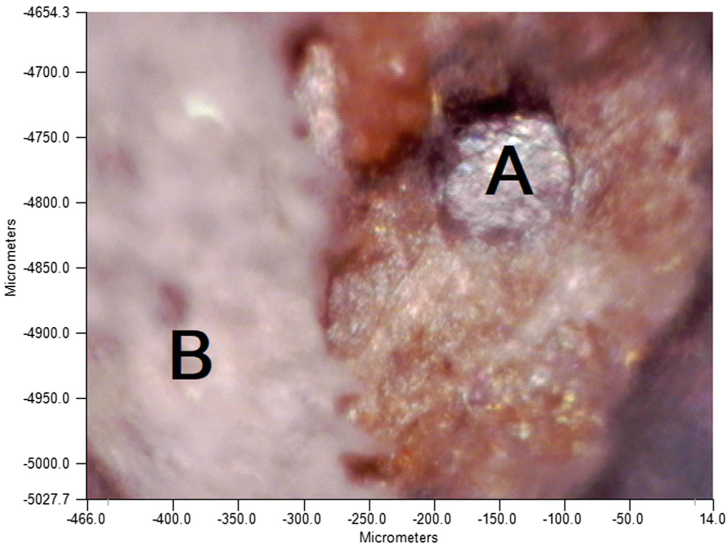

Fig. 2. PE microparticle. Area **A** is the polymer surface and area **B** is cellulose covering the surface of the polymer.

3.2 Discussion

Provisional data reveals a dominant presence of low density polymers, which is normal for surface water samples. Both ATR-FTIR and micro ATR-FTIR techniques are very helpful for polymer identification. Though, in some cases where MPs are covered with biofouling, whether inorganic or organic, it may be difficult to identify them because ATR is a contact method where infrared light penetration is very low. As work progresses, data from both surface water and sediment from all study areas will be used to verify the distribution of MPs.

3.3 Spectroscopic Analysis

Spectrum B from Fig. 1 has a representative broad absorption band at 3346 cm^{-1}, which belongs to the hydrogen-bonded OH stretching present in cellulose [7]. The two peaks at about 2920 and 2851 cm^{-1} are due to C-H stretching from aliphatic saturated compounds [7]. The band at 1643 cm^{-1} is assigned to the bending mode of adsorbed water [7]. The bands at 1427, 1369, and 1314 cm^{-1} belong to the $–CH_2$ scissoring, -OH bending vibration, and C-H asymmetric deformation of cellulose, respectively [7]. The bands at 1158, 1100, and 1031 cm^{-1} are assigned to the C-O-C, C-O, and C-C stretching of cellulose, respectively [7].

4 Conclusions

MPs were found in surface water samples of Mondego estuary. Fragments were the most common type of MPs observed. As dominant polymer type, PE was found in all colour categories reported. An efficient pre-treatment is needed when studying samples with high levels of organic matter. Cellulosic natural fibres in MPs can mislead because a particle with a simple spectrum can have its characteristic bands easily overlapped.

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Qualitative and Quantitative Screening of Organic Pollutants Associated on Microplastics from Ofanto River (South Italy)

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1 Introduction

A key concern of microplastic pollution is whether they represent a risk to ecosystems and human health. However, there is a lot of uncertainty associated with this issue. To evaluate the risk of microplastics to aquatic environments, data on the exposure and effect levels of microplastics are required. The adverse effects on aquatic organisms exposed to microplastics can be separated into two categories: physical effects and chemical effects. The former is related to the particle size, shape, and concentration of microplastics, and the latter is related to hazardous chemicals associated with microplastics. Although data on microplastic exposure levels in marine environments and organisms have rapidly increased in recent decades, limited information is available on chemicals associated with microplastics.

The present study aims to identify and quantify persistent organic pollutants adsorbed on microplastics which then become vectors for these highly toxic pollutants; some of these compounds are added during plastics manufacture, while others adsorbed from the surrounding ambient.

PCBs, OCPs and PAHs have been selected as target compounds to be identified and quantified while a qualitative general non target-screening of plastic additives and related chemicals has been carried out by Gas Chromatography High-Resolution Mass Spectroscopy technique.

2 Sample Collection

Samples were collected from Ofanto river, the most important river in Apulia region (South Italy) for length, area and abundance of water. In order to monitor the trend of microplastic concentrations over a year, five seasonal sampling campaigns have been planned. River surface microplastics samples were collected during February, April,

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October, December 2017 and May 2018; all of them were taken from the same point located at 6 km from Ofanto river mouth following the experimental conditions reported in [1].

In order to compare the concentration of adsorbed environmental contaminants to the amount of natural chemicals originating from plastics three matrix blanks made by virgin colorless polyethylene (PE) pre-production pellets (size 5 mm), virgin green polyethylene (PE) pre-production microparticles (size <500 μm) and virgin colorless polypropylene (PP) pre-production pellets (size 5 mm) were analyzed together with the field samples. Pre-production material was purchased by a local plastic industry.

3 Materials and Methods

3.1 Pollutants Extraction

About 250 mg of plastic material for each sample as well as for the blanks were mixed with previously washed diatomaceous earth to form a free-flowing powder and spiked with known concentrations of an internal standard solution containing the labelled compounds [13C12]PCB 104. The mixture was extracted with 5 ml of n-hexane enough to completely submerge samples and then it was sonicated (Branson 5210R-MT Ultrasonic Cleaner) for 30 min at 40 °C. The extraction procedure was repeated other two times with two additional 5-ml portions of clean solvent to insure complete extraction of analytes from the matrix. The organic extracts were then evaporated to incipient dryness under gentle nitrogen stream (using a Caliper Life Sciences TurboVap II Concentration Workstation) and re-solubilized into 0,5 mL Nonane.

3.2 Target Compounds Analysis

GC-MS analysis of 32 PCBs (#18, 28, 52, 44, 95, 101, 104*, 99, 81, 77, 110, 151, 123, 149, 118, 114, 146, 153, 105, 138, 126, 187, 183, 128, 167, 177, 156, 157, 180, 169, 170, 189), 8 OCPs (α-BHC, β-BHC, γ-BHC, δ-BHC, Aldrin, p,p'-DDT, p,p'-DDE, p,p'-DDD) and 16 EPA-PAHs (acenaphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(123-cd)pyrene, dibenzo(ah)anthracene, and benzo-(ghi)perylene) were performed using a ThermoElectron TRACE GC Ultra coupled with a PolarisQ Ion Trap (Thermo Electron, Austin, TX) mass spectrometer equipped with a PTV injector and a TriPLUS RSH autosampler. The system was managed by Thermo Electron Xcalibur software version 1.4.1. Compound separation was achieved using an Agilent CP8944 VF- 5 ms U (length 30 m, i.d. 0,25 mm, film thickness 0,25 μm) column.

3.3 Non-target Compounds Screening

A non-target qualitative screening of chemicals adsorbed on microplastics was performed by GC-HRMS analyses using a 7890B gas chromatograph (Agilent Technologies, Santa Clara, USA) (Fig. 2.5.4.1.A) and a 30 m J&W HP-5MS (5% Phenyl

Methyl Siloxane) capillary column with an inner diameter of 250 µm and a thickness of 0.25 µm coupled to a 7200 Q-ToF (Agilent Technologies, Santa Clara, USA) operating in electron ionization (70 eV) and full scan (m/z 50–500) mode. Helium was used as carrier gas at a constant flow of 1 ml/min.

Data processing was performed by deconvolution of the chromatographic peaks with the Unknowns Analysis tool of MassHunter Agilent quantitative analysis software (B.08.01). Identification of the compounds was carried out for comparison with NIST 17 library (covering EI MS spectra for 267,376 different chemical compounds) adopting a minimum similarity criterion of 70%. Similarly, peaks that also were detected in the procedural blanks were eliminated.

4 Results and Discussion

4.1 Results

The quantitative analysis of PCBs, PAHs and OCPs underlined the presence of these contaminants on microplastics collected from surface water. PAHs were found in all samples showing values ranging from 30 to 269 ng/g (Table 1). All samples were found to contain PCB (expressed as the sum of 31congeners) in the range from 0.6 to18 ng/g. PCB 52 and five of the 16 EPA-PAHs have also been detected in virgin pre-production pellets (Fig. 1 and Table 1).

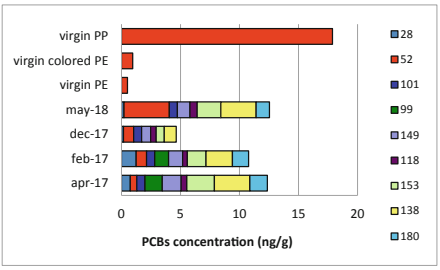

Fig. 1. Composition of OCPs found on environmental microplastic samples (Apr-17, Feb-17, Dec-17, May-18) and on virgin matrix samples (Virgin PE, Virgin colored PE, Virgin PP). Virgin PE and PP were colorless pellets while Virgin colored PE were green microparticles <500 µm).

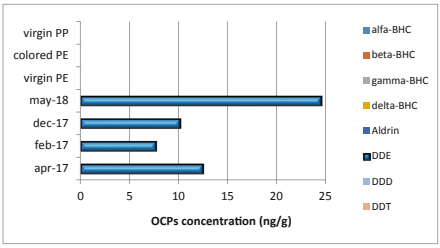

Fig. 2. Composition of OCPs found on environmental microplastic samples (Apr-17, Feb-17, Dec-17, May-18) and on virgin matrix samples (Virgin PE, Virgin colored PE, Virgin PP). Virgin PE and PP were colorless pellets while Virgin colored PE were green microparticles <500 µm).

The only pesticide observed associated with plastic debris was DDE showing for almost all samples values above the Italian regulatory limit (10 ng/g) set for soils (Fig. 2).

The deconvolution of the chromatographic peaks detected by the HRGC-MS chromatograms related to the samples extracted (Apr. 17, Feb 17, Dec 17, May 18), revealed the presence of a mean of 3,674 components, 93 of which were identified by comparison with the spectra obtained from NIST 17 library with a mach factor >70%. Even though several peaks were present in the HRGC-MS chromatograms many peaks were not able to be identified. Table 2 shows a screening of fifty on ninety-three chemicals hypothetically identified divided on the basis of the main source of origin: Hydrocarbons (Alkanes, Alkenes, Substituted and Cyclic hydrocarbons, PAHs) Plastic Additives (UV-stabilizers, Phthalates, Antioxidants), Intermediate, Alcohols, Biofilm and Algae compounds.

4.2 Discussion

Although the amount of PCBs observed in the present work revealed law values, the congeners distribution shown by Fig. 1 underlines a distinct pattern between virgin pre-production microplastics and environmental samples. On 31 congeners investigated, PCB 52 is the only congener found on all virgin pre-production microplastics samples contributing alone for the 100% of the PCBs amount. These results suggest that congener 52 is probably used as an additive in plastic raw materials in particular for PP polymers and colored raw materials.

The monitoring of PAHs using beached resin pellets by the International Pellet Watch Program revealed a concentration of hydrocarbons ranging from values lower the limit of quantification to values greater than 24000 ng/g-pellets with a mean density of about 3000 ng/g-pellets. These results are higher than those found in the present study (30–270 ng/g) although no Italian data on marine pellets and on microplastics of freshwater environments are present in the database to which to compare. The only pesticide found in all environmental microplastic samples was 4,4-DDE ranging from 8 to 25 ng/g exceeding the Italian limit of 10 ng/g in three campaigns (May 18, April 17, December 2017). In nature, p,p'-DDE and p,p'-DDD are the two main products of dechlorination of p,p'-DDT by microorganisms and/or physico-chemical properties of soil [2]. These results suggest that DDT may have been used as a pesticide in that area, probably in past decades when it was legal. Indeed, Apulia is well-known for their large vineyards and olive plantations on their hills and along coastal areas [3, 4], and high DDT residues may originate from agricultural activities in these areas [5]. A detailed study on the variety of chemical compounds present on different types of microplastic was carried out, and the possible origins of these compounds were suggested. A great diversity of compounds, which were probably related to plastic, was found, as well as compounds that can be related both to plastic, biofilms and/or environmental pollution, such as agricultural and industrial activities. The great variety and the limited information available concerning these plastic related compounds and their degradation products illustrate the need for a thorough bioavailability study.

Table 1. Concentrations of 8 OCPs, expressed as ng/g, found on environmental microplastic samples (Apr-17, Feb-17, Dec-17, May-18), on virgin matrix samples (Virgin PE, Virgin colored PE, Virgin PP) and on Environmental blanks. Virgin PE and PP were colorless pellets while Virgin colored PE were green microparticles < 500 μm).

PAHs	Naphtalene	Acenaphtylene	Acenaphtene	Fluorene	Phenantrene	Antracene	Fluoranthene	Pyrene	Benzo (A) antracene	Chrysene	Benzo(B) fluorantene	Benzo(K) fluorantene	Benzo (A) pyrene	Indeno (1,2,3,) pyrene	Dibenzo (A;H) antracene	Benzo (G;H;I) perylene	TOTAL
Unit of measure	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
Apr-17	<LOD	16	12	30	57	<LOD	33	41	<LOD	7.3	<LOD	<LOD	<LOD	3.4	<LOD	16	215
Feb-17	<LOD	9.8	6.6	33	78	<LOD	45	54	<LOD	10	<LOD	<LOD	<LOD	9.8	<LOD	23	269
Dec-17	<LOD	4.7	<LOD	34	46	<LOD	32	15	<LOD	3.3	<LOD	<LOD	<LOD	5.5	<LOD	20	160
May-18	<LOD	1.4	<LOD	<LOD	30	<LOD	32	27	<LOD	10	<LOD	<LOD	<LOD	5.2	<LOD	15	120
Virgin PE	28.43	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.5	<LOD	<LOD	30
Virgin Colored PE	<LOD	<LOD	2.7	<LOD	27	<LOD	26	18	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	73
Virgin PP	15	<LOD	<LOD	29	<LOD	<LOD	17	4.8	<LOD	<LOD	<LOD	<LOD	<LOD	2.4	<LOD	<LOD	69
Environm. blanks	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Table 2. Overview of fifty different types of compounds hypothetically identified on microplastic samples by the general Screening. The components are grouped according to their presumed origin.

Category	CAS#	Compound name	RT	Match factor	Formula	Library molecular weight
Hydrocarbons-Alkanes	544-76-3	Hexadecane	9.73	96.33	$C_{16}H_{34}$	226.266
	593-49-7	Heptacosane	16.42	94.68	$C_{27}H_{56}$	380.438
	630-04-6	Hentriacontane	20.49	93.29	$C_{31}H_{64}$	436.501
	630-02-4	Octacosane	18.99	91.34	$C_{28}H_{58}$	394.454
Hydrocarbons-Alkenes	629-89-0	1-Octadecyne	20.05	88.42	$C_{18}H_{34}$	250.266
	765-13-9	1-Pentadecyne	18.60	87.09	$C_{15}H_{28}$	208.219
Substituted Hydrocarbons	1186-53-4	Pentane, 2,2,3,4-tetramethyl-	7.74	92.25	C_9H_{20}	128.157
	62108-23-0	Decane, 2,5,6-trimethyl-	9.18	89.72	$C_{13}H_{28}$	184.219
	2801-84-5	Decane, 2,4-dimethyl-	8.87	73.02	$C_{12}H_{26}$	170.203
	1002-43-3	Undecane, 3-methyl-	10.74	89.41	$C_{12}H_{26}$	170.203
	53366-38-4	Cyclopentane, (2-methylbutyl)-	14.97	88.11	$C_{10}H_{20}$	140.157
	562-49-2	Pentane, 3,3-dimethyl-	12.54	78.18	C_7H_{16}	100.125
	17301-32-5	Undecane, 4,7-dimethyl-	10.95	85.37	$C_{13}H_{28}$	184.219
	563-16-6	Hexane, 3,3-dimethyl-	14.01	82.51	C_8H_{18}	114.141
Halogenated Hydrocarbons	4292-19-7	Dodecane, 1-iodo-	18.33	85.83	$C_{12}H_{25}I$	296.1
	1000406-32-0	Tetracosane, 1-iodo-	18.16	80.23	$C_{24}H_{49}I$	464.288
	4282-42-2	Nonane, 1-iodo-	17.14	71.06	$C_9H_{19}I$	254.053
	4292-19-7	Dodecane, 1-iodo-	20.19	70.74	$C_{12}H_{25}I$	296.1
Cyclic Hydrocarbons	4457-00-5	Cyclopentane, hexyl-	17.69	70.39	$C_{11}H_{22}$	154.172
	294-62-2	Cyclododecane	13.45	80.15	$C_{12}H_{24}$	168.188
	16538-89-9	Cyclooctane, (1-methylpropyl)-	14.28	77.88	$C_{12}H_{24}$	168.188
	4516-69-2	Cyclopentane, 1,1,3-trimethyl-	11.87	77.39	C_8H_{16}	112.125
PAHs	129-00-0	Pyrene	15.86	75.34	$C_{16}H_{10}$	202.078
	83-32-9	Acenaphthene	9.90	82.13	$C_{12}H_{10}$	154.078
	91-57-6	Naphthalene, 2-methyl-	9.34	81.77	$C_{11}H_{10}$	142.078
	5394-86-5	1H-Indene, 1-(phenylmethylene)-	13.70	76.75	$C_{16}H_{12}$	204.094
	24157-81-1	2,6-Diisopropylnaphthalene	12.11	76.95	$C_{16}H_{20}$	212.157
	2131-42-2	Naphthalene, 1,4,6-trimethyl-	11.08	75.79	$C_{13}H_{14}$	170.11

(*continued*)

Table 2. (*continued*)

Category	CAS#	Compound name	RT	Match factor	Formula	Library molecular weight
Plastic additives-UV stabilizer	1843-05-6	Octabenzone	19.81	94.19	$C_{21}H_{26}O_3$	326.188
	5466-77-3	2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester	16.68	92.45	$C_{18}H_{26}O_3$	290.188
Plastic additives-Phtalates	84-69-5	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	13.37	91.99	$C_{16}H_{22}O_4$	278.152
	117-81-7	Bis(2-ethylhexyl) phthalate	17.98	92.49	$C_{24}H_{38}O_4$	390.277
	84-66-2	Diethyl Phthalate	11.27	88.20	$C_{12}H_{14}O_4$	222.089
	84-74-2	Dibutyl phthalate	14.10	87.10	$C_{16}H_{22}O_4$	278.152
	1000315-48-5	Phthalic acid, bis(2-pentyl) ester	19.91	77.68	$C_{18}H_{26}O_4$	306.183
	1000315-52-2	Phthalic acid, 2-isopropylphenyl methyl ester	10.78	75.33	$C_{18}H_{18}O_4$	298.121
Plastic additives-Antioxidant	3896-11-5	Bumetrizole	18.36	88.01	$C_{17}H_{18}ClN_3O$	315.114
	101-72-4	1,4-Benzenediamine, N-(1-methylethyl)-N'-phenyl-	15.41	86.59	$C_{15}H_{18}N_2$	226.147
	95906-11-9	Tris(2,4-di-tert-butylphenyl) phosphate	17.85	86.12	$C_{42}H_{63}O_4P$	662.446
Intermediate	4337-65-9	Hexanedioic acid, mono(2-ethylhexyl)ester	17.01	91.05	$C_{14}H_{26}O_4$	258.183
Alchols	10042-59-8	1-Heptanol, 2-propyl-	9.03	95.90	$C_{10}H_{22}O$	158.167
	3913-02-8	1-Octanol, 2-butyl-	11.59	86.88	$C_{12}H_{26}O$	186.198
	54004-41-0	1-Pentanol, 4-methyl-2-propyl-	11.73	84.85	$C_9H_{20}O$	144.151
	598-32-3	3-Buten-2-ol	9.02	70.52	C_4H_8O	72.058
	112-43-6	10-Undecen-1-ol	15.97	70.41	$C_{11}H_{22}O$	170.167
Biofilm and Algae compounds	83-47-6	.gamma.-Sitosterol	26.10	82.02	$C_{29}H_{50}O$	414.386
	201358-24-9	24-Noroleana-3,12-diene	26.66	88.59	$C_{29}H_{46}$	394.36
	502-69-2	2-Pentadecanone, 6,10,14-trimethyl-	13.15	88.43	$C_{18}H_{36}O$	268.277
	1617-70-5	Lup-20(29)-en-3-one	27.45	77.11	$C_{30}H_{48}O$	424.371

5 Conclusions

The work reported here is the first study showing an Italian river context. The resulting data provide an initial assessment of the extent and nature of this pollution in Ofanto river. Limited study related to freshwater ecosystems are present in literature and even less about Italian environments.

This topic is subject to ongoing study and future perspectives regard a deepening of microplastic adsorbed pollutants investigating about the presence of heavy metals and herbicides on microplastics. These activities are part of a regional project funded by Apulia region: MICROPLASMA- MIcro and maCRO PLAStic pollution Monitoring with Advanced technologies.

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Assessment of Microplastic Pollution in Sarno River

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1 Introduction

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 [1]. 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 [2].

The occurrence of plastics in marine environments has been widely reported in several papers [3–5]. 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 [6]. 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 main tributaries the Cavaiola and Solofrana torrents [7].

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).

In this contribution, a preliminary study on the presence of microplastics in the Sarno River is presented.

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2 Experimental

2.1 Sampling Procedure

The river chosen for the determination and quantification of microplastics was the Sarno River, in Italy. Water sampling was conducted at three different sites along the river upstream to downstream: site 1, in the city of Sarno, near the river source; site 2, after the input of two important tributaries, the Cavaiola and Solofrana; site 3 near the river estuary. The sampling sites are illustrated in Fig. 1. A manta trawl with a 330 μm mesh net was used for water sampling.

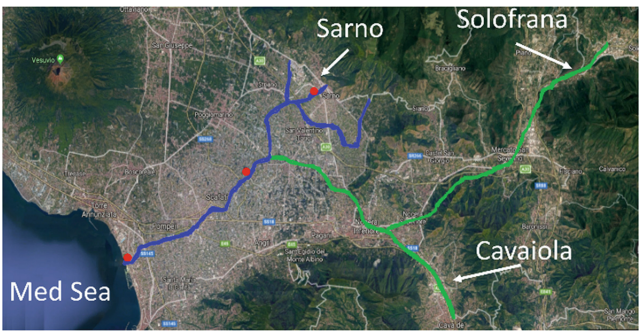

Fig. 1. Map of the sampling sites (red dots) along the Sarno River. (Color figure online)

2.2 Analytical Techniques

Water samples were processed using peroxide oxidation and then filtered. Samples were first filtered through a metal sieve (pore size 1.5 mm), the recovered fraction was washed with Milli-Q water. The water was filtered again through a 180 μm pore sizer filter. The recovered samples were analysed by using a stereomicroscope Leica (OM), a scanning electron microscope (SEM) equipped with EDX analysis and a Fourier transformed infrared spectrometer (FTIR).

3 Results and Discussion

The two step filtration procedure allows the separation of the organic fraction of large dimension, constituted mainly by river flora and shells of different shapes, and the analysis of the smaller fraction filtered on a filter with smaller porosity. The water at the river source appears contaminated by domestic sewages since food residues and a high amount of cellulose fibers were detected together with fat, oil and grease (FOG) deposits and different diatoms.

The water sampled at site 2, after the input of Cavaiola and Solofrana tributaries was characterized by the presence of burnt shrubs, imparting a dark color to the

recovered samples, leaves and shells. In the samples, polystyrene microplastics were detected together with oil and grease (FOG) deposits.

The analysis of the sample of the river estuary, site 3, was strongly affected by the presence of an abundant organic fraction that remains after the peroxide oxidation and obscures the possible presence of small plastic particles (Fig. 2).

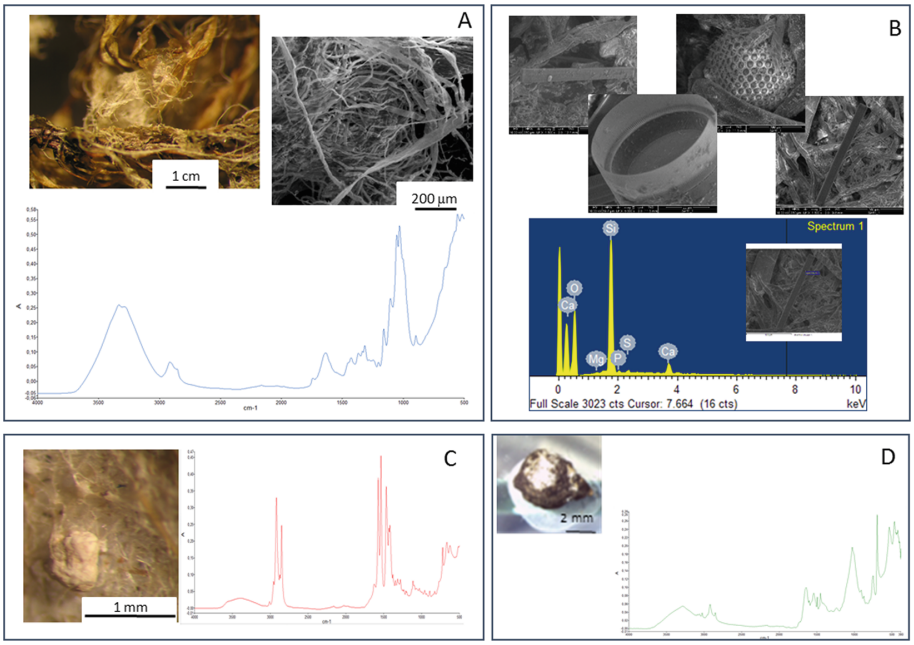

Fig. 2. (a) Detection of cellulose fibers; (b) EDX analysis of different diatoms; (c) fat, oil and grease (FOG) deposit; (d) polystyrene microplastic.

4 Conclusion

This study represents the first approach in the detection of microplastics in Sarno river. Due to the complexity of the water samples recovered from Sarno river in term of presence of organic substance, the presence of microplastics was not easily detectable.

These findings emphasize the urgency to further monitor this river and to develop an *ad hoc* analytical protocol to analyse and quantify microplastics in Sarno river.

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Holistic Approach to the Marine Microplastics: Sampling, Characterization, Consequences

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1 Introduction

The aim of this research is to propose the universal sampling method of pelagial waters to provide qualitative and quantitative data about marine microplastics [1]. It has been already confirmed that the plastic is ubiquitous in the marine environment and easily fragmented by UV radiation and mechanical force of waves and wind [2]. Taking into consideration the increasing production, time of use, and following degradation, one may expect the problem of microplastics debris to be exponentially increasing in the years to come [3]. Microplastics (MPs) are defined as all plastic debris smaller than 5 mm [2].

Contrary to the secondary microplastic, which is the final product of physical and chemical degradation processes, the primary one comes to the ocean from cosmetics microbeads or synthetic cloths fibres. Among various consequences of their presence in the ocean one may point out the negative impact on the sea wildlife [4], possible ecotoxicity [5], significant change in ecosystem and global circulation in the food chain [6] including human health hazard. For more accurate predictions and theoretical modelling of microplastics spatial distribution, accumulation zones, and impact on the biota, the more precise source data are urgently needed [7]. Although several dozen scientific groups around the world are actively involved in this domain of research [8], the obtained results are often incomparable due to the different adopted methodologies. The most frequent inconsistency is related to the filter mesh size, usually from 50–330 μm, resulting in research focused directly on a particular fraction. Other differences are related mainly to the sampling depth, chemical pre-treatment of the material or data extrapolation procedure. Considering that, the comparison of results and so the global model construction is significantly constricted.

Moreover, to cover the vast area of the world ocean, the fieldwork should be scaled up. The involvement of NGO and citizen scientists in data collection is crucial. For these reasons, the sampling device needs to be simple and the measurement easy to carry on, for instance, by sailors. Furthermore, the proposed method should eliminate or at least hinder as much as possible the risk of contamination by every day available plastics or during further laboratory analysis. The control samples reveal the MPs concentration in air that is not to be neglected. That is one of the reasons why the one-step characterization is a significant advantage. In the proposed protocol, the collected material is directly (on the device filter) analysed by IR and Raman spectroscopy

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without any further substrate change. Within this research, only the surface water layer is considered as the beach and sediments are already so far much better studied and the existing protocols do not need to be significantly improved.

2 Materials, Methods, and Comparison with the Existing Solutions

In the project and construction of the proposed in this work sampling device, the various criteria were considered to ensure:

- significant simplicity and efficiency,
- acceptable overall cost,
- minimal samples contamination,
- possibility to collect debris in a precise range of linear dimensions.

Those parameters were chosen as a result of the systematic analysis of all main drawbacks of the existing solutions [9]. The aim was to overcome them. The first large-scale attempts of MPs monitoring in an open ocean system dates back to the beginning of the XXI century [10]. The nets, pumps, sieves, bottles, and buckets were reported. Bongo nets, WP2, plankton and multi nets, water intake pumps were directly implemented as water filters in search of the polymer debris. The neuston material, as a polymer itself, introduces the self-contamination. No standard in terms of their size, efficiency, or operating depth was established, making the quantitative information hard to normalize [11]. The second generation of device consists of the manta trawl, and their main drawback is the large net mesh (around 300–333 μm). They operate on the surface or submerged.

Further filtration and sieving of the material are frequently necessary. The samples pre-treatment, in general, is the one or combination of the acids (e.g. HNO_3, $HClO_4$), alkali (e.g. NaOH, KOH), oxidative (H_2O_2) or enzymatic (e.g. corolase, trypsin, pepsin, papain). For instance, among the methods listed in the latest reviews (2019), one may find the following sampling equipment: WP2, neuston net, net Mesh, manta trawl. All are insufficiently accurate and prone to the contamination. The crucial parameter to be noted is the filter diameter, which determines the cut-off of the smallest fractions and so underestimates final results. In the majority of reported studies, it is $\sim$300 μm, rarely 200 but sometimes even more than 750.

In the case of the proposed device, all of the used materials do not contain any plastics. That ensures minimal self-contamination. The metal parts, stainless steel, and alumina are applied. In that manner, the polymer neuston fibres are excluded from the system. The device is composed of the cylindrical filters support and the front chassis to amplify the laminar flow during trawling and prevent spinning (Fig. 1). The front part length is related to the stainless steel cylinder (from 6 up to 20 cm) adjustable diameter to ensure the accurate buoyancy of the device that should partially remain on the surface and collect the water flow from its first layer (up to the 0,5 m depth). The vertical supporting elements are from 20 up to the 40 cm. The flux might be calculated (estimated from time, speed and water column volume) or directly measured at the entrance.

The flow meter is advisable. That will enable the standardization of the hauling time for the desired water volume to be filtrated. The distance from the ship should be maintained at least 10 m to eliminate the self-contamination. In this construction, the 15 m long standard metal line was used. Between the main body and the wire, the swivel is placed, which is also the weakest part of the system, susceptible to break at the end of equipment lifetime. The fatigue scratch near the shackle was the most common damage observed during the exploration of the device. The shackles connect all other elements. It is recommendable to leave on the deck the free filter to collect the blank sample. In more detailed studies the two sets should be hauled simultaneously from both sides of the hull. The same device is appropriate to filter the water column at the determined location for a specified time (for instance in the harbours, estuary or at the delta of a river). In that case, the material is collected at the depth equal to the linear size (high) of the device. The total wave size is also taken into account.

Fig. 1. The metal filter support with the front chassis at different stages of device construction.

The main element is the system of two filters with different pore sizes: wider on the front (500 or 300 μm) and narrower at the bottom (100 or 25 μm). Both are metal woven chrome-nickel sieves provided by METALEX (Fig. 2). The role of the first is the initial filtering of larger particles and organic matter suspension, whereas the second constitute the collector and the substrate for further analysis. Adjusting the pore size of both filters is possible, and so the specific fraction of the debris might be collected. The commonly observed clogging of the neuston nets is almost eliminated. The chrome-nickel main advantage is its compatibility with IR or Raman spectroscopy, due to that the additional sample transfer or filtering in the laboratory is not needed. That enables the direct material characterization and limits contamination unavoidable during several steps protocol of samples preparation. That is not the case of still popular GFF filters to

which this approach constitutes the alternative. The main disadvantage of the glass fibres is their significant self-luminescence. The microscopic pictures confirm the pore size of 500, 300, 100 or 25 μm respectively. On Fig. 2, the sieves 500 and 25 are presented as after preliminary studies of various size combinations tested, and they were chosen as the best ones.

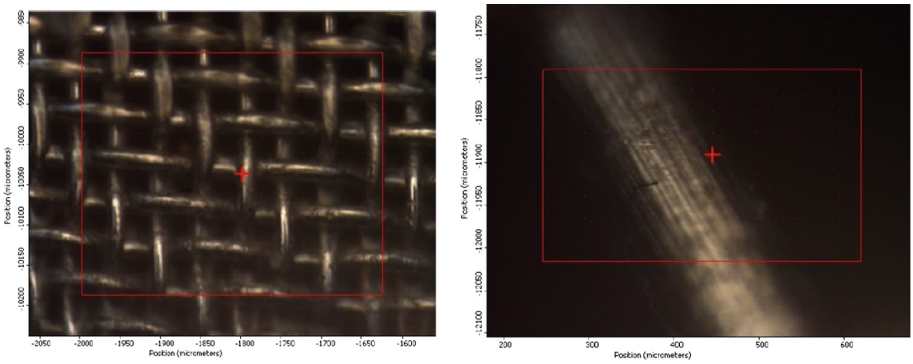

Fig. 2. The microscopic pictures of metal weaved chrome-nickel sieves of 25 and 500 μm pore size.

Although the smaller pore size of the second filter is better to quantify the micro fraction, the availability, costs, and technical issues are critical factors choosing 25 μm the reasonable compromise. U-bolt fits this collecting filter, quickly changed and transported in the aluminium foil. For further laboratory analysis, the following spectroscopy instruments [12] were used: IR microscopy Thermo Scientific Nicolet iN10MX, Raman microscopy with four laser lines accessible: 455 nm, 532 nm, 633 nm, 780 nm. SEM pictures are helpful for surface analysis.

The exploration time in the low weather conditions for the system is limited mainly due to the persistence of the swivel and seawater-induced corrosion, but still, on average, much longer than 72 h. During the conducted studies, some of the devices worked for weeks without any visible sign of the damage.

3 Field Tests, Additional Measurements, and Results of a Sampling Protocol

The proposed devices, with a bottom diameter of 95 mm or 75 mm, has been successfully tested on the following monitored waters:

- Ligurian Sea (part of the Mediterranean Sea, in particular, the Santuario Pelagos),
- West Coast of Svalbard Archipelago,
- Southern Baltic and the North Sea.

That choice of tested area is due to the numerous factors:

- their diversity (regarding ecosystems, abiotic conditions, and susceptibility to pollution sources),
- diagnostic importance (for instance the remote and pristine polar environment),
- the availability of complementary research data, such as the general sources and types of pollution, bio-indicators, physical and chemical conditions, water properties (in case of the long tradition of Polish Polar Scientific Expeditions in Svalbard or the constant monitoring in Santuario Pelagos – protection zone of the marine mammals).

The proposed equipment versatility is proved when considering the variety of chemical, physical and meteorological conditions in the selected area. Some examples of monitored area (by one device) are listed in Table 1 to show its range and durability. In the mentioned zone no debris record was mentioned before, probably due to the loss of the smallest fraction (<300 μm). Within the presented study, the numerous microplastics were found.

Table 1. The example of trawling log.

from 78°54,542′ N, 07°48,946′ E to 78°56,110′ N, 08°32,225′ E	from 78°59,430′ N, 11°24,237′ E to 79°03,14′ N, 11°36,366′ E
from 78°59,160′ N, 09°45,843′ E to 79°02,623′ N, 11°07,713′ E	from 79°01,240′ N, 11°48,759′ E to 78°58,510′ N, 11°39,601′ E
from 78°57,469′ N, 12°01,225′ E to 78°57,488′ N, 12°01,015′ E	from 78°58,510′ N, 11°39,601′ E to 78°55,208′ N, 12°06,103′ E
from 78°59,892′ N, 11°59,006′ E to 78°59,554′ N, 11°33,787′ E	from 78°53,549′ N, 12°28,773′ E to 78°53,078′ N, 12°25,524′ E
from 79°02,262′ N, 11°39,675′ E to 79°02,131′ N, 11°42,360′ E	from 78°53,078′ N, 12°25,524′ E to 78°55,495′ N, 12°22,503′ E

4 Conclusions

While the quantitative studies of collected samples are still in progress, the preliminary research revealed the presence of microplastics debris on the majority of samples. It is possible that the lower-size fraction was omitted during the previous study reported due to the too large neuston nets holes. That confirms the efficacy of the method as well as the scale of the problem. The systematic data evaluation consists of detailed IR and Raman spectroscopy of filtered material correlated with the water volume and the preparation of sampling area map. Although in the literature, the quantitative data and MPs concentration estimations are available sometimes basing on the research of just a few samples, authors found it methodologically erroneous. The more simple, faster and cheaper technique as the one proposed within this study may help to rescale the sampling area and frequency.

The validated, consistent sampling protocol enables the qualitative and quantitative data comparison and is recommendable as presented within this paper. Although the

final analysis has to be performed by a specialized scientific group, the materials collection could be done by social-scientists, sailors and all no-specialists nature lovers due to the simplicity and low cost of the proposed portable device. Finally, the contribution to the database creation is highly recommended.

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Marine Microplastics at Santuario Pelagos

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1 Introduction

The exponentially growing global plastic production (from 21 millions of tonnes in 1950, via 147 in 1993, up to >405 currently) and the increasing pollution caused by marine anthropogenic litter draw attention to the question of the ubiquitous microplastics presence [1], especially in the world ocean. Taking into consideration the fact that more than 40% of plastic products are the single-use-only items and from 6,3 billions of tonnes of plastic litter the 5,7 had never been recycled, the microplastics pollution peak is still to come. Although many models have been proposed to describe the global propagation and accumulation of the debris, there is a significant lack in the validated source data. The aim of this research is to propose a simple and efficient method of pelagial sampling to estimate the marine microplastics (MPs) concentration. The whole protocol is described in detail to make it a standard enabling the qualitative and quantitative analysis and comparison of data from different research. Finally, as it is cheap and simple, it may become popular and frequently used even by no-specialists (for instance in a citizen science projects and campaigns) in order to provide the vast database. It was tested in Santuario Pelagos as this valuable protected area is under increasing anthropogenic pressure due to the numerous primary and secondary sources of MPs.

2 The Pelagos Sanctuary

The Pelagos Sanctuary is a restricted and protected (since 25 November 1999) area (87 500 km^2) characterize by abundant plankton productivity and wildlife (4–18% of marine species worldwide), particular seabed and densely populated by marine mammals. Eight of them are observed regularly, that is: Sperm whales, Cuvier's beaked whales, Long-Finned Pilot whales, Risso's dolphin, Bottlenose dolphin, Common dolphin, Striped dolphin and Monk seal. It includes the costal and pelagial waters of France, Monaco and Italy. Starts from the Giens Pennisula to Fosso del Chiarone including various islands, such as whole Corsica and the northern part of Sardegna and reaches 2022 km of coastline. The Pelagos Sanctury is highly vunerable to the microplastics as it is in contact with the highly urbanized and populated area, moreover being the popular tourist destination.

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3 Monitoring Methodology and Results

The universal sampling protocol consists of:

1. hauling the device (from research vessel or by sailors or volunteers),
2. collecting the sample directly on the exchangeable metal filter,
3. physical and chemical characterization of the debris directly on the metal net by using IR and Raman spectroscopy, SEM,
4. acquiring the qualitative and quantitative data and collecting them in the database complementary with geo-information.

The systematic monitoring of the selected area was done by the pelagial water sampling equipment constructed by author. The measurements are possible to be carried on with the trawling speed up to the 6 knots (with optimal of 2–4 kN). The buoyancy is adjusted by additional to take only the top water layer. The field pictures (Fig. 1) show the working device, its buoyancy, and nautical behaviour during hauling. Moreover, the immersion level enables the orientation about the depth of the sampled water column. During hauling, the water flux might be monitored in different ways. The most precise one is the control at the entrance. Moreover, the GPS data are recorded (KML file) together with meteorological conditions (same format as a standard sailors logbook).

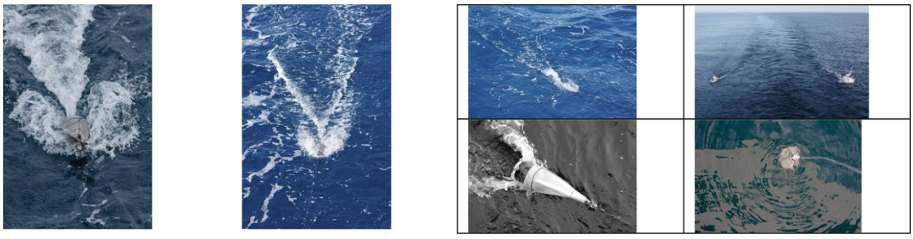

Fig. 1. The different conditions and stages of trawling with the controlled depth of immersion.

The exact sampling time and distance depend on the area and monitored parameters. Although the use of stainless steel, some of the elements are less durable and, whole system lifetime is estimated to be approximately a month of exploitation at high sea and hundreds of nautical miles. The collected filter has been viewed under microscopy and debris identified by IR and Raman spectroscopy (Fig. 2). The spectra might be obtained as the map of the selected area instead of a point, which helps in efficient studies of a large surface. At this stage, the quantitative analysis and estimations are preliminary, but the qualitative study revealed the presence of polymer debris, from at least four different groups, in the majority of collected filters.

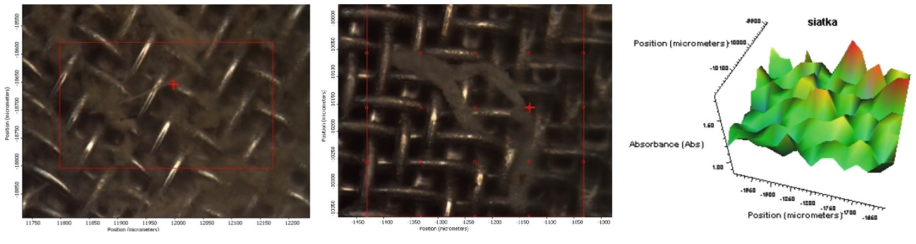

Fig. 2. The microscopic view of the filter after sampling with the proposed devices with the traces of microplastics and plastic fibres.

Measurements with the proposed device can be successfully conducted in waters with different concentration of organic matter. That is due to the preliminary filtration by 500 μm net. In the case of some samples, when the organic matter was not so dense, the direct characterization is possible without an additional pre-treatment. That is an advantage also for the roughness characterization performed to establish to what extent the surface is available for the microfilm. In other cases, the standard procedure of NaCl is used when possible. If not, as last, the 30% H_2O_2 with/without the $FeSO_4$ or $ZnCl_2$ are helpful [2]. How long one shall haul the sampler depends on the particular water basin, one's speed and might be normalized to the amount of water flux. It is recommended that the direct flow meter at the entrance should be added. In another case, the estimation is based on the average speed, log, time, and water volume. Although those parameters are user-dependent, the monitored area is fixed.

As the Mediterranean Sea is one under the most increasing anthropogenic impact, its protected areas need to be systematically monitored. The proposed device was tested in the field of Santuario Pelagos (Fig. 3). During the sampling in 2017 and 2018 season, each time the microplastics pollution was observed and reported. Although the estimation of MPs concentration in the Mediterranean can be found in the literature [3], one should focus on the relatively small, if not insufficient, number of samples used for calculations. For instance, for the West Mediterranean the following numbers of microplastics concentration [items for km^2] 69,161 ± 83,244, 112,000, 82,000 ± 79,000 are based on the 24 [4], 6 [5], 21 [6] samples, respectively. The proposed device can carry on at least a few hundreds of measurements.

Fig. 3. The monitored area of the Santuario Pelagos.

Obtained samples are easily characterized and identified by Raman spectroscopy (Nicolete Thermo Scientific, green line 532 nm) [7]. Figure 4 presents the spectra of prevailing polymers: polyethylene, polypropylene and polystyrene.

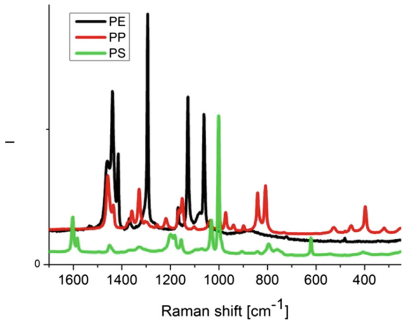

Fig. 4. The debris qualitative identification by Raman spectroscopy.

4 Conclusions and Future Perspectives

As expected the marine microplastics do not respect the border of protected area and is found in nearly all trawled chromium-nickel nets. The proposed methodology is simple and efficient. Collected samples are analysed using SEM, Raman and IR spectroscopy. Eco-toxicological studies are carried on in order to determine the impact of MPs and nanoparticles on a representative species (such as the *Hediste diversicolor* for a Baltic Sea region).

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Microplastics Uptake and Egestion Dynamics in Pacific Oysters, Magallana Gigas (Thunberg, 1793), Under Controlled Conditions

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1 Introduction

Microplastics have been considered to be dangerous for aquatic organisms' health (Alomar et al. 2017). Indeed, their accumulation by ingestion can lead to increased exposure to pollutants and pathogens, and effects on physiological activities linked to nutrient uptake, growth and survival (Browne et al. 2011; Sussarellu et al. 2016; Fendall and Sewell; 2009; Van Cauwenberghe and Janssen; 2014). Von Moos et al. (2012) studied the effect of exposure and ingestion of microplastics (≤ 80 μm) in Blue mussel (*Mytilus edulis,* Linnaeus, 1758). These authors reported that the smallest particle sizes were accumulated in gills and digestive gland with a consequent strong inflammatory response and a lysosomal membrane destabilization. Unfortunately, no information on excretion was provided by these authors and conclusions on the fate of the larger particles cannot be made. Van Cauwenberghe and Janessen (2014), investigated the presence of different microplastics particles (size class 5–10, 11–15, 16–20, 21–25, >25 μm) in farmed blue mussel and Pacific oyster, showing that these were present in both species at concentration of 0.36 ± 0.07 particles g^{-1} and 0.47 ± 0.16 particles g^{-1} soft tissue, respectively. The same authors also depurated animals from the same batches for 72 h observing a significant reduction in the abundance of microplastics, concluding that although depuration was an effective procedure, the consumption of farmed bivalves could potentially represent a risk to consumers' health.

The first aim of this present study was to investigate the adult oysters' egestion dynamics after exposure to known concentration of microplastics under controlled conditions. Moreover, previous studies have so far used microplastics of sizes comparable to phytoplankton cells. However, in the marine environment, microplastics are present in sizes often larger than microalgae cells and there are evidence suggesting that

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bivalves could potentially up-take particles as large as 500 μm (O'Donohe and McDeromtt 2014). Still, no information on the ability of oysters to uptake, retain and egest larger particles is currently available. Consequently, the second aim of this study was to determine whether larger particles had the potential to remain in the marketable product post depuration by employing sizes larger than those commonly used in previous microplastics absorption studies. The size classes of 100 ± 7.42, 250 ± 23.2 and 500 ± 52,34 μm were chosen because Van Cauwenberghe and Janssen (2014), found that *Crassostrea gigas* reared in the Atlantic Ocean (average shell length of 9.0 ± 5.0 cm), showed a prevalence of microplastics size >25 μm, and because studies on mussels and Pacific oysters so far were focused only on microplastics of a size comparable to phytoplankton or in general at size between 0.5 and 90 μm (Sussarellu et al. 2016; Cole and Galloway 2015; Van Cauwenberghe et al. 2015; Farrell and Nelson 2013; Browne et al. 2008; Von Moos et al. 2012), without taking in to account that in the marine environment microplastics are present in different sizes and adults' Pacific oysters can uptake larger size microplastics from the environment.

2 Materials and Methods

Pacific oysters (20 oysters 85 ± 2.3 g/ind.) were collected from a farm in the San Teodoro Lagoon (Italy) (40°48'39.18''N, 9°40'24.42''E), and kept in a cold box until arrival to the laboratory. For the purpose of this study, oysters were individually deployed in 20 glass spherical aquariums of 1.5 L, filled with filtered sea water. Preliminary trials were performed to determine both the level of aeration required and the most suitable type of microplastics polymer. For this purpose, three polymers of the following densities were tested: polystyrene 1.04–1.1 g/cm^3; polyamide 1.12–1.15 g/cm^3; polycarbonate 1.20–1.22 g/cm^3 (Avio et al. 2016; Enders et al. 2015). With the aim to keep the microplastics beads suspended in the water column to maximise their chances to be filtered by the oysters, batches of 30 microplastics per polymer were deployed to an experimental tank and aeration was adjusted by a valve. Once the appropriate aeration was identified by observing the microplastics distribution on the water column, the ability of the chosen polymer to withstand the tissue digestion procedure (Li et al. 2015) was tested. This was conducted using a sterile container containing soft tissues of 3 Pacific oysters (80 ± 3.5 g/ind.) plus 9 plastic beads per size class (100 ± 7.42, 250 ± 23.2 and 500 ± 52,34 μm) of the microplastics chosen for the study (3 replicates). The soft tissue was covered with hydrogen peroxide 15%, this was added until the oyster was completely digested (Avio et al. 2016). Once the oysters were digested the remaining solution was filtered using 47 mm Whatman GF/F filters (0.6–0.8 μm) and then analysed under the dissecting microscope (Leica Mz8).

The experiment was carried out in 2 parts: 24 h exposure (Cole and Galloway 2015) and 72 h depuration (Van Cauwenberghe and Janessen 2014). During the first 24 h experimental individuals (n = 20) were individually exposed to 30 microplastic particles of each size (100, 250 and 500 μm) with a density of 60 particles per litre. This particles density despite being higher than the ones commonly reported in sea water (De Lucia et al. 2014) was chosen for analytical and practical reasons. The

oysters collected after exposure were transferred to a new tank, again filled with 1.5 L of filtered sea water. Aeration was not supplied in order to avoid faeces and pseudo-faeces mixing. The water left in the tanks during the 24, 48 and 72 h after exposition, was filtered and beads counted using the same procedure described above. Finally, at the end of the trial (72 h after exposure) oysters were collected from the experimental tanks and externally washed and dissected taking care that the water contained in the shell cavity was stored in a plastic tray. The digestive gland, gills and mantle of each oyster were dissected, washed and placed in labelled sterile containers. The water contained in the shell and the water used to wash the tissues was collected and filtered as described previously. All dissected tissues of each individual were digested using hydrogen peroxide 15%, at room temperature of 22 °C for 7 days, and the resulting digestate was filtered.

3 Results and Discussion

3.1 Results

At the end of the 24 h exposure, the uptake (% of missing beads) of the different sizes (100, 250 and 500 μm), was 19.4 ± 1.1%, 19.4 ± 2% and 12.9 ± 2% respectively. No significant difference in uptake between the microplastics of 100 and 250 μm was observed, however beads of 500 μm in size had a significant lower uptake when compared with the others sizes (P = 0.009) (Fig. 1).

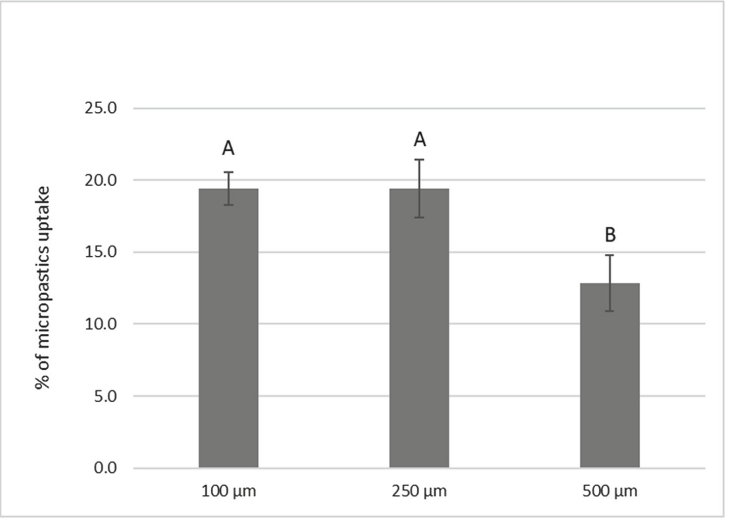

Fig. 1. Uptake of the different microplastic particle size classes from ambient water. Significant differences (P value > 333 0.05) are showed by different letters, results are presented as mean ± SE; n = 20.

Table 1 illustrates the percentage of microplastics recovered from the depuration water, and tissues at the different time points over the depuration period. A significant effect of time ($p < 0.001$) and a significant interaction between time and treatment ($p < 0.02$) was observed. The excretion of microplastics beads of all sizes was significantly higher during the first 24 h in comparison with the later time points. Furthermore, no significant difference was recorded in the excretion of microplastic particles of 100 µm and 500 µm between 48 and 72 h of depuration, whilst significantly more beads of 250 were released after 48 h in comparison to 72 h of exposure (Fig. 2).

Table 1. Summary of the percentages of egested during 72 h depuration, and non-egested post depuration, Microplastics, both divided by sizes and as a mix of beads (100, 250 and 500 µm).

Microplastics beads egested and non-egested in:	100 µm %	250 µm %	500 µm %	Mix %	Mix %
24 h	68.3 ± 3.6	58 ± 4.0	74.9 ± 5.6	63.9 ± 3.0	84.6 ± 2
48 h	12.5 ± 2.2	21.9 ± 3.5	12.6 ± 4.3	17 ± 2.2	
72 h	1.5 ± 1.1	3.4 ± 1.7	7.1 ± 3.1	3.7 ± 0.9	
Internal cavity	17.7 ± 3.8	16.7 ± 2.4	5.4 ± 2.7	15.4 ± 2	15.4 ± 2
Digestive gland	0	0	0	0	
Other soft tissues	0	0	0	0	

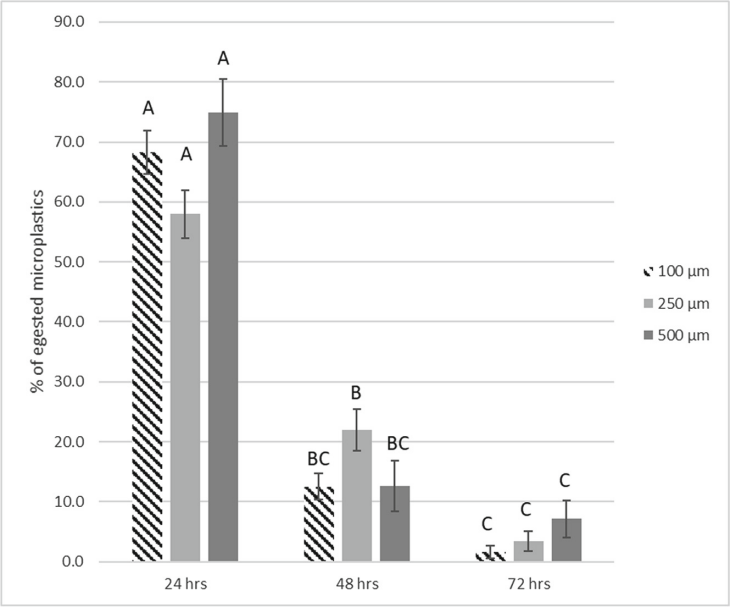

Fig. 2. Egestion dynamics of the different microplastic particle sizes. Significant differences (P value > 0.05) are showed by different letters, results are presented as mean ± SE; n = 20.

Although the vast majority of ingested microplastic particles were released during the 72 h of depuration, 17.7 ± 3.8, 16.7 ± 2.4 and 5.4 ± 2.7% of microplastic particles of 100, 250 and 500 μm respectively were still present in the water contained inside the shell cavity. At this location a significant difference in the abundance of each particle size class was observed, with the largest size class being significantly less abundant than the other two ($p = 0.007$) (Fig. 3). Importantly, no microplastic particles were found in the digestive gland and in the other tissues post digestion. Taking into account each time step there was a decreasing egestion of microplastic particles during the depuration time: 63.9 ± 3%, 17 ± 2.2% and 3.7 ± 0.9% in 24, 48 and 72 h, respectively. Only 15.4 ± 2% of the microplastic particles were retained within the oysters after 72 h of depuration (Table 1).

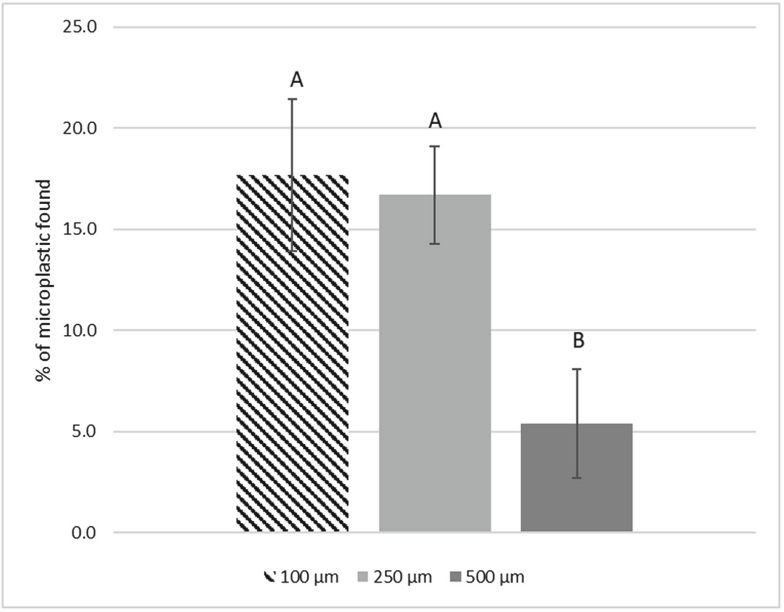

Fig. 3. Residual microplastic particles of the different sizes post depuration. Significant differences (P value > 0.05) are showed by different letters, results are presented as mean ± SE; n = 20.

3.2 Discussion

In this study, Pacific oysters showed an efficient egestion rate, egesting 84.6 ± 2% of the microplastic particles taken up, while only the 15.4 ± 2% of beads taken up were retained within the shell cavity, post depuration. Furthermore, no microplastic particles were observed within the oysters' tissues, while in the Sussarellu et al. (2016) study, microplastic particles were found in the stomach and the intestine of Pacific oysters. This can be attributed to the difference in the particle size used (100, 250 and 500 vs 2–6 μm), and it is possible that the *C. gigas* food sorting mechanisms recognise only

the smaller size as a food source due to similarity in size with phytoplankton (Ward and Shumway 2004).

4 Conclusions

To date, studies on microplastic uptake have been conducted mainly to investigate their potential negative physiological effects on marine live, including bivalves, or to establish whether animals entering the human food chain could be a carrier of these particles and therefore represent a risk for consumers (Sussarellu et al. 2016; Fernández et al. 2018; Von Moos et al. 2012; Pont et al. 2016; Silva et al. 2016; Van Cauwenberghe and Janessen 2014). The main difference between these approaches has been the controlled nature of the studies. The former employed controlled conditions (known density, type and size of the microplastics employed), whilst the latter focused on the abundance of plastics in marketable products without considering levels of exposure, uptake or the nature of the polymers. In contrast, our study investigated both the uptake and egestion dynamics under controlled conditions to more robustly describe the fate of microplastic particles of 100 to 500 μm diameters during exposure and depuration therefore contributing to the collective knowledge on these dynamics in shellfish produced for human consumption. Our results suggest that these larger particles could probably be filtered by the oysters but, instead of being ingested, they are retained within the shell cavity by adhesion. Importantly, during the depuration period, microplastic particles were observed in faeces and pseudo-faeces, but it is not possible to conclude here that the beads have been ingested, because these were not observed within the digestive system. Further work focused on the ingestion and excretion of microplastic particles of different sizes class, including particles larger than microalgae cells, should be conducted to estimate gut transit time of these particles. In conclusion our data, taken together with results from other studies, strongly indicate that *M. gigas* could be a carrier of different microplastic sizes in the human food chain, not only through the absorption and inclusion in tissues (Bricker et al. 2014; Van Cauwenberghe and Janessen 2014; Li et al. 2015), but also through the adhesion of these particles in different parts of the internal cavity of the oysters shell.

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Extraction Protocol Optimization for Detection of Microplastics in Digestive System Contents of Loggerhead Turtle (*Caretta Caretta*)

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1 Introduction

Marine litter is one of the most important human-caused pressures on the marine environment, it is defined as "any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment" [1, 2]. The most abundance part of marine litter is composed by plastic [3]. The main and most remarkable risks for the species inhabiting the marine environment are entanglement and accidental ingestion. Moreover, from the direct release of particles of plastic and as a fragmentation of large items consequence, originate microplastic. The presence of microplastics in the marine environment has raised scientific interest during the last decade. As demonstrated, ingestion of marine litter can have lethal and sub-lethal effects on wildlife that accidentally ingests it, and sea turtles are particularly susceptible. For this reason loggerhead turtle (*C. caretta*) has been identified by the European Commission (EC), in the Marine Strategy Framework Directive (MSFD) [4, 5], as indicator for monitoring the amount of Marine Litter ingested by marine animals in Mediterranean basin. The Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise "G. Caporale" (IZSAM) is contributing to monitor the impact of marine litter on sea turtles and biota for MSFD, thanks to the participation of EC funded-project "Implementation Of Indicators Of Marine Litter On Sea Turtles And Biota In Regional Sea Conventions And Marine Strategy Framework Directive Areas – INDICIT" (https://indicit-europa.eu/). This is an European program to implement novel protocols and indicators of litter impact on marine turtles [6] Data from Adriatic coast are provided by IZSAM that is a member of Abruzzo and Molise stranding network instituted by Regional Government [7, 8], whose main objectives is diagnostic investigation of dead cause of stranded marine animals with the aim conservation of protected species, the indirect control of environmental status and the protection of public health.

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2 Experimental

During the regular activities of Abruzzo and Molise bi-regional stranding network, IZSAM applied INDICIT standardized protocols for the investigation of the presence of litter (including macroplastics) in digestive system (GI) of stranded sea turtles. Furthermore, considering several procedures developed [9], an experimental extraction protocol was applied in randomly selected samples, in order to detect the possible presence of microplastics in GI contents. Possible correlations with GI lesions have been explored.

2.1 Materials

The inspection and biometric measurements of animals foresee individuals safety devices, camera, pens, note sheets and measuring tape. Necropsy examination and the sample collection need of disposable scalpel, knives, scissors, containers for samples, 6 plastic clamps (at least 6 for each sample). Moreover, the analysis of gastro-intestinal contents require precision balance (0.01 g), sieve with 1 mm mesh, and the analysis of ingested litter need petri dishes, stereomicroscope, precision balance (0.01 g).

For the experimental microplastic (<1 mm; ≥ 0.45 μm) extraction protocol, different instruments are needed: oven, a clean air flow cabinet, ultrasonic bath, filtration ramp and a stereomicroscope. All lab materials used for dissection, extraction and analysis (beaker, bottles, glasses for filtration ramp, petri dishes and water) were rigorously sterilized. In addition, all sample processing phases were performed in a clean air flow cabinet to exclude external contamination from fibres which might represent a major source of contamination [10].

2.2 Methods

In the last year, out of all stranded sea turtles intended for *post mortem* examination at IZSAM labs to determine the causes of death, 49 were used to investigate the presence of marine litter in gastro-intestinal contents by INDICIT protocol [11]. Furthermore, 16 random samples of residue contents were used to detect the presence of microplastic (until 0.45 μm) using experimental extraction protocol.

2.2.1 Preparation of Samples

For each individual, a series of parameters like biometric measurements (Curved Carapaces Length – CCL) was carried out according to decomposition status. During necropsy, gastro-intestinal system is exposed and different portions are isolated by clamp: oesophagus, stomach and intestine. Observed anomalies in the GI have been noted (e.g. perforations, inflammation, ulcers). The content of each portion is weighted, washed in freshwater with filter mesh 1 mm. The filtered material equal and greater than 1 mm is rinsed in 70% alcohol, washed in freshwater and dried out (GI content items – phase 1). The filtered suspensions with particles less than 1 mm have been submitted to the following phase 2.

For the analysis of microplastics, each sample was allowed to dry in oven (75 °C, for 2 h), then it was added $ZnCl_2$ solution (1.6–1.8 g/cm^3). The obtained solution was

stirred and decanted for 12 h to obtain a supernatant which contains possible microplastics (GI content extracted – phase 2).

2.2.2 Analytical Techniques

GI content items (phase 1) were investigated by selecting and separating litter from natural food/no food with the aid of stereomicroscope. The number and the weight of different categories (Table 1) of marine litter is reported for each gastro-intestinal portion. Furthermore, the weight of the organic fraction (subdivided into food remain and natural non-food remain) and a descriptive analysis were carried out. Using collected data, the percentage of sea turtle with litter in GI system (which is called FO – frequency of occurrence) was calculated. Also, the difference of FO in each GI tract (oesophagus, stomach and intestine) and the percentage of founded singular category items were calculated.

For the analysis of microplastics (phase 2), the supernatant of each sample was subjected to three sonication cycles, then filtered under vacuum on a cellulose nitrate membrane (0.45 μm pore size), which was washed with 30% H_2O_2 solution for the digestion of residual organic matter and then with sterile water. Finally the filters were microscopically observed for microplastics count. Processed samples, with the newly developed extracted particles protocol, were photographed and categorized according to the shape (fragments, film, line) and colour. Textile fibres were found only occasionally and excluded from the analysis because they could represent airborne contamination from clothing during the sampling or processing [10, 12].

Table 1. Marine litter categories established for monitoring (11).

Acronym	Litter category	Description
IND PLA	Industrial plastic	Plastic pellet and granules, usually cylindrical and round shape, but also oval or cubical shapes
USE SHE	Sheet-like material	Plastic bags, agricultural sheets or plastic foil
USE THR	Threadlike materials	Ropes, filaments and the remains of ghost fishing gear usually made of nylon
USE FOA	Foamed plastics	Polystyrene foam or foamed soft rubber
USE FRA	Fragments	Fragments of hard plastic items
USE POTH	Other user plastic	Elastics, dense rubber, balloon pieces, and soft air-gun bullets
OTHER	Litter other than plastic	Cigarette butts, newspapers, rubbish and hard pollutant
FOO	Natural food	Remain of natural diet
NFOO	Natural no food	Any natural items like stone, wood or pumice

3 Results and Discussion

3.1 Results

Turtle CCL ranged from 29.0 to 80.0 cm. Eight sea turtles with a completely empty GI were excluded from the analysis. Marine debris has been detected in 16 specimens and

the FO was 39.02%. Marine litter has been detected mainly in intestine (FO = 24.49%), followed by stomach (FO = 10.20%) and finally in oesophagus (FO = 2.04%) (Table 2). For all GI tracts, plastic represents the principal detected litter, and "USE-SHE" is the main category in terms of abundance (Table 3).

Table 2. Distribution of the Total mass (Litter + Natural food + Natural no food), the Litter mass (Total mass – Natural food - Natural no food) and the Plastics mass (Litter mass – Other) in the oesophagus, stomach and intestine, and the FO (%) of Litter, Plastic and USE category in those portion.

	Total mass (g)			Litter mass (g)			Plastic mass (g)			Litter	Plastic	USE
	mean ± SD			mean ± SD			mean ± SD			FO (%)	FO (%)	FO (%)
Oesophagus	0,006	±	0,043	0,006	±	0,043	0,006	±	0,043	2,04	2,04	2,04
Stomach	13,002	±	48,324	0,077	±	0,488	0,077	±	0,488	10,20	10,20	10,20
Intestine	71,238	±	101,531	0,079	±	0,244	0,075	±	0,244	24,49	24,49	22,45

Table 3. Results of weight of various categories of marine litter ingested by sea turtles.

	Items	mean ± SD			%
Total	116	2,367	±	5,472	–
IND_pla	8	0,163	±	1,143	6,90%
USE_she	44	0,898	±	3,519	37,93%
USE_thr	15	0,306	±	1,211	12,93%
USE_foa	13	0,265	±	1,036	11,21%
USE_frag	3	0,061	±	0,317	2,59%
USE_poth	32	0,653	±	2,803	27,59%
USE_oth	1	0,020	±	0,143	0,86%

For the extraction of microplastics from GI system, 13 out of 16 samples showed microplastic fragments, namely 81.25% (Table 4).

Table 4. Number of microplastic particles found in GI contents.

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total
No items	4	1	9	5	9	3	0	3	1	6	4	2	0	0	1	6	**54**

The shape of extracted particles was mainly constituted by line (74%), followed by film (19%) and fragment (7%) (Fig. 1-A). The number of isolated particles per colours were: 16 black, 15 blue, 14 transparent, 5 red, 2 grey, 1 yellow and 1 green (Fig. 1-B). The shape is almost always regular (83%) (Fig. 1-C) and the most isolated plastic particles have been found in the intestine (67%) (Fig. 1-D).

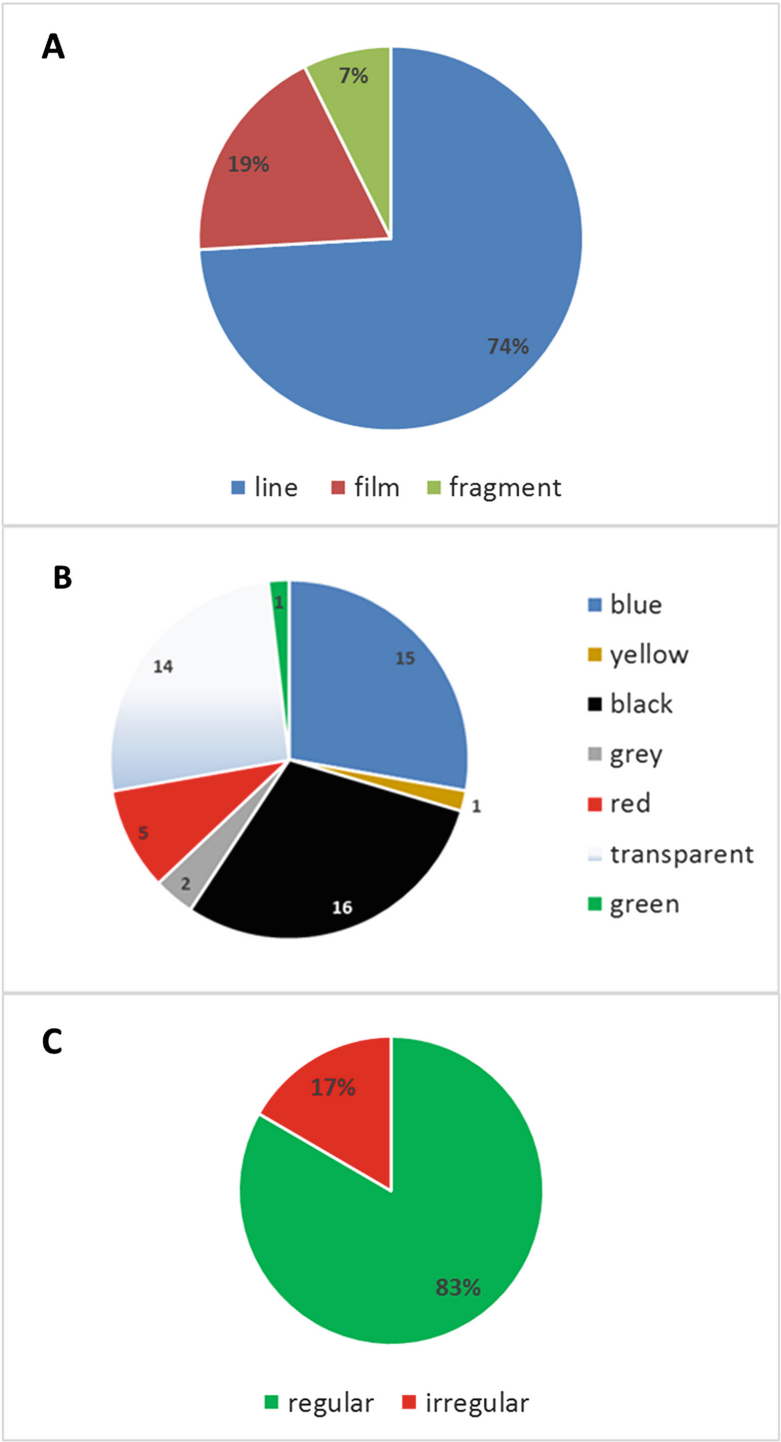

Fig. 1. Characteristics of microplastics extracted from GI contents: typology (A); colour (B); shape (C); intestine and stomach (D).

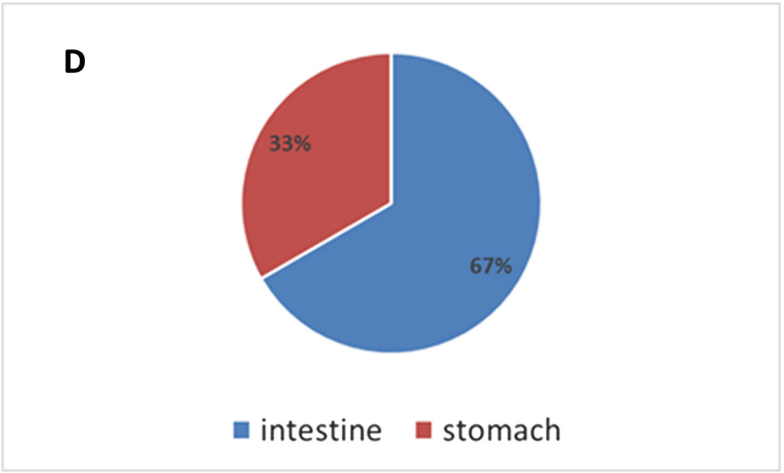

Fig. 1. (*continued*)

3.2 Discussion

The samples, randomly selected for the execution of phase 2, were composed by 7 samples with plastic greater than 1 mm and 9 without this macro items. Considering these data, the FO is 43.75%. The results of experimental extraction protocol highlight a presence of microplastic ($\geq$0.45 μm) in 13 samples equal to the percentage of 81.25% of the total samples.

In the same 7 samples with plastic greater than 1 mm, microplastic extraction protocol was resulted positive (100%) for different types of microplastic (fibres, fragments, filaments, etc.). Furthermore, within the group of 9 samples without plastic items evidence, in 6 samples microplastics were encountered (66.67%).

During necropsy of sea turtle carcasses from which 16 samples were collected, lesions like ulcers, moderate and severe inflammations of intestine and hepatic degeneration were evidenced.

4 Conclusions

The extraction protocols optimization was useful for the detection of microplastics until 0,45 μm in GI contents. Data on presence of these particulates demonstrated that they are almost always present in GI contents of sea turtles, unlike the others greater. It's to be further investigated the possible correlation cause-effect among plastic presence and gastrointestinal system disorders. Moreover, the same extraction protocol could be used in other biota species.

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Study of Plastics Debris Collected on the North Beaches of the Garda Lake After the Severe Storm Vaia in Autumn 2018

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1 Introduction

Research for Facts and Sharing Knowledge are two of the six commitments outlined by Marine Litter Solution in the declaration to reducing ocean pollution [1]. This presentation represents our recent contribution to the study of the Garda Lake contamination with plastic debris after the severe storm Vaia that hit the north side of Italy in Autumn 2018 [2]. The average rainfall in Trentino reached 275 mm during the three days 27–29 October, with the max peak of 600 mm according to Meteotrentino [3]. Moreover, the gales of up to 120 km/h brought down thousands of trees e.g. in the Fiemme Valley. In the following days all the rivers in the area increased their stream bed, which brought about intense "cleaning" of their side banks. It was also the case of the Sarca River which is draining about one fourth of Trentino, and it is the main tributary of the Garda Lake. Consequently, to avoid the danger of flooding, the water of the Adige river was redirected to this lake through the Torbole tunnel (Trento) from October 29th until November 1st with a flow rate of 350 m^3/s. Thus, various organic/vegetable and inorganic materials were continuously transported and dispersed into the lake, and then accumulated on the shoreline, due to the local south wind "Ora".

2 Experimental

The first monitoring/collection of northern beaches of the Garda took place November 2nd–11th, whereby various macroplastics and microplastics were gathered. Zone 1, Zone 2 and Zone 3 correspond to the local Municipalities of Riva del Garda, Arco and Nago-Torbole, respectively (as shown in Fig. 1).

Afterwards, further surveys were also organized in the following period February–July 2019. In particular, more than thirty volunteers from Liceo Maffei checked and retrieved plastics items in the same selected position of Zone 1A and Zone 1B, as on November 15th and March 14th (see identification position by gps coordinates in Tables 1 and 2).

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Fig. 1. Shoreline of the North-side of the Garda Lake and the Zones of plastics survey. Direction of water stream tributary (Sarca River and Tunnel from Adige River) and Ora Wind are indicated by full and dot arrows, respectively.

2.1 Methods of Survey and Collection

First inspection was performed in the first days after Vaia storm along the easily accessed beaches of Riva del Garda, Arco and Nago-Torbole, that are usually frequented by tourists for bathing and/or surfing in the summer season. In agreement with the suggestion of recent Gesamp Report, several positions were fixed for the collection of samples in the selected areas, initially 1 m^2 in November 2018, and then in 2 m^2 [4].

2.1.1 Collection of Samples

The selected positions of survey on gravel shoreline of the Garda beaches were registered by the gps coordinates, and collected plastics specimens were distinguished by position and date. Two types of collection were carried out: (1) collection of large items mainly from Piles (see Figs. 2) and collection of small items as typical microplastics (Figs. 3).

Various plastics were counted and weighted. Progressive characterization was primarily focused on the identification of polymers, and then specific testing procedures were used for the evaluation of sample weathering, which were in agreement with literature [4] and with the procedures adopted in our laboratories [5].

Fig. 2. Example of waste Pile in Zone 1A Sabbioni Beach (left) and Zone 2A Baia Azzurra (right – in distance). The selected area of 1 m^2 for collection of small size samples is also evidenced (left) (November 4th, 2018).

Fig. 3. Example of large size (left) and small size (right) plastic wastes collected in Zone 1B. (Purfina Beach, November 4th, 2018)

2.2 Materials and Testing Techniques

2.2.1 Materials

Representative compositions and types of various plastic materials retrieved in several survey (Zone 1A and Zone 1B) are well corresponding to marine plastic wastes as reported in literature by Gesamp 2016 [6]. In particular polyethylene, polypropylene, polystyrene, polyurethane, polyethyleneterephthalate, polyvinylchloride, polyamide, rubber, and so on.

2.2.2 Analytical Techniques

Analytical techniques were selected in accord with the previous literature survey [5]. An analytical balance Gibertini E42 (sensitivity 0.1 mg) was used for evaluation of sample

weight and density following ASTM D-682. Density measurement of foamed PS and PU samples was carried out by means of the forced immersion in distilled water [7].

Fourier Transform Infrared (FTIR) spectroscopy was employed in the range 4000–650 cm^{-1} by using PerkinElmer Spectrum One in order to identify the polymer composition and to evaluate the level of oxidation by detecting the carbonyl peak [5].

Differential Scanning Calorimetry (DSC) was performed from 0 to 300 °C at 10 °C/min (flushing air at 100 ml/min) by using a Mettler DSC30 to evaluate the glass transition temperature (Tg), temperatures of the crystallization and melting peaks, the degree of crystallinity, and to monitor the Oxidation Onset Temperature (OOT) [8, 9].

The melt flow index (MFI) was measured according to ASTM D 1238 standard, by means of the Kayeness Co. model 4003DE capillary rheometer, at the temperature of 190 °C with an applied load of 2.16 kg [10].

Vicat softening temperature VST defined by ASTM standard was indicated by ATS-FAAR mod. MP/3 machine (Milan, Italy) at heating rate of 2 °C/min and 10N of loading [11].

SEM analysis (Carl Zeiss AG Supra 40 field emission scanning electron microscope FESEM) was performed on fracture surfaces.

Mechanical tests were carried out in both longitudinal and transversal directions with the dumbell specimens ISO 527-2 (gauge length 25 mm; width 5 mm;) by using an Instron testing machine mod 4502 at crosshead speed of 2 or 20 mm/min.

3 Results and Discussion

During the first survey in Nov-2018 several larger size samples were found (see Figures). In particular Piles of wastes localized in Zone 1A (Sabbioni Beach) and Zone 1B (Purfina Beach) were investigated for retrieval of plastic residues before Municipal Waste Collection Service. Collected samples were counted and weighed. Results from Pile collection and relative position are reported in Table 1.

Table 1. Positions of lake-waste Piles for plastics retrieval on the Riva beaches Zone 1A (Sabbioni) and Zone 1B (Purfina) of the survey on Nov 15th 2018. Number of particles, total and average weights are given.

Position Lat N/Long E	Macro-pieces/Weight/ (g)	Average weight (g/p)	Small-pieces/Weight/ (g)	Average weight (g/p)
Sabbioni Pile1 45.88140/10.84864	9/155.3/	17.3	115/228.8/	2.0
Sabbioni Pile2 45.88133/10.84896	21/651.0/	31.0	109/115.8/	1.1
Sabbioni Pile3 45.88062/10.84963	38/463.7/	12.2	46/68.0/	1.5
Purfina Pile1 45.87918/10.85344	41/1824.0/	44.5	101/401.7/	4.0
Purfina Pile2 45.87907/10.85356	8/78.6/	9.8	37/154.8/	4.2
Purfina Pile3 45.87896/10.85408	44/1291.3/	29.4	35/89.4/	2.6
Purfina Pile4 45.87874/10.85470	45/300.0/	6.7		

Moreover, in selected areas of 1–2 m^2, an initial survey of sample collection was repeated after 4 months and 8 months. The data on number, weight, average weight of collected samples are reported in Table 2.

Table 2. Positions for Plastics retrieval on the Riva beaches of the initial and two subsequent surveys after 4/8 months. Place size of 2 m^2 (N-S oriented). Number of particles, total and average weights are given.

Position Lat N/Long E	1st Survey (1 m^2)	2018 Nov 15th	2nd Survey (2 m^2)	2019 Mar 14th,	3rd Survey (2 m^2)	2019 Jul 20th,
	Pieces/Weight/ (g)	Average weight (g/p)	Pieces/Weight/ (g)	Average weight (g/p)	Pieces/Weight/ (g)	Average weight (g/p)
Sabbioni Beach1 45.88144/10.84870	22/5.8/	0.26	176/37.2/	0.21	7/0.3/	0.04
Sabbioni Beach2 45.88114/10.84876	7/3.9/	0.56	57/19.2/	0.34	12/0.8/	0.07
Sabbioni Beach 3 45.88135/10.84890	20/5.3/	0.27	62/15.5/	0.25	21/2.7/	0.13
Sabbioni Beach 4 45.88127/10.88906	3/0.7/	0.23	47/3.6/	0.08	14/0.7/	0.05
Sabbioni Beach 6 45.88113/10.84925	18/4.6/	0.26	42/5.5/	0.13	9/0.1/	0.01
Sabbioni Beach 7 45.88104/10.84933	5/1.9/	0.38	11/5.9/	0.53	6/1.0/	0.17
Purfina Beach 1 45.87909/10.85332	19/3.4/	0.18	25/13.8/	0.55	10/3.8/	0.38
Purfina Beach 2 45.87910/10.85344	27/8.0/	0.30	26/5.5/	0.21	5/1.3/	0.26
Purfina Beach 3 45.87909/10.85332	22/33.3/	1.51	9/6.0/	0.67	7/1.5/	0.21

It is worth noting that the number of pieces collected in the 2nd survey was higher than that in the first one, probably due to the cumulative enrichment and deposition of plastic wastes during the winter season [12]. On the other hand, during the 3rd survey after 8 months the number of pieces and their average weights were considerably reduced.

3.1 Results for Specific Plastic Wastes

3.1.1 Foamed Products

Various types of foamed plastics have been collected, typically polystyrene (EPS) and polyurethane (PU) with fragment dimensions in the range of the macroplastic and microplastics.

In the case of EPS the effect of fragmentation and compression it is well evident. Due to the specific production process, foamed EPS products easily break and form single or multi-aggregate sferoids (Fig. 4). The density of various samples depends on the initial production for instance 0.039 + 0.003 g/cm^3, and on the level of aging and compression. Damaged EPS specimens with density 0.062 + 0.007 g/cm^3 and even higher were found.

Fig. 4. EPS particles at different level of fragmentation

Fig. 5. Selected EPS specimens used for density measurements (left) and ESEM image (right) of PS fragmented particles. Evident precursor of EPS-microplastics can be seen.

ESEM micrography evidences the damage extent of foamed particles, with a clear indication of microplastic formation (Fig. 5b). Single sferoidal particles tend to detach after ageing due to the low level of adhesion energy.

Density of aged samples of light blue extruded polystyrene (XPS) was measured 39 ± 6 kg/m^3. Mechanism of fragmentation and compression of aged XPS was different from that of EPS.

The densities of PU foams were found in the typical range 0.039 ± 0.003 g/cm^3 (Fig. 6-left). As they are based on polyether and/or polyester precursors, PU foams are differently susceptible to hydrolytic and/or oxygen/light induced degradation. Their physical aspect, which is very similar to that of the river stones, is derived from the progressive erosion and smoothing of the surface, due to the relatively easy micro-fragmentation during abrasion and friction in water waving.

Some sample evidenced an increased density in the range 0.150–0.715 g/cm^3 due to the adsorption of various amounts of present contaminants (Fig. 6-right)

Fig. 6. Example of aged PU foam samples.

3.1.2 Case Study 1 - Macroplastic HDPE Pallet-Box

In the first fortnight after Vaia, various fragmented part of a large fruit pallet-box were retrieved in different shorelines. The main part #1 was found in Zone 2A (Fig. 7), and other large pieces easily recognized by light green colour were retrieved in various beaches Zone 1A (fragment #2 of 3.7 kg) and 1B (fragment #3 of 544 g); Zone 2B (fragment #4 of 788 g; see Fig. 8). The thickness of various sections is ranging between 3.3 and 7.5 mm. The impact multi-fracture evidences high energy produced by Vaia storm.

Fig. 7. Main part #1 of HDPE pallet-box after extraction from water in Zone 2A. (Baia Azzurra November 4th, 2018).

Fig. 8. HDPE-fragment #4 (weight 788 g; length 53 cm) retrieved in Zone 2B. (November 3rd, 2018). Three specimens for Vicat test and grinded parts for MFI analysis are showed.

Analysis of HDPE pallet-box showed the OOT value of 217 °C (much lower than 260 °C, the reference value of new HDPE). Moreover a crystallinity of 62% found for aged HDPE (66% on the external surface layer) was slightly higher than 56-59% of virgin HDPE. Similarly, Vicat temperature 129 ± 1°C was even higher than that of common polyolefin plastic; the reason could be attributed to the surface stiffening, as documented by the higher crystallinity. The found melt flow (MFI) of 7.6 ± 0.1 g/10 min is typical of injection molded product. This type of thick HDPE plastic waste exhibit good properties and it could be considered for direct recycling.

3.1.3 Case Study 2 – PE Pluriball (Zone 1B)

Pluriball is a packaging foil made of low density polyethylene with various sizes of air balls for light impact protection. An aged Pluriball sample was found in Zone 1B on spring 2019 (Fig. 9) with evidence of the cut specimens for mechanical testing (longitudinal and transversal direction).

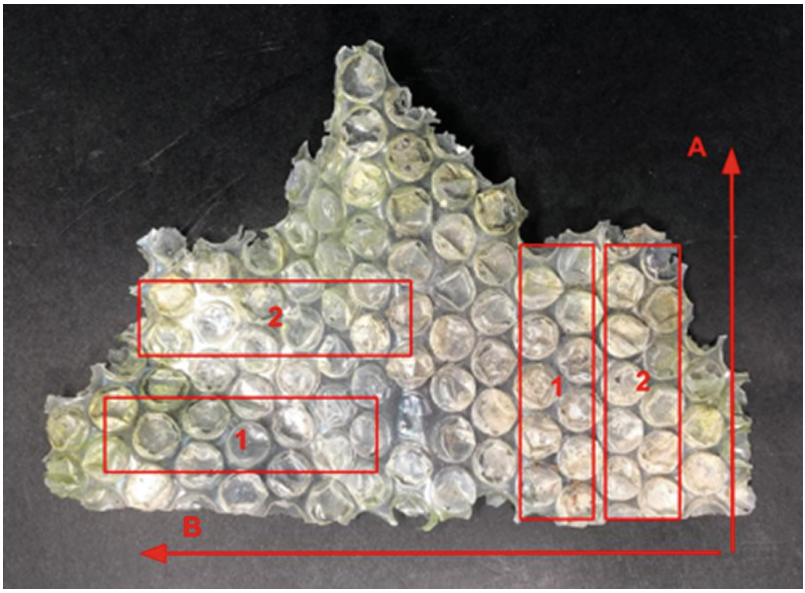

Fig. 9. Aged Pluriball with the evidence of many points of fragmentation of the balls (internal diameter of 8 mm). See magnification in Fig. 10.

FTIR spectra and the decrease of OOT (198 °C with respect of 215 °C of new Pluriball) confirmed the high level of oxidation and degradation. Both optical and electronic micrographs evidenced fragile fracture (Fig. 10). Correspondingly a significant decrease in mechanical properties was also observed. Mechanical tests of aged samples were compared with those of a New Pluriball of similar geometry (Fig. 11).

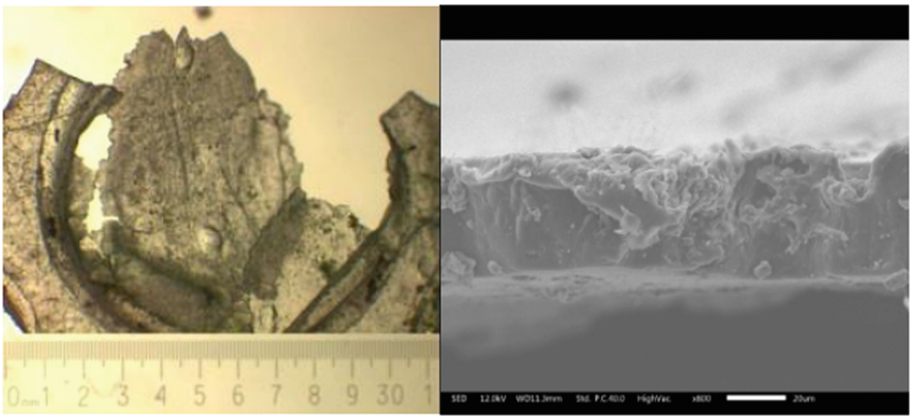

Fig. 10. Magnification of fragmented ball in aged Pluriball. View of broken ball (left) and micrograph of cross-section along the thickness after fragile fracture (right).

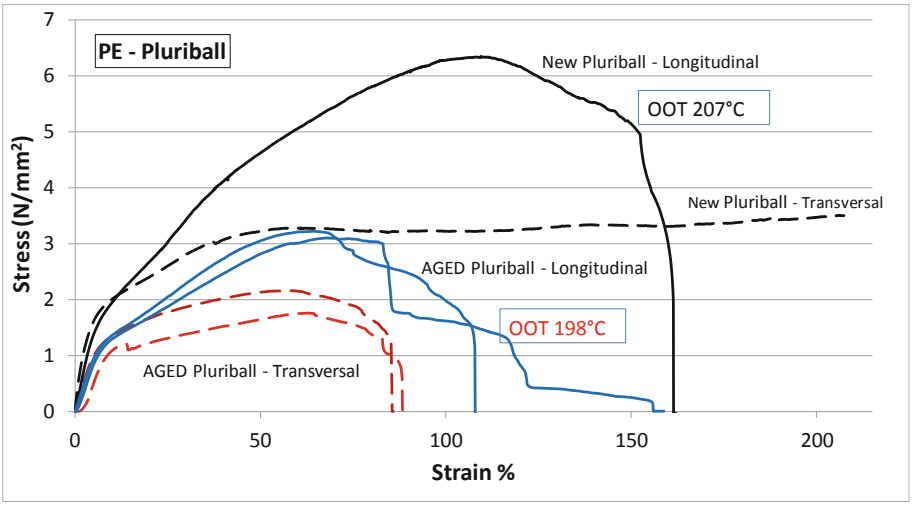

Fig. 11. Stress-strain curves of Aged and New Pluriball (Longitudinal and Transversal direction).

3.1.4 Case Study 3 - PE Sack Bag (Zone 3B)

The case of an aged polyethylene Sack-bag is similar to that of PE Pluriball. Both FTIR and OOT analyses showed the effect of oxidative action in aged sack-bag. Stress strain curves in longitudinal direction are reported in Fig. 12 and relative results of longitudinal/ transversal properties are summarized in Table 3. An aging factor has been proposed for comparing the weathering effects.

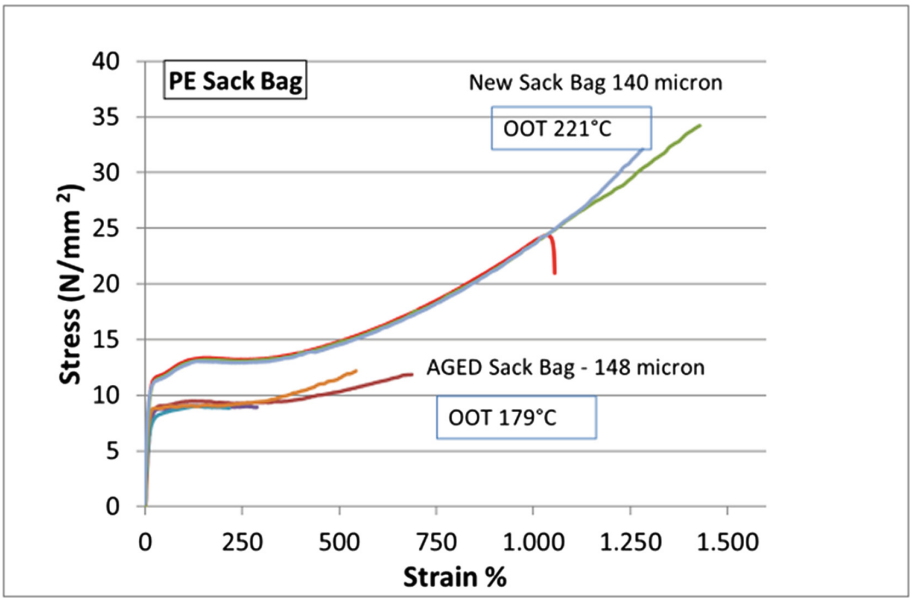

Fig. 12. Stress-strain curves of Aged and reference New Sack-bag (longitudinal direction). T

Table 3. Strength and strain at break of the PE Sack bag. New sample and aged sample (Zone 3B).

PE sack-bag sample	Strength [MPa]	Strain at break [%]	Aging factor*
NEW Longitudinal	30 ± 5	1250 ± 197	1.00/1.00
AGED Longitudinal	10 ± 2	482 ± 270	0.33/0.39
NEW Transversal	28 ± 2	1576 ± 42	1.00/1.00
AGED Transversal	12 ± 2	399 ± 313	0.43/0.25

*calculated as the ratio of the correspondent values of aged and new samples.

3.1.5 Case Study 4 - Polypropylene Jar (Remained Fragmented Bottom) PP95

An interesting case of micro-fragmented plastic was found for the residual bottom part of polypropylene cup, a disk of diameter 190 mm.

The sample had been produced by injection molding as documented by the printed information (see Fig. 13a). It is very interesting to note the date of production (March 1995), the name of producer (ISI Plast, Correggio RE-Italy), the type of polymer (PP), and the application (food contact – EURO V.6).

FTIR and OOT documented the high level of oxidation and degradation.

The sample appears to be a fragile multilayer system due to the long time of aging (Fig. 13b). This large debris (45.157 g) represents a potential source for the fragmentation in more than 1000 micro-plastic pieces of 40 mg. Its original and unmodified

thickness was about 1.15 mm, whereas the thickness of pre-fragmented portions was found in the range of 0.15–0.55 mm, thus confirming a high level of weathering and degradation.

ESEM micrograph evidences the fragile fracture (Fig. 14).

Fig. 13. Bottom part of fragmented plastic jar "PP95" (left). Residual lateral border with various fragments (right). Precursor of PP microplastics.

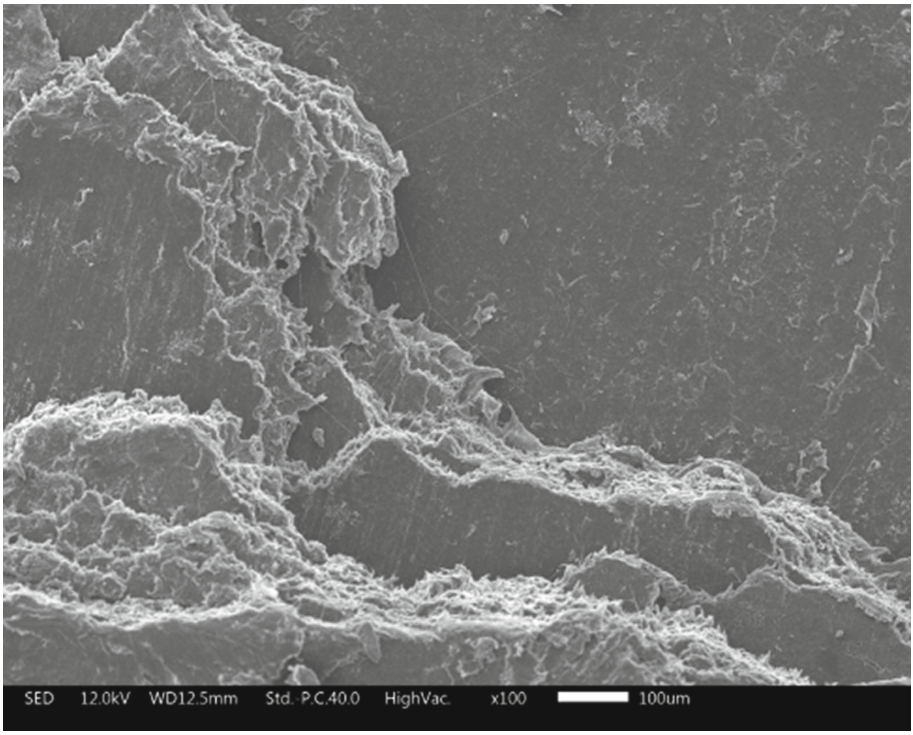

Fig. 14. ESEM image of fragmented surface of the aged sample "PP95" after fragile fracture.

4 Conclusions

The exceptional meteorological event (Vaia 2018) provided unique opportunity to evaluate the level of plastic wastes diffusion in the local sub-alpine region. In particular, the high extension and intensity of the weather perturbation determined the way of cleaning/collection of heterogeneous debris on various water-ways banks Their consequent accumulation in the main rivers, and finally their partial deposition on the lake shores was distinguished by an enormous amount of wooden-detritus. The immediate/rapid survey in the first November fortnight and the repeated inspections along the year 2019 allowed the collection of macroplastics, mesoplastics and microplastics. The various collected items directly derived from the local rivers Sarca and Adige

Specific classification and laboratory characterization of the various aged plastic objects provided interesting information related to the effects of aging. Geometrical size (thickness) has been evaluated as the key-factor for potential formation of polyolefin microplastic as a function of the time of aging.

Mechanical properties were compared with those of corresponding new products, and their relative variation was used as the ageing factor for evaluation of the weathering. Complementary information was obtained by means of morphological observation, FTIR, OOT, and other analyses.

A promising result of this research is related to the activity of dissemination and of the active recruitment of young volunteers for evaluation of the phenomenon. Subsequently, regular surveys in cooperation with local high schools and local/regionale administration have been planned and carried out.

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Microplastics and Polycyclic Aromatic Hydrocarbons Occurrence in a Demersal Fish (*Solea solea*) in the Adriatic Sea

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1 Introduction

In recent years, microplastics (MPs), normally defined as plastic particles with a diameter less than 5 mm, are emerging contaminants ubiquitously present in all the compartments of the aquatic ecosystem from surface water to benthic sediment, including aquatic biota [1, 2]. In the marine environment, MPs can be originated from two sources: manufactured products that contain microplastics, e.g. cosmetic products, and fragments released form larger plastic debris through photooxidation, mechanical action and biodegradation [3]. Once entering into the aquatic system, MPs pose serious hazards to marine organisms, causing damage by contact, ingestion and uptake. Evidence of MPs ingestion is well documented in marine organisms [4] and harmful consequences of MPs to biota may also derive from the possible transfer of chemicals associated to the plastic debris, especially persistent organic pollutants (POPs), such as polycyclic aromatic hydrocarbons (PAHs) [5, 6]. In this respect, the hydrophobicity and lipophilicity of the POPs and the high surface-volume ratio of the plastic particles are the main responsible factors of the interaction between MPs and POPs [7].

To date, many studies are focusing on the interaction between MPs and POPs, concerning sorption processes and competitive behaviour of chemicals onto MPs [5], however most of them are experimental studies [8] and very few field studies have been carried out on wild organisms [9]. Combining two different studies [2, 10], both carried out in the same period and on the same geographical area (Adriatic Sea) but one focused on MPs and the other one focused on PAHs, in the present field study the most commonly found plastic polymers (polyvinyl chloride, polypropylene, polyethylene, polyester and polyamide) and PAH congeners (phenanthrene, fluoranthene and pyrene) [7] were analysed. PAHs were evaluated in sediments and several fish tissues (gills, liver and fillet) of wild sole (*Solea solea*) and MPs were evaluated in the

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gastrointestinal tract of the fish. The aim of the analysis was to characterize the MPs and PAHs spatial distribution as well as any correlation among the contaminants.

2 Materials and Methods

2.1 Study and Field Area

The Adriatic Sea is the most continental basin of the Mediterranean Sea, it is strongly influenced by anthropogenic contributions predominantly from the Po Valley, a highly entropized basin with rich nutrient loads and pollutants that are transported in the open sea in all northern and southwestern basin. Individuals of *Solea solea* and sediment specimens were sampled in the GFCM Geographical Sub-Area 17 (GSA 17: Northern Adriatic Sea) in correspondence of seven sampling stations. In Fig. 1 is shown the sampling area and the sampling stations. The fishes were obtained from rapido trawl surveys (SoleMon, Grati et al. [11]) carried out in fall (November–December) 2014. Fish were dissected fresh on board and whole gastrointestinal tracts, fillet, liver and gill were collected and frozen at −20 °C until the laboratory analyses. Out of 105 fishes, 64 were analysed for MPs while the remaining (n = 41) for PAHs analysis. In addition, surface sediments (n = 7) were collected by a box-corer.

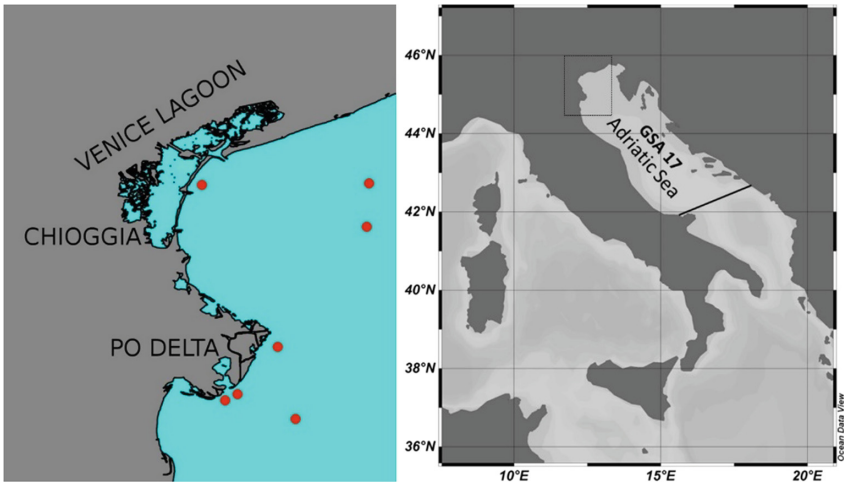

Fig. 1. Sampling area and the sampling stations in the Adriatic Sea (Geographical Sub-Area, GSA 17)

2.2 Laboratory Analysis

2.2.1 MPs Extraction and Characterization

All the material and reagents have been carefully prepared and filtered. The content of oesophagus, stomach, and intestine of each specimen was weighed and digested with a

10% KOH solution. The supernatant containing the floating plastic particles was subsequently collected and filtered, as previously described. Extracted particles were microscopically observed, photographed, measured at their largest cross section through an ocular micrometre, and categorized according to three size classes (<100 μm; 100 μm < × <500 μm; >500 μm) and fibers. Plastic particles were counted per individual fish. Particles were then characterized by μFT-IR spectrometry for polymer composition. The methods refer to the work of Pellini et al. [2].

2.2.2 PAH Extraction and Quantification in Fish Tissues and Sediments

The Quechers (QUick Easy CHeap Effective Rugged and Safe) method was applied and developed for the extraction and purification steps of PAHs from fish tissue (gill, liver and fillet) [10]. At the same time, the ultrasonic bath and liquid-liquid separation were performed for the PAH extraction from surface sediments [12]. PAH identification and quantification in fish tissues and surface sediment samples were performed by the same methods using an HPLC system (Ultimate 3000, Thermo Scientific) equipped with a fluorescence detector (RF-2000, Thermo Scientific). The whole analytical procedure was validated by analysing the reference materials (IAEA code 106, code 408 and code 383) and the recovery fell within the confidence interval of 95%.

2.3 Statistical Analysis

All the graphs and the statistical analyses were performed using the free statistical software R ver. 3.6.0 64-bit for Windows [13]. As an indication of the linear relationship between variables, the Pearson correlation coefficient (ρ) was considered with a reference p-value of 0.05 for significance. In case of ANOVA, firstly the constant variance assumption was evaluated according to the Levene test. In case of homoscedasticity, the usual parametric ANOVA was considered; if the ANOVA p-value was significant, we carried out the post-hoc Tukey comparisons. In case of heteroscedasticity, we resorted to a nonparametric ANOVA analysis followed, if significant, by games-howell post-hoc comparisons [14] as offered by the R package "userfriendlyscience" (ver. 0.7.2).

3 Results and Discussion

3.1 Results

3.1.1 Distribution of MPs and PAHs

Among the 64 fishes sampled for MPs, 95% has evidence of MPs in the gastrointestinal tract. Of all selected MPs particles, polyester and polyamide contributed 25% and 21%, respectively, followed by polypropylene and polyvinylchloride (20%) and finally polyethylene (14%); of these MPs particles 95% are fragments and 5% fibers. Fish samples close to the coastal area of the Po Valley and Venice Lagoon had the highest values of MPs abundance with an average number of particles for fish in the range 12–12.5, while lower values were recorded off Chioggia and Po Valley stations, 10–10.5.

The three analysed PAH congeners (phenanthrene, fluoranthene and pyrene) have been found in all sediment samples. The total PAHs (Σ_3 PAHs) in the sediment sampling stations ranged from 20 ng g^{-1} (off Chioggia) to 673 ng g^{-1} (Venice Lagoon).

Among the 41 fishes sampled for PAHs, phenanthrene and pyrene were observed in 98% of the all fish tissues and fluoranthene in 80% of them. The average of Σ_3 PAHs in the fish samples (gills, fillet and liver) ranged from 38 ng g^{-1} (off Chioggia) to 94 ng g^{-1} (Venice Lagoon).

In Fig. 2, the bubble plots report the mean as a function of the seven sampling stations, of the PAH concentration in fish (Fig. 2a) and sediment (Fig. 2b) as well as the MPs abundance in fish (Fig. 2c). As can be seen, the higher concentrations of PAH and MPs are all located near coast.

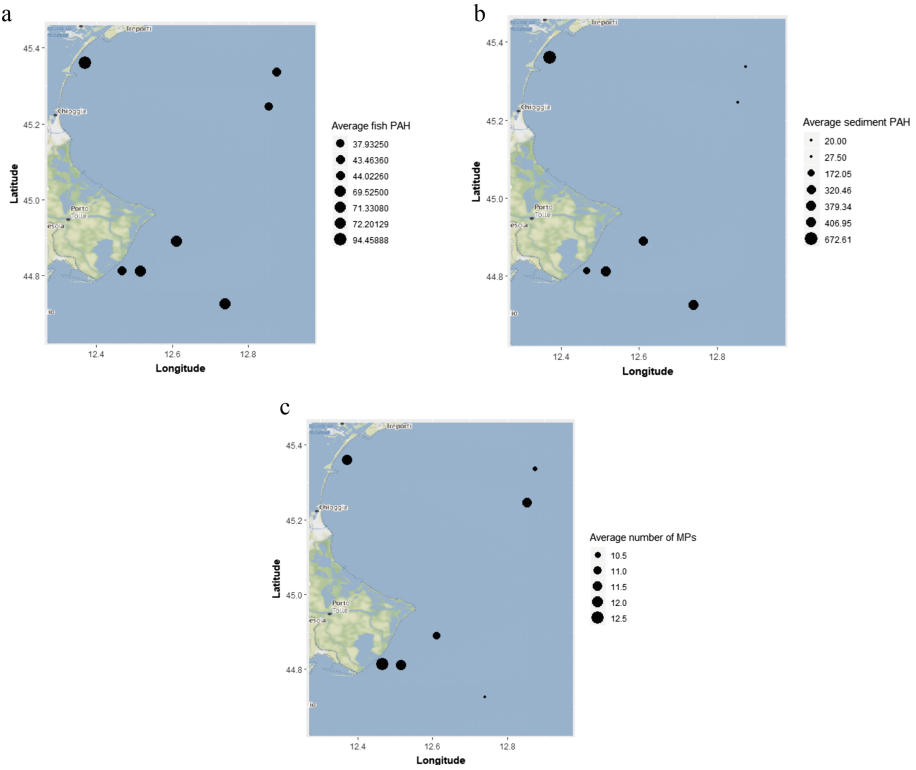

Fig. 2. Average concentration as a function of geographical coordinates for: PAH concentration (ng g−1) in fish (a), PAH concentration (ng g−1) in sediment (b) and average number of MPs particles in fish (c).

3.1.2 Correlation Between PAHs and MPs

In Fig. 2, an apparent correlation, as a function of sampling station, can be spotted among the PAH concentration in fish, the PAH concentration in sediment and number

of MPs particles. However, the only significant correlation coefficient was between the PAH concentration in fish and the PAH concentration in sediment ($\rho = 0.970$, p-vales < 0.001), see Fig. 3. Correlation between the PAH concentration in fish and the number of MPs particles in fish ($\rho = -0.104$, p-vales = 0.825) and between the PAH concentration in sediment and the number of MPs particles in fish ($\rho = 0.046$, p-vales = 0.921) does not indicate any linear relationship for these variable couplings.

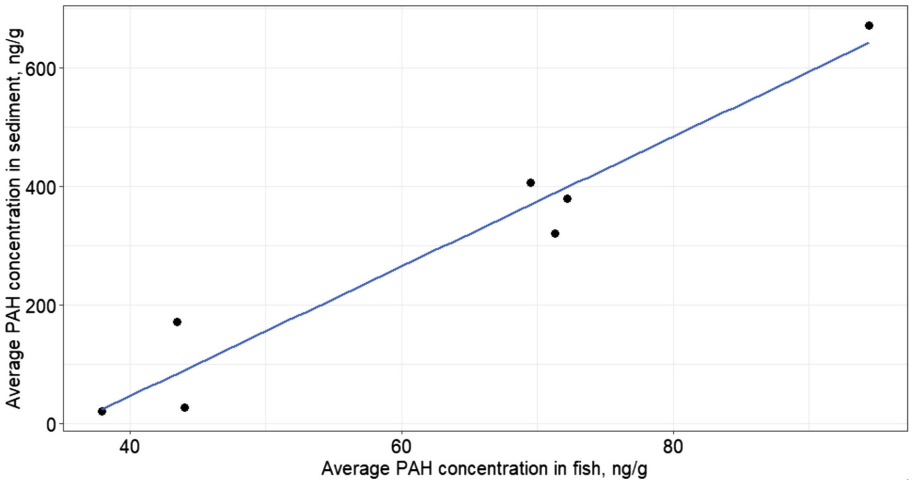

Fig. 3. Relationship between average PAH concentration in fish and sediment for the seven sampling stations.

For each sampling station, a specific Pearson correlation coefficient was calculated coupling each PAH to each MPs type and size, considering four different scenarios: (1) the PAH concentration in sediment, (2) the PAH concentration in gill, (3) the PAH concentration in fillet and (4) the PAH concentration in liver. Regard PAH congeners and MPs type, most of the correlation coefficients were not statistically significant (p-value > 0.05), except for few cases in scenario (2): fluoranthene – polyamide; and scenario (3): phenanthrene – polypropylene, phenanthrene – polyethylene and pyrene – polyvinyl chloride). As regard PAH congeners and MPs size, the only statistically significant correlations (p-value < 0.05) were found between pyrene – MPs size <100 μm and fluoranthene and MPs size $100 < \times < 500$ μm in fillet and liver tissues, respectively.

3.2 Discussion

The spatial distribution of PAHs (phenanthrene, fluoranthene and pyrene) in the sediment samples and in the sole tissues and the spatial distribution of MPs (polyvinyl chloride, polypropylene, polyethylene, polyester and polyamide) in gastrointestinal tract of sole caught in the North Adriatic Sea have shown a higher contamination of PAHs and MPs in stations close to coast compared to offshore stations. Moreover, a

significant relationship between PAH in fish tissues and sediments has been found. In fishes, the three PAH congeners are mainly concentrated in the gill tissue than in liver or fillet (Fig. 4a), with a statistically significant difference between gill and fillet (Fig. 4b).

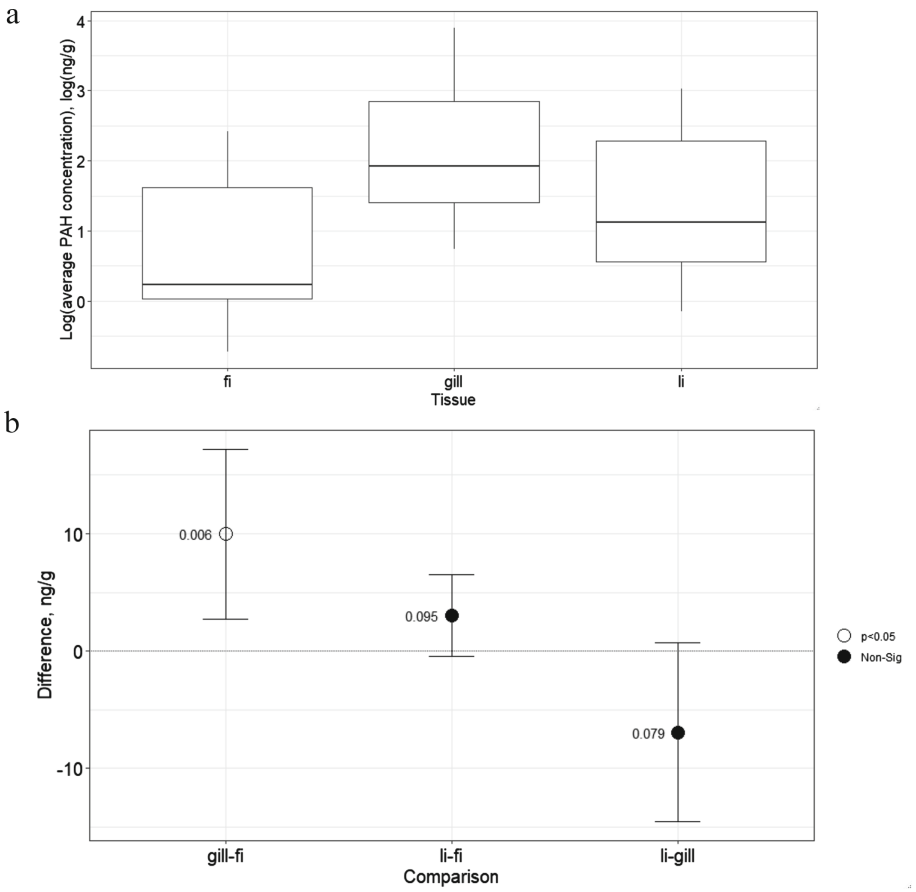

Fig. 4. (a) Box-plot of the natural logarithm of the PAH concentration as a function of fish tissue: fi = fillet, gill = gills, li = liver. (b) Post-hoc comparisons of PAH concentration fish tissue. Symbols represent the difference between the respective group means reported on the x-axis and the error bars at the 95% confidence intervals. The horizontal line at y = 0 is added for reference. The number close to the symbol reports the corresponding p-value. Significant differences (p-value < 0.05) are indicate by empty symbol.

The strong relationship between PAH concentration in sediments and fish, the higher PAH concentration in gill tissue, in addition to almost no correlation between single MPs and PAH congeners, see Sect. 3.1, strengthened the hypothesis of a dominant contribution of environment more than that from MPs on fish PAH level, in line with published literature data [15, 16].

4 Conclusions

The present study is the first carried out in field on *S. solea* in Adriatic Sea that highlights the possible relationship between MPs and PAHs. Preliminary results show that MPs are not PAH carriers. Further, the main source of PAH fish contamination comes from the surrounding environment, i.e. marine sediments, where the sole lives. Such findings are validated by the strong relationship between PAH concentration in the marine sediments and fish, by the missing relationship of the PAH fish concentration with the MPs and by the different PAH concentration in the three fish tissues considered. Further field studies are already planned in order to improve the understanding of the relationships between MPs and PAHs in marine environments.

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Ecotoxicological Effects of Microplastics in Marine Zooplankton

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1 Introduction

Marine microplastics are recognized as an emerging pollutant accumulating in marine environment, throughout the water column and on the seabed [1]. Over 300 million tons of plastic are produced globally each year [2], and almost 10% of the annual production ends up into the oceans, where degradation of plastic objects can take several hundred years. Microplastics (MP) are particles with sizes below 5 mm that can derive from the breakdown of larger debris or can enter the environment as microscopic fragments. Because of their small size, MPs are of concern especially because they can be ingested by a variety of marine organisms, and possibly be transferred along the food web [3]. Marine invertebrates are among the primary biological targets of MPs, being exposed both to polymeric particles in suspension, as planktonic larvae, and to the fraction in sediments, as adults [4]. Planktonic stages of marine invertebrates are very sensitive to environmental stressors, including MPs [5]. MP ingestion has already been documented in the planktonic stages of several invertebrates, including crustaceans, echinoderms, rotifers and mollusks [4–10]. Such ingestion can affect feeding behavior, development, reproduction and growth of marine invertebrate early stages [11]. Despite the increasing number of studies available on ecotoxicological impact of MPs on aquatic organisms [12–14], yet, there is very little research investigating behavioral responses. Due to their rapidity and sensitivity, behavioral analyses are often used to establish effects from contamination in comparison with the standard LC_{50} approach used in ecotoxicology and may be ideal for studying the effects of different pollutants, including MPs [12].

In this study, we used low-density polyethylene MPs to perform (i) experiments with planktonic stages of crustaceans, sea urchin, rotifers and ephyrae jellyfish by investigating ingestion, lethal and sub-lethal responses.

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2 Materials and Methods

2.1 Microplastic

Fluorescent green (FMG-1.3, nominal size 1–5 μm, 1.3 g/cm^3 density, 414 nm excitation/515 nm emission) low-density PE microplastics (hereafter PE-MPs) purchased from Cospheric (USA) were used for toxicity test.

2.2 Ingestion

Fluorescently labelled particles were employed for evaluating PE-MP ingestion by planktonic stages of marine invertebrates. Early life stages of crustaceans (*Tigriopus fulvus, Artemia franciscana),* sea urchin (*Paracentrotus lividus*), rotifers (*Brachionus plicatilis*) and ephyrae jellyfish (*Aurelia* sp.) were exposed at different MP concentrations (0.01–0.1–1–10 mg/L). After 24 and 48 h, the organisms were removed and washed with fresh FSW three times to remove MP bound to the exoskeleton. Organisms were fixed in 4% paraformaldhehyde solution in phosphate-buffered saline (PBS, pH 7.4) and observed under a Leica DMRB light and epi-fluorescence microscope. Images were acquired using a DFC420C Leica CCD camera and Leica software (Leica Application Suite V3). The resulting images were stored and displayed with Leica software program, using TIFF image format.

2.3 Toxicity Tests

Early life stages of crustaceans, sea urchin, rotifers and ephyrae jellyfish were used as model organisms to assess PE-MP acute (mortality and immobility) and behavioral (swimming speed alteration) responses. Toxicity tests were performed by exposing organisms at different PE-MP concentrations (0.01–0.1–1–10 mg/L), in triplicates. After 24 and 48 h of exposure, percentage (%) of mortality and immobility was evaluated by observation under a stereomicroscope (Stereo Discovery V.8, Zeiss, Germany); % Swimming Speed Alteration (SSA) was measured using Swimming Behavioral Recorder system (SBR system).

2.4 Statistical Data

All data are expressed as means ± standard error of the 3 replicates. Lethal (LC_{50}) and Effective median concentrations (EC_{50}), i.e. concentrations causing a 50% reduction in survival or the sub-lethal endpoint recorded, and their 95% confidence limits, were calculated using Trimmed Spearman Karber analysis [15]. When significant differences ($p < 0.05$) among groups were found using ANOVA then each treatment was compared to the control using Tukey post hoc test to calculate the lowest observed effect concentration (LOEC). When data failed to meet the assumption of normality, non parametric Kruskal Wallis test and Mann Whitney test were used. For SSA test, statistical analysis has been performed using swimming speed data. Data were considered significantly different when $p < 0.05$. SPSS statistical software (Statistical Package for the Social Sciences, Version 20) was used for data analysis.

3 Results

3.1 PE-MPs Ingestion

Microscopy observations showed that PE-MPs were actively ingested by all tested planktonic stages within 24 and 48 h. PE-MPs were accumulated in the gut of *T. fulvus, A. franciscana, P. lividus, B. plicatilis* and around the mouth and lappets of ephyrae jellyfish (Fig. 1).

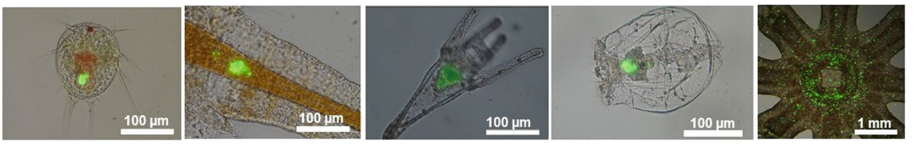

Fig. 1. PE-MP ingestion in all the tested species at 1 mg/L after 24 h of exposure. From the left: *T. fulvus, A. franciscana, P. lividus, B. plicatilis, Aurelia* sp.

3.2 Toxicity Tests

No acute toxicity in term of LC/EC_{50} was observed in any species exposed to MPs. Significant differences between control and MP concentrations were found by analysing sub-lethal responses.

PE-MP exposure significantly affected immobility of *T. fulvus* (LOEC = 0.01 mg/L) and *Aurelia* sp. (LOEC = 0.1 mg/ L), as shown in Table 1. Swimming speed of all the tested species (*A. franciscana, P. lividus, B. plicatilis, Aurelia* sp.) was significantly inhibited at low PE-MP levels (0.01 mg/L).

Table 1. Lowest Observed Effect Concentrations (LOEC; mg/L) for mortality, immobility and swimming speed alteration tests of the tested species after exposure for 24 (*P. lividus*) and 48 h (*T. fulvus, A. franciscana, B. plicatilis, Aurelia* sp.).

Organisms	Mortality (mg/L)	Immobility (mg/L)	Swimming speed alteration(mg/L)
T. fulvus	>10	0.01	Not available
A. franciscana	>10	>10	0.01
P. lividus	>10	>10	0.01
B. plicatilis	>10	>10	0.01
Aurelia sp.	>10	0.1	0.01

4 Discussion and Conclusions

This study demonstrated that planktonic stages of crustaceans, sea urchin, rotifers and ephyrae jellyfish actively ingest PE-MPs in laboratory conditions. The ingestion of plastic by marine organisms is a common and widely documented phenomenon in marine environment; in 1972 Carpenter documented plastic polystyrene pollution of surface waters of the northwest Atlantic and ingestion by fish [16]. Nevertheless, the

demonstration in laboratorial condition of MP ingestion and their effects on marine organisms, as well as the possible transfer along the food web have only recently become an important subject of research.

Early life planktonic stages of crustaceans, sea urchin, rotifers and ephyrae jellyfish are extremely sensitive to chemical pollutants, that explain their wide use as model organisms for ecotoxicological purposes [5, 17, 18]. Due to their low density, PE and other MP polymer accumulate in the surface layer of the water. Both weathering and biofouling may affect the buoyancy of these particles, which can be found also in the water column. Therefore, planktonic organisms are readily exposed to MPs.

In the last few years, knowledge of MP ecotoxicological impacts on marine organisms, including producer and consumer levels, has steadily increased. PVC-MPs have been found to inhibit the growth of the marine microalgae *Skeletonema costatum* [19]. On the contrary, no significant growth rate inhibition has been noted in *Tetraselmis chuii* after exposure to PE-MPs [20]. Zooplanktonic stages of several marine invertebrates have been shown to ingest MPs [21], including crustaceans, echinoderms, rotifers and cnidarians [4, 7, 22], and sub-lethal responses have already been documented [5, 17, 23, 24].

Our results confirm MP ingestion by early life stages of planktonic invertebrates, since MPs were found in the gut of all the tested species. No lethal effects were reported after ingestion, whereas sub-lethal endpoints (immobility and swimming speed alteration) were effectively impaired according to the investigated organisms. A significant impact on the immobility was found in *T. fulvus* and *Aurelia* sp., whereas swimming behavioral alteration was shown for all the species, starting from the lowest concentration tested (0.01 mg/L).

Although our study did not report negative consequences in planktonic stages of marine organisms, it clearly showed that small plastics can be ingested by early life stages of zooplankton and that MPs can potentially reached high level in the food chain. Further studies are needed evaluating different endpoints in battery of model organisms in order to elucidate MP effects and mechanisms, thus increasing our knowledge on plastic pollution and its interaction with biota.

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Occurrence of Microplastics in the Gastrointestinal Tracts (GITs) of the Common Dolphinfish, *Coryphaena Hippurus*, from the Western Mediterranean Sea

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1 Introduction

Plastics are the most common marine litter typology found on the beaches and seafloor or floating on the sea surface and water column [1–3]. When these plastic items reach micro size (<5 mm) they are commonly known as microplastics (MPs) [4]. In marine environment MPs can derive from direct introduction as microbeads used in consumer products (primary MPs) [5] or from degradation and erosion processes caused by abiotic and biotic factors (secondary MPs) [6]. Regardless of their origin, once these particles are in the environment, they have several negative impacts on marine life. As a matter of fact, due to small size, chemical and physical structure, MPs are resistant and their lifetime is very high. For this reason, MPs are found in different compartment of marine ecosystem and they also can affect marine food web [7–11]. In particular, plastic ingestion by marine organisms could cause mechanical and chemical injuries blocking the gastrointestinal tract and feeding appendages, causing internal lesions or pseudo-satiation and facilitating the direct (i.e. additives) and indirect transfer of toxic substances [12, 13]. Plastics could be ingested accidentally during feeding activity or voluntarily (i.e. predators may mistake them with prey) but also as a result of secondary ingestion [9, 14].

In the Mediterranean Sea, the occurrence of microplastics in marine species such as bivalves [15], fishes [7, 9, 11], other invertebrates and marine mammals [8] was already reported in several studies. However, information regarding some pelagic species is still limited. Whit regard to *Coryphaena hippurus* Linnaeus, 1758 some papers on the

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feeding habits of this species also reported information on litter ingestion [16]. This study was focused on the occurrence of MPs debris in the GITs of *C. hippurus*, caught under Fish Aggregating Devices (FADs) in the western Mediterranean Sea [17, 18]. This species is highly migratory fish and an opportunistic and voracious pelagic predator. It is an important resource of commercial value and represents a target species of small-scale fishing in several areas of the Mediterranean Sea.

2 Experimental

A new experimental digestion method basing on a combination of basic-acid agent was applied for the processing of *C. hippurus'* GITs. Two digestion steps were performed, including potassium hydroxide (KOH) and nitric acid (HNO_3), in order to remove most of the organic and inorganic material, respectively.

2.1 Fish Collection and Sample Preparation

A total of 27 juvenile common dolphinfish was sampled by purse-seine and trolling lines around FADs. Specimens were measured (fork length, FL in mm) and weighted (total weight, W in g) and the gastrointestinal tracts (GITs) were removed and frozen at −20°C until analysis.

2.2 Materials

Potassium hydroxide (KOH, Labochimica srl Padova, Italy) and nitric acid ($\geq$65% HNO_3, Honeywell Fluka™, Rodano, Italy) solutions were used to digest the GITs of fish. In particular, 10% (w/v) KOH was prepared by dissolving pellet in filtered water. While 20% (v/v) HNO_3 was prepared by diluting the stock solutions with filtered water. Glass fibre filters (pore size 1.6 μm, GF/A Whatmann, GE Healthcare, United Kingdom) were used for the filtration steps after digestion.

2.3 Microplastics Extraction Protocol

2.3.1 Digestion Method

In laboratory GITs were weighted and placed into glass beakers in 1:3 (w/v) with 10% KOH and incubated at 60 °C for 6 h according to Rochman protocol [19] with minor modifications. Digestive solutions were filtered under vacuum on glass fibre filters GF/A. Immediately after the filtration, the filters generated from the first digestion step were allowed to react with 40 mL of 20% HNO_3 for 60 min at room temperature and then were cleaned with filtered water.

2.3.2 Microplastics Identification

After digestion, filters were examined under a stereomicroscope Zeiss Discovery V.8 coupled with AxioVision AxioVs40 version 4.8.2.0 digital image processing software. Plastic particles founded in GITs of *C. hippurus* were counted and measured (length, width, thickness). Then, they were photographed and classified by shape (fibres,

fragments, sheets and sphere), colour and size according to the protocol of Marine Strategy Framework Directive [20]. To avoid airborne contamination of samples, especially from textile fibres, all equipment was cleaned and washed with filtered water before use and one blank sample for each battery of digested GITs was processed. MPs with same shape and colour of those presented in blank samples were excluded from the results.

2.3.3 FT-IR and Data Analysis

All plastics samples were analysed by Fourier transform infrared (FT-IR) spectroscopy technique (Agilent Cary 630 spectrophotometer) to identify the polymer composition. Only spectra matching more than 80% with reference polymers present in databases were accepted as plastics [21, 22].

The frequency of occurrence (F%) of plastics debris in the GITs of *C. hippurus* was calculated as the proportion of individuals containing plastics on the total of samples.

3 Results and Discussion

After the digestion of 27 GITs of *C. hippurus*, plastic particles were found in 17 samples and the frequency of occurrence was 63% (Table 1). Among them, fragments and fibres as microplastics were the most abundant categories (Fig. 1). The most common colours observed were transparent, yellow, blue, and white. The polymers identified by FT-IR were polyethylene (PE), ethylene propylene diene monomer (EPDM), polyethylene terephthalate (PET), polystyrene (PS), polyvinyl chloride (PVC), neoprene, styrene butadiene rubber (SBR), and polyacrylate polyester. Above all, PE was the most abundant plastic compound founded. It represents one of the most common polymers widespread in marine environment [23]. Actually, more than 50% of floating litter in the Western Mediterranean Sea was represented by high- and low-density polyethylene (HD-PE and LD-PE) [10]. Indeed, PE is mainly used for packaging as well as fishing gear manufactures with polypropylene and nylons [23].

In general, the occurrence of plastics in *C. hippurus* may be due to its generalist feeding behaviour and predation on schooling prey (as observed for other pelagic predators) [9, 11]. However, it could be also influenced by the presence of the artificial structures consisting in the attractive fishery devices of FADs. As a matter of fact, FADs are equipped with palm leaves connected to a float (usually empty plastic bottles tied together) anchored to the bottom with a synthetic rope to a large stones or blocks [24]. For this reason, FADs are identified as a potential source of marine debris and the degradation of their plastic components may increase the chance of introduction of synthetic materials into the marine food web [19].

Table 1. Mean value and standard deviation of fish length and weight. The number of GITs containing plastic debris and the frequency of occurrence of plastics are also reported

Species	Number of examinated stomachs	FL (mm) (mean ± SD)	W (g) (mean ± SD)	Number of stomachs with plastics	F (%)
Coryphaena hippurus	27	266.6 ± 31.8	203.1 ± 55.8	17	63

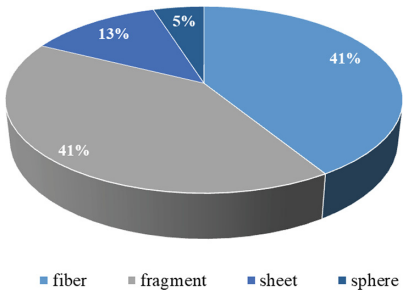

Fig. 1. Type of plastics litter detected in the GITs of *Coryphaena hippurus* (n = 27).

4 Conclusion

The results obtained confirmed the newly developed digestion method as a reliable approach to detect MPs in the GIT of opportunistic pelagic predators, feeding on a wide variety of prey items. Therefore, this protocol could be adapted also for other fish species. Finally, this species can be considered as a good bio-indicator for the impact of litter ingestion (micro and macro litter) in open waters at medium-spatial scale (Mediterranean UN Environment/MPA sub-regions). For this reason, it was included as bioindicator in the project "Plastic Busters MPAs: preserving biodiversity from plastics in Mediterranean Marine Protected Areas".

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Effects of Polymethacrylate Nanoplastics on Lipid Metabolism in *Sparus Aurata*

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1 Introduction

Small plastic particles are considered emerging pollutants, and this has motivated research to establish their ecological and environmental consequences. Currently, the study of the effects of nanoplastics (NPs) in aquatic organisms is still scarce, especially in organisms of higher trophic levels, such as fish. The Mediterranean Sea is considered a region of high accumulation of plastics, due to the high plastic load from 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.

The objective of this study was to evaluate the effect of short-term exposure to polymethylmethacrylate (PMMA) NPs on lipid metabolism in *S. aurata*, using molecular and biochemical endpoints, considering that lipids are important source of energy for fish. Parameters related with the antioxidant response were also studied as a marker of general toxicity.

The mRNA levels of the following transcripts related with the lipid metabolism or antioxidant response were evaluated in the liver of fish exposed to NPs: peroxisome proliferator-activated receptors (*pparα*, *pparβ* and *pparγ*), retinoid X receptor (*rxr*), apolipoproteins (*apo*) and lipoprotein lipase (*lpl*), glutathione peroxidase 1 (*gpx1*), glutathione reductase (*gr*), superoxide dismutase (*sod2*), glutathione-S-transferase 3 (*gst3*) and catalase (*cat*). Biochemical biomarkers, such as cholesterol, triglycerides, glucose, total oxidative status (TOS) and esterase activity (EA) were measured in fish plasma and liver.

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Considering that the reliability of molecular results is directly influenced by the housekeeping genes chosen for normalization, the stability of three potential reference genes was evaluated, to determine the most suitable genes to be used in RT-PCR analysis. These genes included glyceraldehyde 3-phosphate dehydrogenase (*gapdh*), elongation factor 1α (*ef1α*) and α-tubulin (*tub*).

2 Experimental

2.1 Animals, Experimental Design and Sampling

Juvenile gilthead sea bream (8.7 ± 2.5 g) were obtained from an aquaculture facility and acclimatized to laboratory conditions, with aerated ASW (salinity 30), 19 °C and natural photoperiod. During this period, fish were fed daily with commercial fish food until satiation. Following the acclimatization period fish were exposed to increasing concentrations of PMMA NPs (0.001, 0.01, 0.1, 1 and 10 mg/L). After 24 and 96 h of exposure liver and blood were sampled. Blood was collected from the caudal vein with heparinized syringes and plasma isolated (1500 rpm for 10 min, 4 °C). Plasma samples were immediately stored at −20 °C until further analysis. Liver was divided in two parts (one for gene expression and the other for biochemical analysis), immediately frozen in liquid nitrogen and stored at −80 °C until further analysis.

2.2 Synthesis and Characterization of Polymethylmethacrylate (PMMA)

PMMA NPs were prepared by microemulsion polymerization, adapted from Roy and Devi [27] of MMA, with sodium dodecyl sulphate as stabilizer. After polymerization, particles characterization was performed in ultrapure water by assessing hydrodynamic size by dynamic light scattering (DLS) and suspension stability by zeta potential (Zetasizer Nano ZS, Malvern). Morphological characterization was performed by transmission electron microscopy (TEM) (Hitachi, H9000 NAR), where particles did not have a uniform circular shape and presented an average size of 45 nm. The hydrodynamic size determined by DLS was 40 nm in ultrapure water and particle stability was assured by the relatively high surface charge of the particles (−26.4 mV). A stability test revealed that these particles immediately display an increase in the hydrodynamic size to 58.6 nm when placed in artificial seawater (ASW - salinity 30). After 1 h, the average hydrodynamic size reached 97.3 and after 24 h, the particles displayed an average size of 120.3 nm.

2.3 Total RNA Extraction and Complementary DNA (cDNA) Synthesis

Total RNA was extracted from each liver (n = 9) using Tri Reagent® (Sigma-Aldrich T9424). All procedures were performed following the manufacturer's protocols. RNA quantification was done using a NanoDrop Spectrophotometer (Thermo Fisher Scientific, USA) and RNA quality checked with Experion, using the Experion Standard Sens RNA chip (Bio-Rad Laboratories, USA). Reverse transcription was performed

using 1 μg of the total RNA using the iScript™ cDNA synthesis kit (Bio-Rad, USA) according to the manufacturer's instructions.

2.4 Transcriptional Analysis

Efficiency of the amplification was determined for each primer pair using serial 5- fold dilutions of pooled cDNA and calculated as E = 10(−1/s), where s is the slope generated from the serial dilutions. RT-qPCR was run in a Bio-Rad CFX384 Real-Time PCR Detection System (Bio-Rad, USA). Reactions were done using iTaqTM Universal SYBR® Green Supermix (Bio-Rad, USA) according to the manufacturer's instructions. Briefly, 1 cycle at 95 °C for 5 min, 40 cycles at 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s were run; samples were performed in triplicates. Expression data, obtained from three independent biological replicates, were used to calculate the threshold cycle (Ct) value. After checking for primers efficiency, RT-qPCR analysis of all the individual samples was determined following the same protocol described above. NormFinder was used to identify the most appropriate housekeeping gene among. The expression of the target genes was normalized using the best combination of two housekeeping genes and relative gene expression calculated with the ΔCt method [1]. Primers information is given in Table 1.

2.5 Biochemical Analysis

Cholesterol, triglycerides, glucose, TOS and EA were determined in the plasma of fish using commercially available kits (Olympus Systems Reagents; Olympus life and Material Science Europe GmbH, Hamburg, Germany) following manufacturers indications, with some modifications as already described elsewhere. All parameters were performed with an automatic analyser (Olympus Diagnostica, GmbH, Freiburg, Germany).

2.6 Data Analysis

Results were expressed as mean ± standard error (SE, n = 9 per treatment). Data were tested using the Sigma Plot 12.0 software package. For gene expression and biochemical parameters, different treatments were compared using one-way analysis of variance (ANOVA), followed by Dunnett's method whenever applicable.

3 Results and Discussion

Results demonstrated that the exposure of fish to PMMA NP induce an increased expression of the pparβ after 24 h of exposure, which returned to control levels at 96 h. On the other hand, pparα and pparγ presented an increased mRNA levels for some concentrations at 96 h but not at 24 h after NP exposure (Fig. 1).

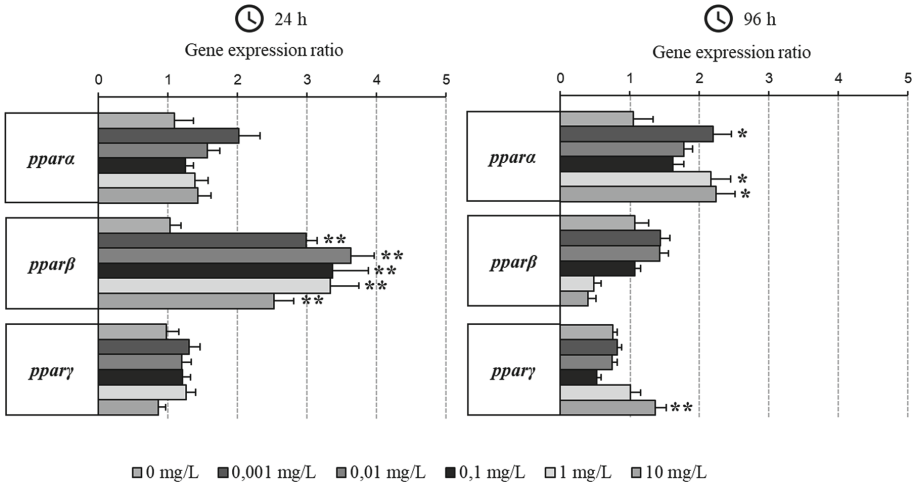

Fig. 1. mRNA levels of selected genes determined in the liver of fish after exposure to polymethylmethacrylate (PMMA) nanoplastics (NPs).

Table 1. Primers used for quantitative real-time PCR amplification of the genes studied in *S. aurata*.

Gene name	Acronym	GenBank accession No.	Forward	Reverse	Efficiency (%)
Elongation factor-1α	ef1α	AF184170	CCCGCCTCTG TTGCCTTCG	CAGCAGTGTGGT TCCGTTAGC	105,33
α-tubulin	tub	AY326430	AAGATGTGAA CTCCGCCATC	CTGGTAGTTGA TGCCCACCT	117,97
Glyceraldehyde 3-phosphate	gapdh	DQ641630	TGCCCAGTAC GTTGTTGAGTCCAC	CAGACCCTCAA TGATGCCGAAGTT	95,12
Peroxisome proliferator-activated receptor Alpha	pparα	AY590299	GCAGCCTGTGAG TCTTGTGAGTGA	CTCCATCAGGTC TCCACACAGC	97,36
Peroxisome proliferator-activated receptor beta	pparβ	AY590301	CGTGTTCGGGA TTCGGGACT	CACCCTGTCGTG CTGCTCTGTA	96,07
Peroxisome proliferator-activated receptor gama	pparϒ	AY590304	CGGAGAGAGAA GCAAGAACAAGAA	GAGGAGGAGGAGA TGGAGGTGTA	105,09
Retinoid X receptor	rxr	HS092100	GGGCTTCTTCAA GAGGACAGT	TGCACCGCTTC TCTCTTCAT	117,89
Apolipoprotein AI	apoa1	AF013120	GAATACAAGGA GCAGATGAAGCAGATG	TGGTGACGGA GGCAGCGATG	83,68
Lipoprotein lipase	lpl	AY495672	CGTTGCCAAGTT TGTGACCTG	AGGGTGTTCTGG TTGTCTGC	92,24
Glutathione peroxidase 1	gpx1	DQ524992	GAAGGTGGATGTGA ATGGAAAAGATG	CTGACGGGACT CCAAATGATGG	105,73
Glutathione reductase	gr	AJ937873	TGTTCAGCCACCC ACCCATCGG	GCGTGATACATCGGAG TGAATGAAGTCTTG	104,87
Superoxide dismutase [Mn]	sod2	JQ308833	CCTGACCTGACCTA CGACTATGG	AGTGCCTCCTGATA TTTCTCCTCTG	105,73
Glutathione-S-transferase3	gst3	JQ308828	CCAGATGATCAGTA CGTGAAGACCGTC	CTGCTGATGTGAGGA ATGTACCGTAAC	104,57
Catalase	cat	JQ308823	TGGTCGAGAACTT GAAGGCTGTC	AGGACGCAGAAA TGGCAGAGG	105,07

Reference

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Measuring the Size and the Charge of Microplastics in Aqueous Suspensions With and Without Microorganisms Using a Zeta-Sizer Meter

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1 Introduction

According to recent models trillion microplastics over 66 tons, are currently floating in the ocean [1, 2]. On top of that, microplastics have been identified interacting with many aquatic organisms. More specifically, microfibers inside mesopelagic organisms have been found from 334 – 1783 m depth in the equitorial mid-Atlantic and 954 – 1062 m in the SW Indian Ocean. Previous studies have found microfibers in the bathypelagic and abyssopelagic sediments (2000 m in the subpolar North Atlantic, 2200 m in the NE Atlantic, 3500 m in the Mediterranean, and 5768 m in the West Pacific) [3].

There are many studies on the effect of microplastics on organisms (reviewed by [4, 5]). They have been linked to diverse reported biological effects, ranging from reduced feeding, swimming and assimilation efficiency to altered size, impaired reproduction as well as tissue damage [2].

Microplastic pollution can have an effect on marine microbial communities, i.e. in all biogeochemical processes in the oceans [6]. Surfaces exposed to seawater are colonized by microorganisms which form a biofilm [7]. However, plastic has a longer half-life than most autochthonous substrates naturally floating in the upper layers of the ocean, as well as a hydrophobic surface that promotes microbial colonization and biofilm formation [8]. Biofilm is one of the major factors affecting the properties of microplastics [9]. In specific, on polymer surfaces biofilm changes the surface characteristics and may lead to degradation [10]. Therefore, the composition of the biofilm community and its activity in relation to environmental settings, plastic degradation [7] as well as biofilm effects on sub-mm microplastic, which are easily ingested by small aquatic organisms, remain to be investigated further [11]. However, there are several limitations to take into consideration in future research, such as lack of harmonization of methodologies for sampling and analysis of microplastics and nanoplastics, limitations in the accuracy of sizes of the detected particles, as well as biased calculation of the potential concentrations in the environment due to analytical instrumentation [12].

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Recent investigation, examined different cases of fluorescent labeled polystyrene beads and *H.alkaliphila* to calculate the Zeta potential in sterile seawater [13] while another study used the same technique (Zeta-sizer) to estimate the size of polystyrene (PS) samples [14].

The aim of the present study is to provide the zeta-sizer meter as a useful technique to measure the size of microplastics in aqueous suspension. The specific objectives of the present study are to measure (a) the size and (b) the charge of the surface of microplastics in the (i) absence and (ii) presence of microorganisms.

2 Experimental

2.1 Materials

PE powder was provided by Greek plastic industry (www.tsianakas.gr). Synthetic Sea Water (SSW) was created by NaCl (31 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (10 g L^{-1}), $NaHCO_3$ (0.04 g L^{-1}), NH_4Cl (109 mg L^{-1}), K_2HPO_4 (5 mg L^{-1}), $CaCl_2$ (5 mg L^{-1}), $FeCl_3$ (0.25 mg L^{-1}) (e.g. [15, 16]).

2.2 Bioreactor Set-Up

To simulate the marine environment in the laboratory, a 1.5-L glass beaker was used as bioreactor. The total solution volume in the reactor was 1 L (12 cm in depth) at room temperature operating in 4-days subsequent cycles [17]. An air pump was providing constant air flow. On the first day of the bioreactor set up, 1 L of sea water was used directly from the marine environment (Patras Marina, Greece) into the reactor. Every five days, SSW was used to refresh the aquatic solution (300 mL of the supernatant liquid solution was discharged and 300 mL of SSW with 320 mg L^{-1} $C_6H_{12}O_6$ were added). 1 g of microplastics (1 g L^{-1}) was added in the bioreactor on the first day. A magnetic stirrer was used for stirring the liquid in the bioreactor. The process lasted for a month.

2.3 Characterization Techniques

After the samples were coated with carbon, Scanning Electron Microscopy (SEM) JSM 6300 of the company JEOL was used to observe the virgin sample of powder PE.

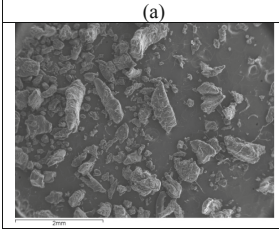
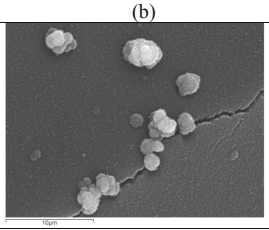
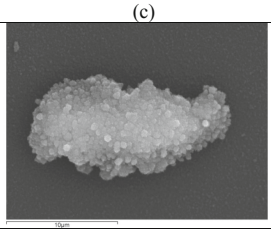
Zetasizer Nano ZS (Malvern Instruments Ltd.) was used to calculate the size and the charge of the samples. A glass cuvette was used to measure the size of the samples: (a) synthetic sea water without microorganisms using only virgin PE microplastics and (b) supernatant liquid from bioreactor due to the floatation of PE. In both cases, ethanol was added before the measurement (1:3) to create neutral buoyancy in microplastic particles. The maximum and the minimum particle sizes that can be detected from the technique are 10 μm and 0.3 nm (radius), respectively. A folded capillary zeta cuvette was used in the same instrument to measure the charge in the above-mentioned samples.

3 Results and Discussion

3.1 Results

In Table 1, the distribution of particle size fraction can be seen. According to 20X-magnification SEM images, different sizes of particles including agglomerates were observed. Based on the agglomerate in magnification 5000X, particles as spheres were observed to form the agglomerate.

Table 1. Different particle sizes of the PE powder (a) scale bar 2 mm, (b) scale bar (10 μm) and (c) an agglomerate particle compare to scale bar (10 μm).

(a)	(b)	(c)
		

In Table 2, the values of surface charge and size of virgin PE without and with microorganisms are illustrated. There was high divergence in the value of the surface charge: (a) -1.8 ± 0.2 mV for the virgin PE while (b) -14.9 ± 1.9 mV for microplastics which were for a month in the bioreactor.

There are also differences in size values. Main size-fraction is higher in PE with microorganisms than in virgin PE, in a difference at $\sim$191 nm. However, virgin PE sample has higher minor size-fraction (5199 nm) than PE with microorganisms sample which also includes particles that are 117 nm in size (Table 2).

Table 2. Concentration table of surface charge and size data of the samples.

Properties	Virgin PE	PE with microorganisms
Main size-fraction (nm)	Peak 1: 658 ± 182 Area of peak: 83%	Peak 1: 849 ± 180 Area of peak: 88%
Minor size-fraction (nm)	Peak 2: 5199 ± 475 Area of peak: 17%	Peak 2: 117 ± 22 Area of peak: 12%
Surface charge (Zeta potential in mV)	−1.8 ± 0.2	−14.9 ± 1.9

3.2 Discussion

Based on SEM images, big size agglomerates of the virgin microplastics were observed. Moreover, using a zeta-sizer meter different smaller plastic-particle sizes (with e.g. [14] or without microorganisms) were calculated that are difficult to observe with the magnification provided by SEM. Finally, the neutral charge of the PE SSW

suspension changes to high negative surface charge (two orders of magnitude higher) due to the presence of microorganisms as in another study [13] that also refers to a negative charge due to microorganisms in a suspension. These alterations can change microplastic behaviour in the environment that e.g. maybe eaten easier by higher trophic level organisms when they are in interaction with microorganisms (Fig. 1).

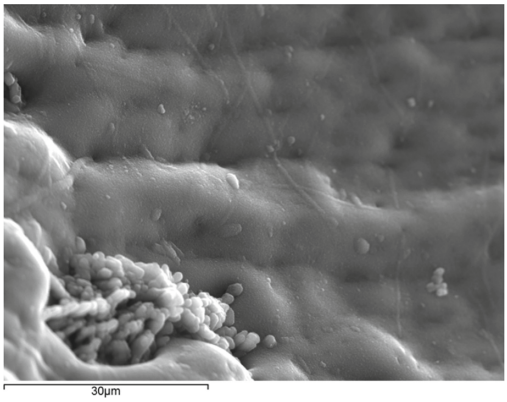


Fig. 1. SEM image (1600X) with the plastic inside the algae flocs.

4 Conclusions

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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1 Introduction

Plastic debris and microplastics (MPs) evolve into an urgent problem for environmental contamination. Until now, many sources have been identified and nearly all the environmental compartments are subject to MP contamination. Plastic pollution has become of concern as potential threat to human health, too, as MPs were also in food and drinking water. Sources of MP ingestion by humans have already been discussed in several studies [1–3]. Moreover, recent studies [4–6], attracted the attention of researchers and the general public as they confirmed the presence of MPs in bottled drinking water.

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 MP concentrations in single-use polyethylene terephthalate (PET) mineral water bottles. We further considered the release of MPs (wear particles and fragments from breakage) from the bottle upon exposure to mechanical stress (squeezing treatment; none, 1 min, 10 min) to better evaluate single-use plastic bottles as one of proven sources of MP intake by humans, especially considering the effects of daily use on these bottles such as the abrasion of the plastic material. For that, we performed a morphological analysis of the PET bottle inner wall surface after a squeezing/crushing treatment, and subsequently counted particle increase of filtered water and identified MPs in the PET bottled water with the two methodologies.

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2 Experimental

2.1 Materials

For the analysis of particle release from bottle material upon exposure to mechanical stress, three different mineral water bottles (0.5 L) were chosen based on the bottle texture (plastic thickness and, hence, bottle weight). We selected three brands representative for light (Brand 1), medium (Brand 2) and heavy (Brand 3) bottles, to test their reaction to mechanical stress. Mineral water bottles of the selected brands were purchased in Italian supermarkets, in a set of 6 in order to have replicates within the same lot of bottles. All samples were single-use bottles made of PET with screw caps made of high-density polyethylene (HDPE).

2.2 Methods

2.2.1 Preparation of Samples

To identify a potential MP release from bottle inner wall surface upon mechanical stress, bottles of the three brands were treated the following; no treatment at all, 1 min and 10 min of rolling the bottles on a smooth surface under a tray carrying a weight of 5 kg at the speed of one complete bottle round per second. This treatment was performed to mimic the squeezing/crushing effects that plastic bottles are subjected to during handling and use, particularly when re-using the bottles. Each water sample (250 mL) was filtered on a mixed cellulose ester membrane (0.45 μm pore size) via vacuum using a glass filtration apparatus (analysed area/filter area ratio of 0.67%). The filters were carefully removed and attached onto standard SEM stab/mounts within a glass container and dried for 48 h. In order to identify a potential MP source from the bottle inner wall, a piece of bottle wall was taken from the same area of each bottle after the water filtration. The plastic piece was cut out of the bottle with a scalpel and mounted on standard aluminium stubs for SEM analysis.

For the analysis of polymer identification with μ-FTIR, the preparations were performed using silver membrane filters (0.8 μm pore size) with new bottles, because filters used for SEM analyses were gold-coated and therefore not applicable for μ-FTIR. The filters were carefully removed and placed directly into a glass dish for the μ-FTIR analysis. To assess sample contamination during the procedure in the lab, we included procedural air blank samples using the same filter used for water analysis.

2.2.2 Analytical Techniques

The combination of SEM and EDX provides high resolution imaging of material surface structures as well as elemental composition signatures in order to confirm the nature of particles. After samples were attached onto standard SEM stubs and coated with a thin film of evaporated gold, they were placed in a Zeiss LEO 1430 SEM coupled with an Oxford detector for EDX analysis.

Particles of filtered bottle water were analysed for elemental composition by EDX. The smallest analysable particle size was 3 μm. Preoperational EDX tests on bottle PET material resulted in an elemental composition ratio of C:O = 73:27, with variability of 5% for each element. Hence, particles on filters were identified as PET when

analysed C:O ratio lied within this variability range and when measured peaks matched those from spectra in the preoperational tests. SEM images of analysed areas were taken and area and shape of all detected particles were measured with the image process program ImageJ. For the morphological analysis of the inner surface of bottle material (bottle inner wall), SEM images were taken from different areas of a sample.

FTIR microscopy was performed using a Thermo Scientific Nicolet iN10 MX Infrared Imaging Microscope. Measurement of all particles >10 µm located directly on the silver filter substrate was carried out in reflection mode in a wavenumber range of 4000–600 cm^{-1} controlled by OMNIC Picta software. The entire filter surface was analysed for MP particles. A total of 128 scans were taken for each spectrum, with a spectral resolution of 8 cm^{-1}. Once a particle with polymeric origin was identified, microscope images were taken and size measured by image analysis. Data were analysed without post processing software.

3 Results and Discussion

3.1 Results

Against our assumptions, brands and treatments had no significant influence on particle concentration in water (two-way ANOVA: $F_{2,4} = 0.918$, $p = 0.54$ and $F_{2,4} = 0.728$, $p = 0.47$) (Fig. 1a). Considering the particles observed in the analysed area by SEM in relation to the total area of the filter, we calculate a mean total particle number per litre of 11,154 ± 1,625 Standard Error (SE). Particle size ranged from 1 µm to 48 µm and did not differ noticeably between brands and treatments (Fig. 1b). The majority of particles in water were in the range of 1–5 µm (70–85%, Fig. 1b).

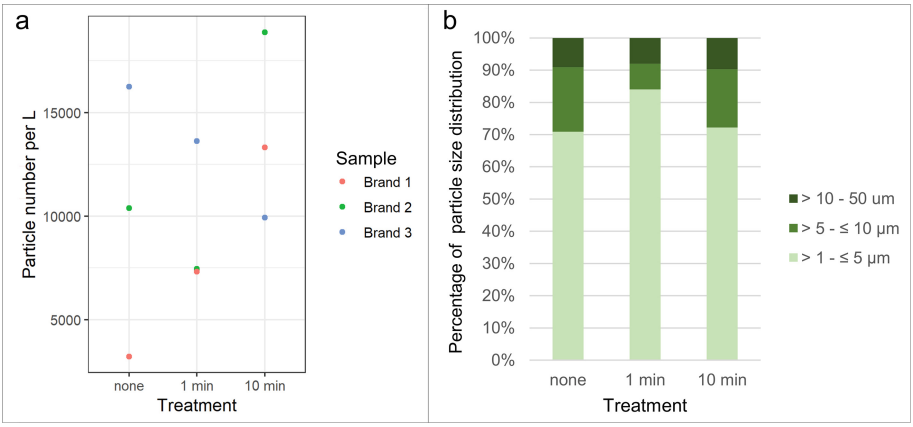

Fig. 1. Particle number in filtered bottled water of the three treatments and brands (a) and size distribution of particles in filtered bottled water from different treatments (b)

More interesting is the number of MP particles identified by their elemental composition. Elemental analyses performed on 58 particles revealed that only two particles had a C:O ratio corresponding to that of PET. The two PET particles derived from water of no-treatment bottle from Brand 3 (3.3 μm), and from water of the 10 min treatment bottle from Brand 2 (25.4 μm). Analysis by μ-FTIR identified in total four MPs of three different polymer types; one PVC fibre was detected in water of the 10 min treatment bottle from Brand 3, and three MPs were detected in water of the 10 min treatment bottle from Brand 2 (one nylon, one polyamide particle and one PVC particle (Fig. 2a). Micro-FTIR spectrum of the latter particle (Fig. 2b) matched better the PVC spectrum of the library when two additives were added, namely kaolin and polyadipate (Fig. 2c). No polymer particle was detected in procedural blanks.

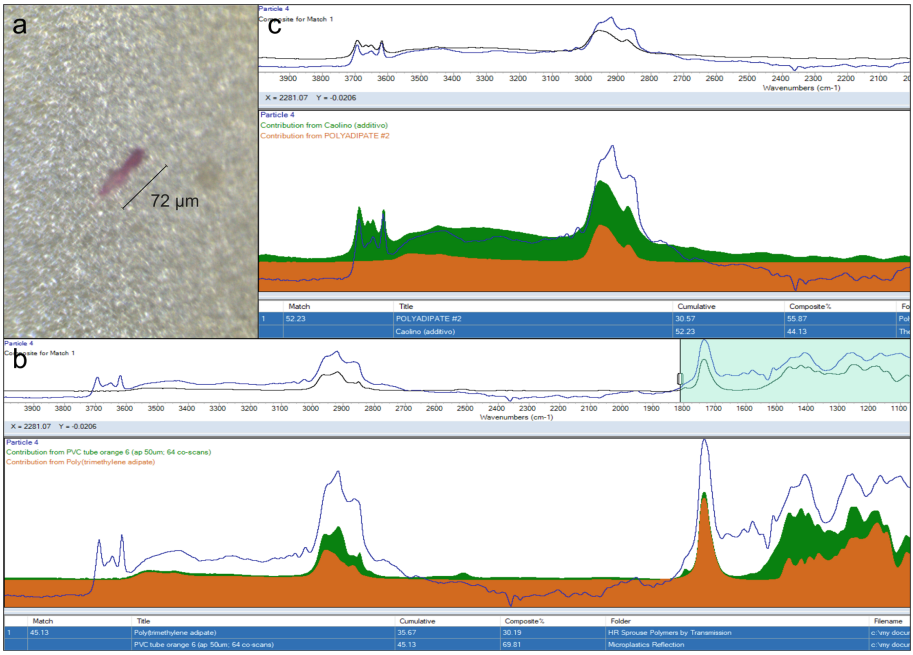

Fig. 2. Particle of filtered water on silver membrane (a), and a section of its respective μ-FTIR spectrum (blue line in box b and c). In box b, the FTIR spectrum of the particle is compared with that of PVC (in black). In the lower part of boxes b and c, the additional contribution of two PVC additives (kaolin and polyadipate) is shown together with the match values of their correlations

Morphological analyses by SEM of the bottle inner wall after the squeezing treatment was performed. No evidence of micro-breaks and wear particles were observed (Fig. 3), revealing the PET resistance towards the treatment procedure.

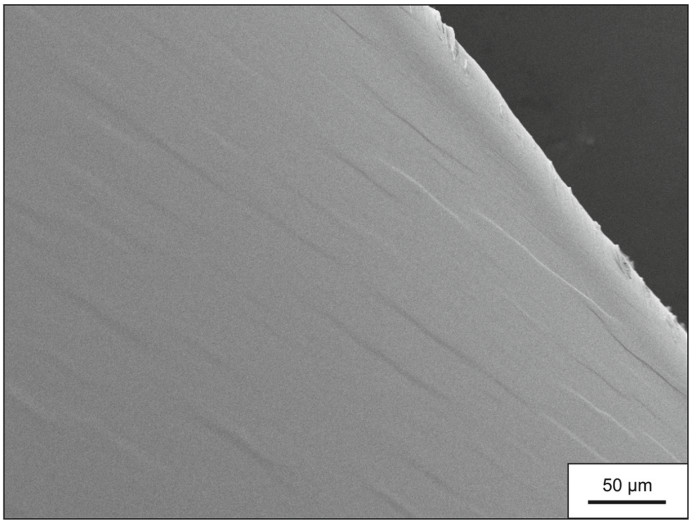

Fig. 3. SEM image of bottle inner wall surface after 10 min squeezing/crushing treatment showing no evidence of breaks or abrasion

3.2 Discussion

The presence of MPs in water from single-use PET bottled was already considered in literature. However, a comparison of MP content in bottled water with results from these studies is difficult due to the different experimental setup and targeted plastic polymer. Both applied analytical methodologies found MPs in the water samples. SEM-EDX has the advantage of delivering high-resolution images which enables quantification and detection of even smaller particles on a surface. EDX analysis is suitable for detecting PET particles and allowed us to perform this MP release study, but cannot distinguish other polymers well. Nevertheless, one of the two detected PET particles was only 3.3 µm in size and could not have been identified by applied FTIR as the limit was >10 µm. The use of a non-metallic filter would have lowered analysable particle size. All MPs found by FTIR were from water of bottles treated for 10 min. However, a correlation of MP concentration and treatment could not be determined, as no PET particle were amongst the MPs detected via FTIR and as the morphological analysis of bottle inner wall (PET surface) showed no signs of abrasion. Interestingly, FTIR analysis of the particle identified as PVC suggested the presence of two additives: kaolin, which is a common additive for this polymer and can be used to increase resistance [7], and polyadipate, which is a plasticizer commonly used for PVC films [8].

Since SEM-EDX analysed area of filters was very small, we prepared a third set of water samples and filtered them on the same silver membranes applied for FTIR analysis in order to obtain a better ratio between the SEM analysed area and the filtered one. With this silver membrane filter (0.8 µm pore size) we obtained an analysed area/filter area ratio of 5.54%. The result reflected those of the cellulose filter; no significant differences in particle number existed between brands and treatment. Out of

curiosity, EDX was performed on 50 particles on the filter from water of bottles from Brand 3 treated for 10 min. Four MP particles were found. Three particles showed a C: O ratio corresponding to that of PET (Fig. 4a and b). One particle showed an elemental composition of 100% carbon, indicating polymeric origin, such as polyethylene (PE, in its high-density form HDPE or low-density form LDPE), polypropylene (PP) or polystyrene (PS), but could not be further specified since these polymers consist of no other elements besides carbon and hydrogen to distinguish them from each other.

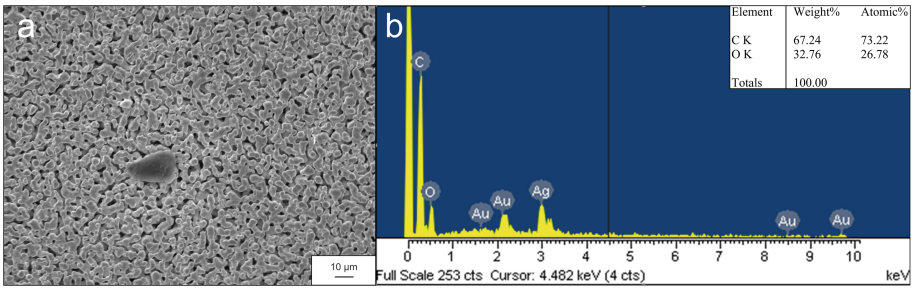

Fig. 4. SEM image of PET particle from filtered water on silver membrane (a) and its respective EDX spectrum and elemental composition of the polymer PET (b). Element Au (gold) derives from the gold cover and Ag (silver) from the silver membrane

4 Conclusions

The comparison of the two methodologies for the analysis of MPs in water samples highlighted their advantages and disadvantages: SEM-EDX is able to lower the size of detected particles, while FTIR spectroscopy gives a better identification and characterisation of the polymer. Micro-FTIR analysis appears to be suitable also for revealing the presence of additives added to the plastic polymer.

A crucial factor is the size resolution, as the majority of found particles were of small size. Even with SEM-EDX, there are several analytical problems for analysing them. A problematic issue rising from this work, even if preliminary, is the difference between the plastic polymers found by the two methodologies in the same water samples. An essential point is the analysed area of the filter for assessing both the particle number and their composition. In fact, by analysing a small fraction of the filter most of the particles are missed out. Therefore, different plastic polymers can be found repeating the analysis also with the same methodology.

Results of the morphological analysis of bottle inner wall after the treatment goes in line with the findings of particle counting in filtered water; PET bottles did not release MP particles into the water after mechanical stress.

In conclusion, the two methodologies are rather complementary than alternatives. Using them in tandem, they can give different information useful for confirming polymer identification in water samples.

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Yellowness Index Determination Using a Mobile App

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1 Introduction

Microplastics are one of the most abundant category of marine rubbish, and given the pervasiveness and persistence of the material, they are a growing threat to marine organisms and ecosystems [1], with very much higher concentrations of POPs in them than in the surrounding waters [2].

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 [1, 3]. 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) [4–6].

These microplastics can be present in marine environment for long periods of time, being transported thousands of kilometers before reaching the coasts [7]. 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) (%) [1], 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.

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2 Experimental

2.1 Materials

The pellets studied were collected with gloves and metal pincers [8] in different beaches from the inter-tidal zone on the coasts in Canary Islands, previously cleaned.

An infrared spectroscopy FTIR 6300 brand *Agilent Technologies*®, and a video spectrocomparator VSC5000 brand *Foster+Freeman*. Moreover of the app *Pantone*® *Studio* on the mobile.

2.2 Methods

Visually, yellowness is associated with scorching, soiling, and general product degradation [9–11]. According to ASTM D 1925-70 or E313-15el, Yellowness Index (YI) is a mathematical expression that allows to quantify the colorimetry in solids.

The color is quantified using coordinates in a three-dimensional colour space proposed by CIE (Fig. 1) where the vertical axis (L) corresponds to the clarity of the colour, the position of the colour on a two-dimensional surface is defined by "a" and "b", where the gray corresponds "a" = 0 and "b" = 0 [12].

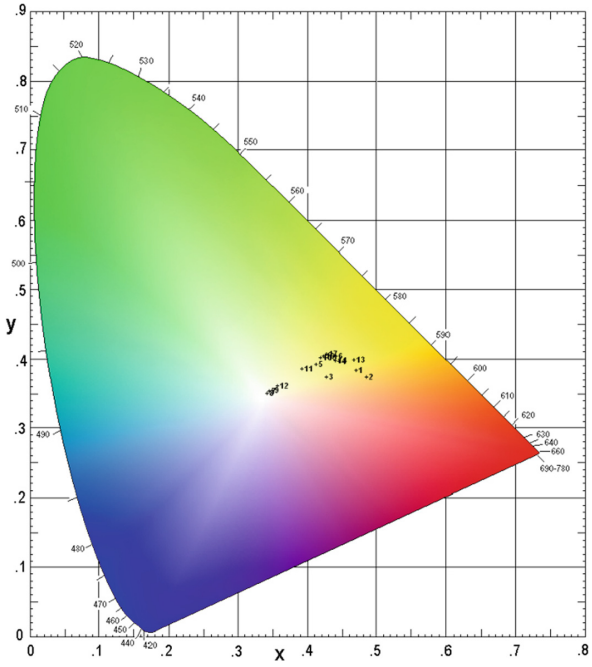

Fig. 1. Example of colour space proposed by CIE 1931 with some samples studied pellets

The CIE L a b represents the Cartesian coordinates of the colours in space, where L is the luminosity and is separated from the chromatic component of colour such as hue, saturation.

L varies from the darkest black to the lightest white, taking values from 0 to 100 respectively. The chromatic components are determined by two Cartesian coordinates a and b. It is described in the range from green (−a) to red (+a) and from blue (−b) to yellow (+b) [13].

Yellowness Index (YI E313) according to the method ASTM E131, it is calculated [9]:

$$YI\,E313(\%) = 100\frac{C_X X - C_Z Z}{Y}$$

The values are the following (Table 1):

Table 1. [9]

Coefficient	C/2º	D65/2º	C/10º	D65/10º
C_X	1.2769	1.2985	1.2871	1.3013
C_Z	1.0592	1.1335	1.0781	1.1498

Where, X, Y and Z are values of CIE Tristimulus and the coefficients depend on the luminosity [9]. The values of X, Y, Z are the perfect reflective diffusion (or clean air) of the combination of observation/luminosity;

$$X = \frac{Y}{y}x$$

$$Z = \frac{Y}{y}(1 - x - y)$$

x, y = The luminosity factor and the chromatic coordinates of the sample [11]. Y = luminance [14].

3 Results and Discussion

3.1 Results

Were studied 120 pellet samples, which were identified as polyethylene by FTIR. After, were studied on VSC5000 to know the Yellowness index. The results were between 10 y 140% de YI, this was related to the Pantone value.

The pellets with the same Pantone values showed a similar YI level, due to this, a reference scale was created as a laboratory tool to determine YI values easily without the need for special equipment.

4 Conclusions

This study shows an easy way to identify the YI level by comparing the known pantone through a mobile application with the YI scale obtained in this study. Simplifying the analysis of microplastics and offering an alternative to the quantification of the YI.

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Cigarette Butts as a Source of Microfibers to the Environment

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1 Introduction

Several clean-up campaigns have reported that cigarette butts (CB for now on) are the largest item littered worldwide [1]. In 2007, the annual global consumption of cigarettes ascended to 6 trillion [2–4], from where approximately 4.5 trillion CBs were carelessly dumped into the environment [5, 6]. Hence, CBs constitute a worldwide and severe toxic litter disposal problem [7]. Yet, little attention is put onto this pollutant.

Cigarette filters (CF for now on) are composed of >12,000 fibers of cellulose acetate. In 2008, a production of 690,000 tons of cellulose acetate filter tows was reached [8]. This material is obtained from reacting cellulose with acetic anhydride and acetic acid. Specifically, the filters are made from cellulose 2–2.5 acetate, which is soluble in organic compounds (e.g., acetone) [9]. This material has a slow degradation rate and can remain in the environment for several years [10–12]. In addition, CBs might suffer a quick release of the fibers and their subsequent fragmentation into smaller and easily ingestible microfibers [6]. On the other hand, cigarette carry more than 4,800 chemical compounds with at least 70 carcinogens and over 200 toxic to humans and to the environment [13]. A proportion of these compounds is adsorbed by the CFs when the smoking process takes place, whereupon some will be leached into the environment and the other will remain in the fibers for an indefinite time [14]. Hence, CBs act as vectors for many hazardous substances too. In this way, microfibers (MF from now on) from CBs are an environmental pollutant that must receive further interest.

In this paper, the releasing rate of MFs from CBs was determined in stirred water. Also, the degradation of the CBs was evaluated with respect to polyester and cotton by applying the Fenton Reaction. Furthermore, specific impacts of MFs from CBs were measured on Daphnia Magna. In this way, CBs were demonstrated to be an important source of persistent and toxic MFs to the environment.

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2 Experimental

2.1 Microfiber Detachment

Cigarette packs from different commercial brands were purchased. These were artificially smoked with a device regulated for a consumption rate of 3 min per cigarette. Afterward, the wrapping papers were removed from the CBs to avoid interferences in the MFs measurement. The CBs were placed into slightly stirred water (1 CB per liter) in order to simulate the movement of natural water bodies. Subsequently, the MFs release was evaluated by counting them in a 10 mL aliquot of sample. In addition, the filters were dried and weighed before and after the experiment to estimate the loss of mass.

2.2 Cellulose Acetate Degradation

Identical masses (0.1 g) of cigarette filters and textile fabrics (100% polyester and cotton) were submitted to a modified NOAA oxidation process, [15] which was proposed for the identification of MPs. The oxidation takes place by applying the Fenton Reaction (20 mL of Fe(II) 0.5 M and 20 mL of H_2O_2 at 30%) at 75 °C. The experiment consisted in submitting 3 series of each material to the reaction. The 1st series were introduced for 15 min of reaction. The 2nd series were treated equally but adding 20 mL of H_2O_2 in minute 15 and maintaining the temperature for 5 min more. The 3rd series were treated equally than the 2nd but adding 20 mL of H_2O_2 in minute 20 and maintaining the temperature for 5 min more. The experiments were run by triplicate. The filter samples were dried and weighed before and after the reaction to measure the lost mass.

2.3 Impacts on Daphnia Magna

A sample of 1 CB per liter of water, without wrapping paper, was continuously stirred for 24 h. Afterward, aliquot samples with concentrations of 10, 4.8, 1, 0.48, 0.1, 0.048 and 0.01 cigarette butts per liter were taken (4 replicates). For each replicate, 2 sets of experiments were conducted: one with the leachate of the CBs and MFs, while the other was filtered and left only with the leachate of the CBs. The *Daphtoxkit F Magna* bioassay was used to evaluate the specific impact that the MFs from cigarette butts might generate on Daphnia Magna. The observations were made 48 h after exposure.

3 Results and Discussion

3.1 Microfiber Detachment

Regarding the MFs detachment from CBs, it was found that **within 15 days**, a CB with low wave action applied onto them can lose about **10% of its mass** in small microfibers (0.5 mm). These MFs were also visually counted by using a stereomicroscope. In this way, more than **100 MFs** can be detached **per CB per day**. However, the detachment

was seen to slow down with time. Nevertheless, it is expected that over time the entire CB will eventually separate and break down into small MFs.

3.2 Cellulose Acetate Degradation

The Fenton Reaction is one of the Advanced Oxidation Processes (AOPs), and it can be used to oxidize the vast majority of organic matter into carbon dioxide [16–19]. Hence, this reaction was selected to create an extreme condition of accelerated oxidation. The degradation of cellulose acetate shows a peak at the beginning of the reaction, but then it reaches a plateau (see Fig. 1). This strengthens the findings reported by previous studies about the low degradability of this material [20]. In addition, from Fig. 1 it can be stated that the polyester samples did not suffer any changes, while the cotton showed continuous and constant degradation.

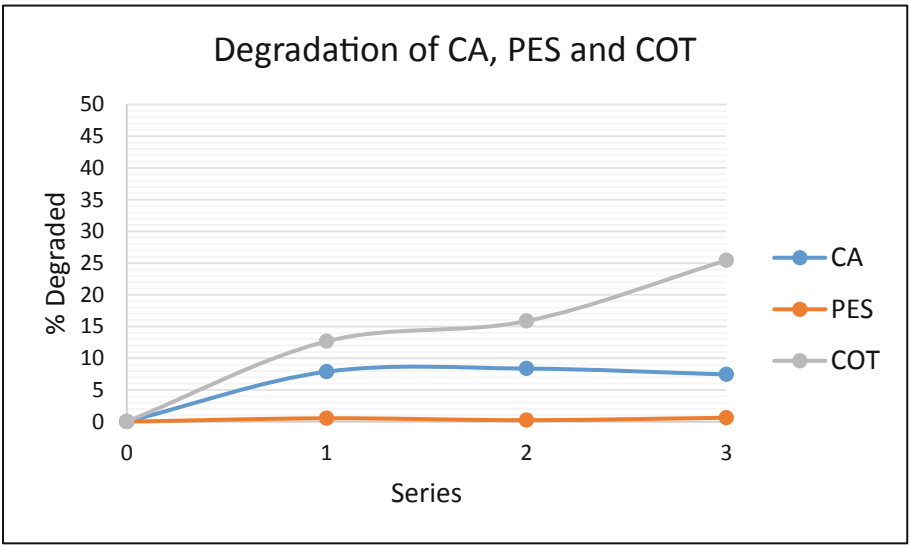

Fig. 1. Degradation of cellulose acetate (CA), polyester (PES) and cotton (COT) when submitted to the Fenton Reaction. Series 1, 15 min of reaction. Series 2 and 3, progressive addition of reactant (H_2O_2) in minutes 15 and 20, respectively.

In this way, the MFs released from a cigarette butt will probably remain in the environment for a few decades [12]. Henceforth, one of the main features of a microplastic, the slow degradation rate, is also exhibited by the MFs detached from cigarette filters.

3.3 Impacts on Daphnia Magna

The effects of the cigarette butt's leachate, with and without the detached MFs, were measured on Daphnia Magna. As explained before, one set of the experiment was

conducted with the filtered leachate (blue line in Fig. 2), and the other with the leachate containing the MFs detached from the CB (green line in Fig. 2).

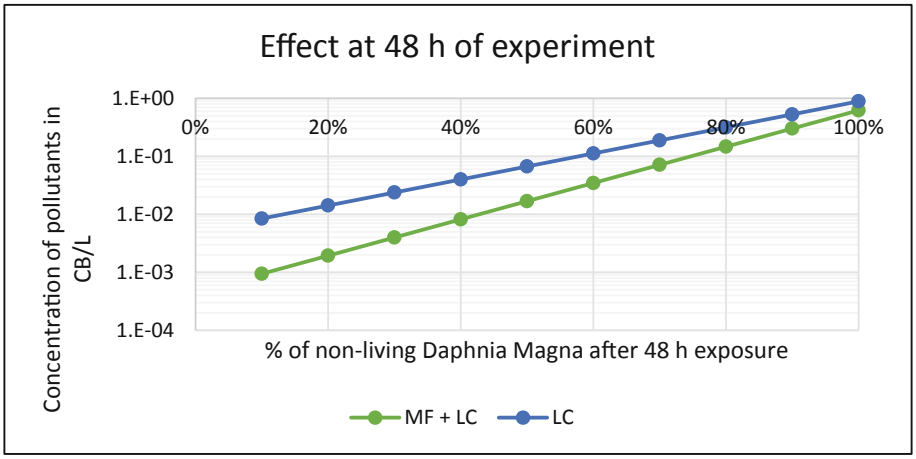

Fig. 2. Percentage of non-living Daphnia Magna after 48 h exposure to different concentrations (y-axis, logarithmic scale) of leachate from CBs (blue) and leachate plus MFs from CBs (green).

As seen in Fig. 2, the addition of MFs to water polluted by CBs' leachate enhances its negative impacts on Daphnia Magna. Moreover, at lower concentrations, a boost in the impacts can be seen when there are MFs polluting the water.

4 Conclusions

The microfiber detachment, the impacts and the degradation rates of the cigarette butts were evaluated. Regarding the detachment of microfibers to water environments, it was found that only one filter can release more than 100 microfibers per day or 10% of their initial mass in the first 15 days. Although this releasing slow down with time, it is expected that finally a total separation and break down of the 12,000 fibers that compose a cigarette filter will occur.

In relation to the impacts, it was found that when the microfibers are present, the effects on Daphnia Magna organisms were augmented up to an order of magnitude with respect to water contaminated by CBs' leachate only. Meaning that the microfibers not only release toxic compounds but also pose an intrinsic risk.

Finally, the degradation of identical masses of polyester, cotton, and cigarette filters were compared by submitting them into extreme conditions (Fenton Reaction). It was found that the acetate of cellulose than constitute the filters suffers an initial degradation (of the order of 10% of its mass) that is quickly halted. On the other hand, cotton gets degraded until fully disappears, while polyester remains unaltered.

In conclusion, microfibers detached from cigarette butts are a last-longing and common litter that can cause similar impacts than synthetic microfibers. They fulfill all but one of the features of a microplastic, the "synthetic" origin. These particles are not contemplated under the definition of a microplastic, and scarce attention has been put onto them. However, microfibers from cigarette butts should not be neglected, as their impacts on the environment are a relevant subject that must receive concern.

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Modified Hyper-crosslinked Resins for Textile Wastewater Treatment

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1 Introduction

Nowadays, textile industry is widely recognized as an important contributor to environmental pollution, for different reasons. Textile production processes require high consumption of water and energy, usage of harmful chemicals, and generate a lot of waste [1]. In the last decade, it was also discovered that not only the production, but also the usage of textiles, has a role in the overall environmental impact of this industry. In fact, the washing processes of synthetic textiles cause the release of microplastics in aquatic environments [2] in quantities that strongly depend on washing conditions and textile characteristics [3, 4]. From the production to the washing of textiles, not only microplastics are released in the wastewater, but also chemicals like synthetic dyes. Recent works report that consumption of textile dyes worldwide is more than 10,000 t/year and approximately 100 t/year of dyes are discharged into water streams [5]. These substances are of the highest concern, since they are relatively stable and therefore difficult to degrade in the currently available wastewater treatment plants based on physical, chemical and biological treatments [6].

Indeed, among the several methods to treat dye-containing wastewater, such as adsorption/absorption, flocculation, electrolysis, and biodegradation, adsorption is preferred for the treatment of polluted water for its cheapness, simple design and versatility. When designing an effective adsorbent, it is of primary importance to improve its adsorption capacity and selectivity to the target pollutants. Among the most investigated adsorbents, hyper-crosslinked resins (HCLR) are very relevant. They are high surface area materials characterized by the extensive crosslinking of a precursor polymer. High specific surface area (SSA) and adsorption properties of HCLR are combined to uncommon chemical an thermal stability, conferred to them by the elevated hyper-crosslinking degree [7]. Furthermore, they are particularly interesting for the wide possibility of tailoring their porosity and adsorption properties through different synthetic procedures [8, 9]. These materials have been successfully embedded in different macroporous matrices in order to obtain hierarchical porous systems with enhanced adsorption properties [7, 10].

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In this work, in order to increase the adsorption properties of HCLR towards polar organic compounds, a new route has been exploited to functionalize vinylbenzyl chloride (VBC) - divinylbenzene (DVB) based HCLR by introducing amino groups into the hyper-crosslinked aromatic structure. The as-obtained amino-modified HCLR have been tested for decontamination of water from Indigo Carmine, used as model polar dye.

2 Experimental

2.1 Materials

Vinylbenzyl chloride (VBC, $\geq$95.0%, mixture of isomers, $\sim$70% meta + $\sim$30% para), p-divinylbenzene (DVB, 85%, meta isomer $\sim$10 wt %), 2,2′-azobis (2-methylpropionitrile) (AIBN, >98%), $FeCl_3$ ($\geq$97%), nitric acid (ACS reagent, 70%), sulfuric acid (ACS reagent, 95.0–98.0%), NaOH ($\geq$97.0%, pellets), stannous chloride (reagent grade, 98%), hydrochloric acid (ACS reagent, 37%), indigo carmine (IC, dye content $\geq$80%) and all solvents were purchased by Sigma-Aldrich (Milan, Italy) and used without further purification.

2.2 Methods

2.2.1 Synthesis of the Hyper-crosslinked Resin

Hyper-crosslinked poly (VBC-DVB) was synthesized through a reported procedure [7]. DVB and VBC were mixed, under nitrogen, in the 2/98 molar composition, then AIBN was added and the mixture was brought to 80 °C and kept at this temperature for 24 h. The obtained precursor resin (named DV) was purified from the unreacted reagents and then underwent Friedel-Crafts reaction to obtain the hyper-crosslinked polymer named XDV.

2.2.2 Amino-Modification of the Hyper-crosslinked Resin

A mixture of $HNO_3/H_2SO_4/H_2O$ in the 75/20/5 volumetric composition was added to a round-bottomed flask, under nitrogen, in an ice bath. Then, XDV was added and the reaction mixture was kept stirring for 1 h. After that, the mixture was poured slowly to a 10 M NaOH solution. The product was filtered and washed with distilled water until neutrality and dried in a vacuum oven at 80 °C.

Then, the NO_2-modified resin (XDV-NO_2) was subjected to a reduction reaction [11] with $SnCl_2$ in HCl/ethanol solution (1/1 vol/vol). The reaction mixture was stirred for 2 h, under nitrogen, at 60 °C. The product was washed with a diluted H_2SO_4 solution, neutralized with distilled water and dried under vacuum at 80 °C. The amino modified resin was coded XDV-NH_2.

2.2.3 Characterization of the Amino-Modified Hyper-crosslinked Resin

In order to confirm the functionalization, XDV, XDV-NO_2 and XDV-NH_2 were analysed by means of Fourier transform infrared spectroscopy (FTIR) in attenuated total reflectance (ATR) mode.

2.2.4 Adsorption Tests

Batch adsorption tests of IC from water were performed on XDV and XDV-NH_2, at 25 °C. IC water solutions in concentrations from 25 to 200 mg/L were prepared; then, about 10 mg of hyper-crosslinked resins were introduced in vials containing 10 mL of IC solutions and, after equilibrium was reached, the IC solution concentration was evaluated through UV-vis spectroscopy using a previously recorded calibration curve. Measurements were performed on a Jasco V570 UV spectrophotometer.

3 Results and Discussion

3.1 Results

A DVB/VBC-based hyper-crosslinked resin was functionalized by inserting amino-functional groups on the highly crosslinked aromatic network by an optimized procedure based on two steps of reaction, one consisting of a nitration of the hyper-crosslinked resin, the second consisting in the reduction of the nitrated resin. The products of the reactions were analyzed by means of FTIR analysis, and compared to the pristine hyper-crosslinked material, XDV. FTIR spectra, reported in Fig. 1, show, after nitration, the insurgence of the characteristic N-O absorption bands (asymmetrical and symmetrical stretching vibration modes, 1530 cm^{-1} and 1350 cm^{-1}) and of the aromatic C-N absorption band (stretching vibration mode, 1277 cm^{-1}) [12]. After reduction, the N-H absorption bands (stretching and scissoring vibration modes, 3442–3360 cm^{-1} and 1603 cm^{-1}) and the aromatic and aliphatic C-N absorption bands (stretching vibration modes, 1277 cm^{-1}, 1166 cm^{-1} and 1049 cm^{-1}) arise in the XDV-NH_2 spectrum [13].

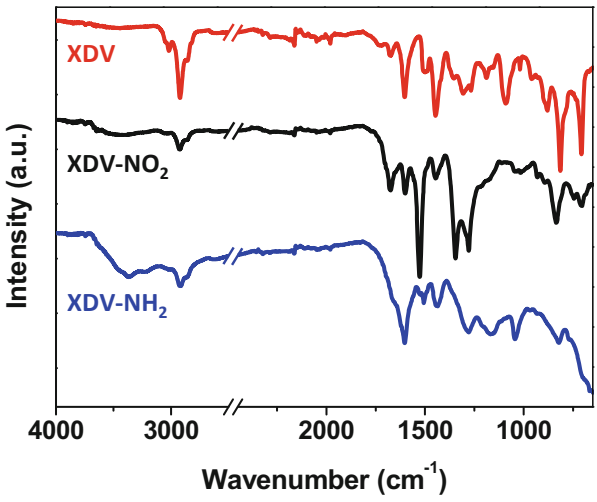

Fig. 1. FTIR spectra of XDV, XDV-NO_2 and XDV-NH_2

XDV-NH_2 show highly enhanced adsorption capacity towards indigo carmine, reaching the 100% of dye removal for IC concentrations up to 50 mg/L, and more than tripled adsorption with respect to XDV capacity at higher concentrations (see Fig. 2).

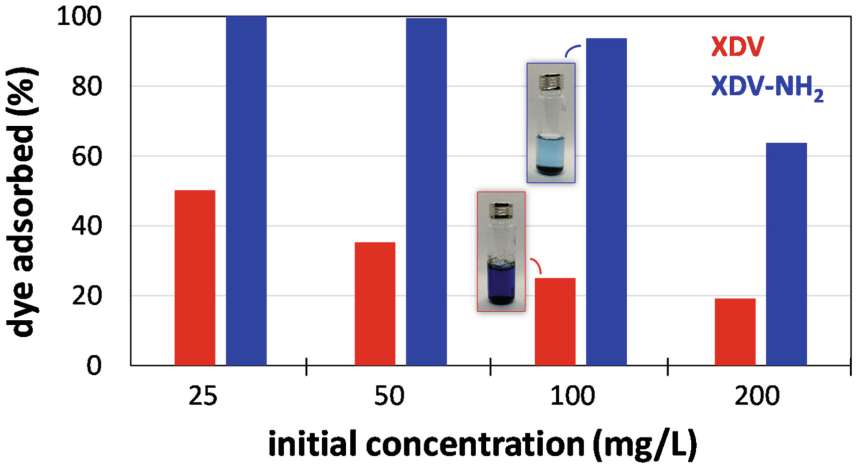

Fig. 2. IC equilibrium adsorption capacity of XDV and XDV-NH_2 (inset photos show the test vials containing the 100 mg/L IC solution and the adsorbents after adsorption)

3.2 Discussion

A general approach to enhance hyper-crosslinked resins affinity towards specific pollutants is their chemical functionalization, either using previously modified precursor monomers/polymers or by post-functionalization of the hyper-crosslinked product. With the first approach, for example, we grafted ethanolamine on the DVB-VBC precursor resin, and exploited the remaining chloromethyl groups for hyper-crosslinking [10]. This approach led to a significant lower extent of crosslinking in the final product, and therefore to the obtainment of a lower surface area material. Nevertheless, the hydrophilic nature of the ethanolamine-functionalized resin resulted crucial for the enhanced adsorption of phenol from water and for CO_2/N_2 selective adsorption. Similarly, also the amino-functionalization of the hyper-crosslinked polymers usually leads to a reduction of the resin SSA. For example, Xu et al. nitrosation and reduction on hyper-crosslinked styrene-based beads led to a reduction of about 10% of the resin SSA and a 30% improvement of the resin adsorption capacity towards nitroaromatic compounds [12]. Wang et al. also synthesized amino-modified hyper-crosslinked resins which displayed decreasing SSA with increasing the degree of functionalization and they also demonstrated the importance of the hyper-crosslinked resins functionalization in enhancing a specific pollutant adsorption [14].

The nitration-reduction process adopted in this work led to obtain an amino-modified hyper-crosslinked resin that shows much-enhanced adsorption capacity towards polar organic pollutants with respect to the neat resin, as demonstrated for indigo carmine. The effect of the extent of functionalization of XDV-NH_2 on the SSA of the resin is under

investigation and will be the subject of a further work. Nevertheless, the results obtained so far demonstrate that XDV-NH_2 show a dramatically enhanced adsorption capacity towards the selected model dye that can be ascribed to the ion-dipole interaction between the adsorbate and the polar functional groups of the resin.

4 Conclusions

A new functionalization route to insert $-NH_2$ functionalities on a styrene-based hyper-crosslinked resin was explored. The $-NH_2$ modified HCLR showed significant enhanced adsorption of Indigo Carmine from water solution, reaching complete dye removal at 25 and 50 mg/L concentrations. This improvement in the adsorption properties demonstrated the efficiency of the functionalization for the removal of polar dyes from water.

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First Investigation of Microfibre Release from the Washing of Laminated Fabrics for Outdoor Apparel

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1 Introduction

The washing process of synthetic textiles represents one of the major source of microplastic pollution in world oceans [1]. It has been estimated that millions of microfibres can be released by a single wash, depending on the detergent used, washing conditons and textile characteristics [2, 3]. Wastewater treatment plants are not completely effective in blocking microplastics and, considering the daily high volumes discharged, microfibres can actually reach aquatic environments [4]. In fact, microfibres were found in beaches worldwide, in the water of Pacific Ocean, North Sea, Atlantic Ocean and even in the Artic and in deep sea sediments [5]. Moreover, they were also found in fishes and shellfish on sale for human consumption, sampled from markets in Makassar, Indonesia, and from California, USA [6].

From 2000 to 2014, the number of garments purchased each year by the average consumer increased by 60%, mainly as a consequence of low prices and easy access to fashion [7]. In parallel, the request for high-tech functional fabrics has been increasing for the application in outdoor apparel.

Combining this information, unless mitigation and prevention measures will be soon adopted, it is likely that the amount of microplastics released by the washing of synthetic textiles will increase dramatically in the next years. An effective solution could be the intervention at the very beginning of this source of pollution, that is the textile itself. Several works have already investigated which textile characteristics may influence the release [2, 3, 8, 9]. Such investigation is of critical importance to provide indications to the textile industry on the ways to mitigate the environmental impact of their products. The present work is the first to investigate the peculiar structure of functional fabrics applied in outdoor apparel. The main properties of these fabrics are waterproofness, windproofness, breathability and climate-regulating performances.

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These properties are obtained through the combination of high performance materials in a laminate structure.

For this purpose, washing tests at lab scale were performed on several types of laminated fabrics, which differ for raw materials used, textile construction and characteristics. For each type of laminated fabric, samples obtained from different steps of the laminate production were tested, from raw materials to laminates with durable water repellent (DWR) treatments.

2 Experimental

2.1 Materials

N. 6 laminates produced by Sympatex Technologies GmbH were analysed, all of them made of 100% polyester. Each laminate was composed by a raw material for the face (F), a monolithic copolyester-based membrane and, for some laminates, also by a raw material for the backing (B). In addition to the samples of the different raw materials (F and B), the experimental tests also considered samples coming from different processing steps: laminate with PFC-free durable water repellent (DWR) treatment (C0), laminate with C6-based DWR treatment (C6). The tested samples are summarized in Table 1.

Table 1. Summary of the tested samples

Laminate code	Raw materials	Processing steps
L1	F1	C0, C6
L2	F1r	C0, C6
L3	F1, B1	C6
L4	F1, B2	C6
L5	F2	C0
L6	F2r	C0

F1r and F2r are the counterparts with recycled fabric of F1 and F2, respectively. F1 and F1r have the same twill weave structure, whereas F2 and F2r are plain weave fabrics. B1 is composed by fleece fabric and B2 is warp knitted.

2.2 Washing Tests and Evaluation of Microplastic Release

The experimental protocol used to quantify the microplastics released by the different samples, is described in precedent works [2, 10]. In brief, simulations of domestic washing processes were performed using the standard machine Gyrowash (James H. Heal & Co, UK) and a commercial detergent dosed as indicated on the label. The

washing effluents were filtered on 5 μm pore size filters, that were observed by a field-emission scanning electron microscope (SEM) to quantify the number of microfibres released. Each test was carried out in four replicates (n = 4), so the average number of microplastics released per gram of washed fabric (N_a) was calculated for each type of sample tested, along with the standard deviation (SD). Statistical analysis on the number of microfibres released by the raw materials was carried out by using IBM® SPSS® Statistics software. One-way Analysis of Variance (ANOVA) with a Student–Newman–Keuls (SNK) post hoc test was performed to assess significant differences at a 5% significance level.

3 Results and Discussion

Starting from the analysis of the raw materials, their releases are summarized in Fig. 1. It is clear that the raw material B1, used for the backing of L3, is responsible for the greatest release of microfibres, whereas F2r, used for the face of L6, released the lowest amount of microfibres. compared to all the other materials tested. The behavior of B1 may be due to its fleece structure that is characterized by fibers that could more easily slip away from the fabric during washing. The recycled fabric F2r released less than its counterpart F2, but the same trend did not occur for F1 and F1r that released quantities in the same range. Therefore, more investigations are needed to better understand the

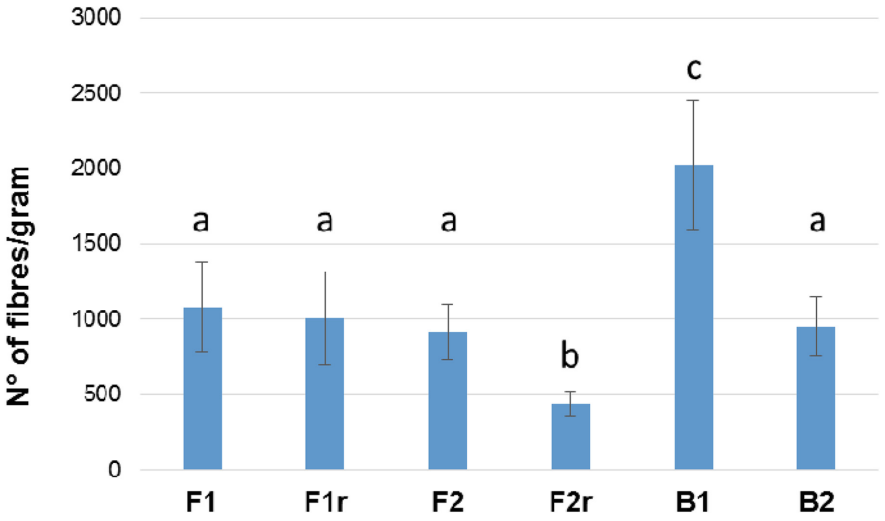

Fig. 1. Number of microfibers ($N_a \pm SD$) released per gram of washed fabric of the raw materials (n = 4). Different letters (a, b, c) indicate significant difference among the groups of data.

influence of recycled fabrics on the release.

Figure 2 summarizes the results obtained in terms of number of microfibres released per gram of washed laminated fabric. In general, it appears clear that lamination was able to reduce the amount of microfibres released. Moreover, such reduction seems independent from the type of DWR treatment applied, since the releases of C0 and C6 samples are very close both for laminate L1 and laminate L2. The laminate L1 released the lowest amount of microfibres, whereas the laminate L3 is the one responsible of the greatest release. Such trend is quite surely due to the raw material B1 used as backing of L3, whose usage should be avoided in order to decrease the release of microfibres. Regarding the laminates with recycled fabrics, L2 released slightly more micofibres than its non-recycled counterpart L1; whereas L5 and its recycled counterpart L6 released a very close amount of microfibres even if the raw material F2r of L6 released much less than the F2 used for L5. Comparing the releases of L1, L2, L5 and L6, no particular trends can be observed and lamination rather than recycling seems to have the main mitigation effect on the releases. Once again, as already observed for the raw materials, the investigation of the release of microfibres from the laminates did not provide conclusive information on the influence of recycled fabrics

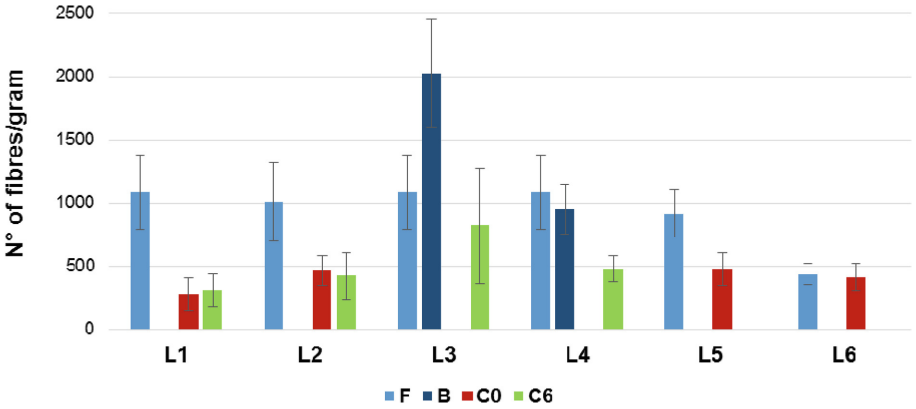

Fig. 2. Number of microfibers ($N_a \pm SD$) released per gram of washed laminated fabric for the samples analysed (n = 4).

on the release.

4 Conclusions

The outcomes of this work allowed to identify trends in the microfibres release from laminated fabrics, pointing out which factors in the choice of the materials and production may have an influence on the release. In any case, the assembling of fabrics in a laminated structure reduce the amount of microfibres released, regardless of the type of

raw material used. The choice of raw materials with a fleece structure should be avoided in order to reduce the amount of microfibres released. Such results are of striking importance to provide mitigation solutions of microfibres pollution to the textile industry.

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Oil Extraction as Separation Method for Microplastic in Sediment Samples

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1 Introduction

Microplastic (MP), plastic particles smaller than 5 mm [1], is widely distributed in the environment [2] and has not only been detected in the aquatic environment [3] but also in terrestrial areas [4]. To understand the whole life cycle of MP it is important to analyse samples from different areas. With the detection of MP in different environmental compartments, it is possible to define sources, entry and transport paths and temporary and ultimate sinks of MP.

To analyse environmental samples, it is necessary to separate MP and other matrices in a first step. Right now, there is no standardized method to separate MP from different matrices in environmental samples, like water, sediment or wastewater [5–7]. Especially for sediments it is an important preparation step as the sample volume, compared to water samples, cannot easily be concentrated on a filter [8]. An easy and effective method is the separation with oil due to the lipophilic characteristics of plastic [9]. Oil does not have toxic properties, like for example zinc chloride, used for separation, and does not depend on a specific density, what is fundamental for commonly used density separation methods with sodium chloride, sodium iodide, potassium formate or others [8]. Since there are only a few publications about this method [9, 10], what is regarded as disadvantage [8], it is extensively validated in this study by using spiked samples in over 200 experiments.

2 Experimental

Different polymers and types [11], describing shape and consistency (fragment, fibre, pellet, microbead, film, foam, filament, rubber), with different densities were investigated in two ways. Firstly, the experiments were conducted with canola oil added to each polymer type. In a second step, natural sediment was used in addition to the polymer type and canola oil. The used materials and methods are explained in detail in the following part.

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2.1 Materials

Based on a standardised protocol for monitoring MP in sediments [11] where the most common MP types were specified the materials used for validation were chosen. Eight different types and additionally nine different polymers were investigated in eleven test implementations. A list of the polymers and their densities is shown in Table 1. The densities are higher and lower than those of water (ρ = 1000 kg/m^3) and consistently lower than those of sediment (ρ = 2650 kg/m^3) [12]. Additionally, the shapes of the used polymers are shown in Fig. 1.

Table 1. Polymer types and their densities used for validation

Test number	Polymer	Type	Density [kg/m^3]
1	PET (Polyethylene terephthalate)	Fragment	1290
2	PA (Polyamide)	Fibre	1101
3	PA (Polyamide)	Pellet	1110
4	PVA (Polyvinylchloride)	Fibre	1315
5	PE (Polyethylene)	Microbead	913
6	PE (Polyethylene)	Film	917
7	PP (Polypropylene)	Pellet	838
8	PS (Polystyrene)	Pellet	1021
9	EPS (Expanded Polystyrene)	Foam	11
10	Carbon	Filament	1760
11	Synthetic Rubber	Rubber	1051

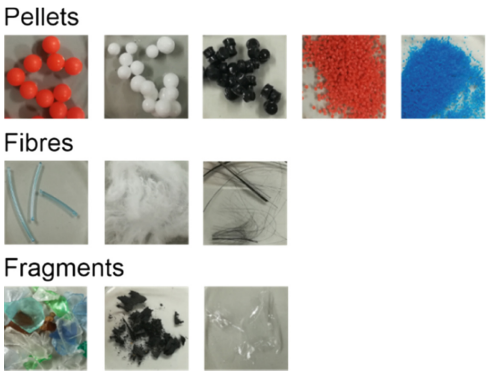

Fig. 1. Shapes of used polymer types

2.2 Methods

The used method is based on a separation unit [13] combined with oil separation [9]. The separation unit was built after Coppock et al. [13] without using any plastic component to prevent cross contamination. It consists of an aluminium tube with an

aluminium gate valve that separates the upper and lower part of the aluminium tube. The tube is based on a plate with a detachable closure so that the apparatus can be cleaned easily afterwards. The whole unit is about 500 mm high (cf. Fig. 2).

To carry out the experiments, ten particles of each material (cf. Table 1) were added with 10 ml canola oil into the tube. It was filled with 1,000 ml distilled water to get the water level higher than the gate valve. The polymers, oil and water were homogenized manually with a steel stirrer over 30 s. After a settling time of 15 min the gate valve was closed to separate the lower part with distilled water and sediment and the upper part with distilled water, oil and the polymers. During settling time, a cover was used on top of the separation unit to prevent cross contamination. Afterwards, the upper layer was decanted in a beaker glass where the particles were counted. With the counted particles in the beaker glass the recovery rate was defined.

If the separation unit is used for normal sediment preparation the upper sample volume can easily be vacuum filtrated on a filter. To prevent an interfering signal during a possible analysis with infrared spectroscopy the filter needs to be rinsed with ethanol [9]. The evaluation of the filter residue can be carried out subsequently without any further intermediate step. This prevents not only cross contamination but also the loss of material. Figure 2 shows the separation unit which was used for the test implementation.

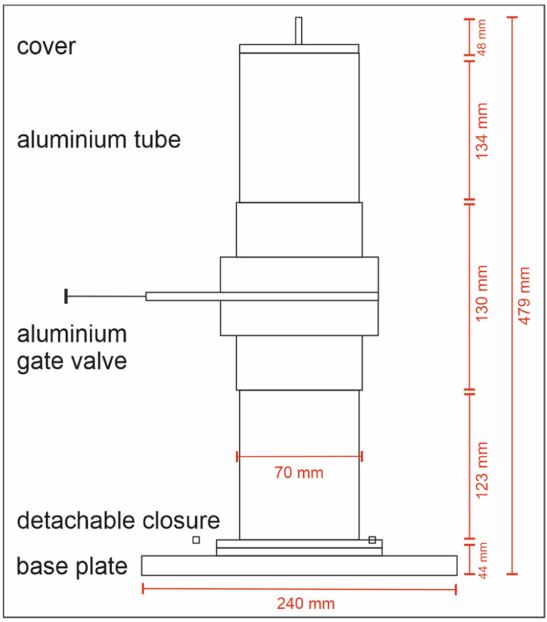

Fig. 2. Separation unit

All investigations were conducted for each test number ten times with

1. **10 ml** canola oil and
2. **10 ml** canola oil and **10 g** fluvial sediment.

The fluvial sediment was sampled before from a regional river catchment area to prove the concept in two approaches. Due to the characteristic appearance of the spiked MP in shape and colour (cf. Fig. 1) it was not necessary to analyse the used natural sediment before concerning a possible MP pollution. Including preliminary tests 290 experiments were conducted. To determine the mean recovery rate 230 experiments were used.

3 Results and Discussion

The mean recovery rate of MP for all investigations is 91.7%, which can be divided into the mean recovery rate of 91.9% for polymers and oil and of 91.5% for polymers in combination with oil and sediment.

Compared to Crichton et al. [9] the recovery of oil is significantly higher than a separation with sodium iodide (83.3%) or calcium chloride (69.0%). Investigations from Mani et al. [10] using castor oil and reaching a recovery rate of 99.0%. This separation method works without the density characteristics of MP. By using density separation the solution need to be prepared for a specific density, like $\rho = 1700\ kg/m^3$ [14]. Therefore, Table 1 shows that a specific density does not cover the densities of all common MP which supports the separation with oil. In addition, the treatment is reduced to one step to separate MP and sediment and is less time-consuming. Finally, MP is not degraded by an oil treatment like it has been reported by alkaline or acidic digestion and has no corrosive or hazardous effect [8].

4 Conclusions

The oil-separation is an easy and quick method to recovery MP and separate sediment and MP particles. With a mean recovery rate of 91.7% it has the highest recovery rate compared to other extraction methods. Furthermore, there are less materials and chemicals needed and it is therefore cost-effective. The lipophilic characteristics makes it possible that all polymers regardless of their density, shape or consistency are detectable. All in all, the sample preparation for analysing sediment of MP is reduced to one treatment step that leads directly to a subsequent evaluation with a microscope or infrared spectroscopy. If the method is also applicable for other matrices, like wastewater, need to be investigated in further research.

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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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1 Introduction

Over the last few decades, plastic contamination has become a major cause of concern among scientists, politicians, and the public. Global annual production of plastic materials currently exceeds 320 million tons, most of which is intended for packaging. It is estimated that between 5 and 13 million tons leaks into the World's oceans every year and can accumulate in both terrestrial and marine environments forming vast areas of plastic debris. Once released, larger materials may be subjected to degradation by solar radiation, mechanical forces, and microbial action, fragmentation and breakdown into microplastics (particles less than 5 mm) and nanoplastics (1–100 nm) [1, 2].

Microplastics are highly persistent in the environment and may be accumulated in different marine ecosystems at increasing rates. The particles can be uptaken by a wide range of marine organisms, and the ingestion is believed to be the main microplastics exposure route for several marine species [3]. Direct consumption of microplastic is prevalent in suspension feeders, including zooplankton, oysters, and mussels, and deposit feeders, owing to their inability to differentiate between microplastics and prey. Predators and detritivores may indirectly ingest plastic through prey (i.e. trophic transfer) containing microplastic. After the ingestion, microplastics absorption, distribution through the circulatory system, and entrance into different tissues and cells can occur [4, 5]. As a consequence, potentially adverse effects may be caused by the particles (e.g. physical damage or reaction to their components) or chemicals added during the particle manufacturing or sorb to the microplastics during their use or permanence in the environment. Moreover, microplastics, as well as the chemicals they contain, can be transferred from marine prey to predators [5, 6].

1.1 Occurrence of Microplastics in Shellfish and Fish Species

Microplastics have been detected in the stomachs of commercially important fish (Table 1) consumed by humans (e.g. Atlantic cod, Atlantic horse mackerel; European

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pilchard, red mullet, European sea bass), and also in bivalves (mussels, oysters), crustaceans (shrimp), and in the gastrointestinal tract and liver of anchovies and sardines that are totally consumed [7]. Bivalves and crustaceans also pose a greater threat to seafood contamination than gutted fish or peeled shrimp. However, the presence of microplastics in the eviscerated flesh (whole fish excluding the viscera and gills) of two commonly consumed dried fish species (Chelon subviridis and Johnius belangerii) was significantly higher than excised organs (viscera and gills), evidencing that the evisceration does not necessarily eliminate the risk of microplastics intake by humans [8]. Microplastics were also found in canned sardines and sprats, salt, beer, honey and sugar (Table 2) [5].

Also in the aquaculture systems, the farmed species can ingest microplastics (e.g. bivalves cultured in estuaries and coastal lagoons) because of the contamination of the water and sediments. Furthermore, when fish, shrimps or other farmed species are fed with feeding materials produced from fish and other animals (e.g. fishmeal), these may be contaminated with microplastics [5, 9].

The trophic transfer of microplastics suggests that these contaminants can be transferred within different food webs (Fig. 1) [5, 10].

Table 1. Summary of studies reporting the occurrence of microplastics in fish of commercial interest. The reported species are included in the list of the most commonly caught marine species worldwide according to FAO, 2016 [5]

Species name	Levels of mp	Size range μm	Parts	Types of debris	Location
Clupea harengus	566; 2%	>1000	Gastrointestinal tract	Fibers, fragments	North Sea
Decapterus macrosoma	17; 29%	>500	Gastrointestinal tract	Fragments, styrofoam	Indonesia eastern from local market
Decapterus muroadsi	20; 80%	5000	Gut	Fragments	South Pacific
Engraulis japonicus	64; 77%	10–500	Gastrointestinal tract	Fragments, bead, filament, foam	Tokyo Bay
Gadus morhua	80; 13%	>1000	Gastrointestinal tract	Fibers, fragments	North Sea
	74; 1.4%	<5000	Gastrointestinal tract	Fibers, fragments, film	Baltic Sea
	205; 2.4%	2800–4200	Gastrointestinal tract	Fragments	Coast of Canada
	302; 18.8%	<5000–>20,000	Stomach	Fibers, fragments, granule, film	Norwegian coast

(*continued*)

Table 1. (*continued*)

Species name	Levels of mp	Size range μm	Parts	Types of debris	Location
Micromesistius poutassou	27; 51.9%	1000–2000	Gastrointestinal tract	Fibers, fragments, beads	English Channel
Sardinella longiceps	10; 60%	500–3000	Gut	Fragments	Indian Coast
Sardina pilchardus	99; 19%	10–5000	Gastrointestinal tract	Fragments, line, film, pellet	Adriatic Sea
Scomberomorus cavalla	8; 62.5%	1000–5000	Stomach	Pellets	Northeastern Brazil
Scomber japonicas	7; 71%	>9.07	Gastrointestinal tract	Fibers, hard plastic, nylon	Mediterranean Sea
	35; 31%	217–4810	Gastrointestinal tract	Fragments, fibers	Portuguese Coast
	30; 3.3%	≤ 2100	Gut	Fragment	Southeast Pacific Ocean
Scomber scombrus	13; 31%	217–4810	Gastrointestinal tract	Fragments, fibers	Portuguese Coast
Sprattus sprattus	515; 18.8%	100–>5000	Gastrointestinal tract	Fibers, fragments	Baltic Sea

Table 2. Occurrence of microplastics in different food items and drinking water [5]

Item	Levels of mp	Size range μm	Types of debris	Location
Beer	24; 100%	Not specified	Fibers, fragments, granules	Germany
	12; 100%	100–5000	Fibers, fragments	USA
Honey	19; 100%	10–20	Fibers, fragments	Germany, France, Italy, Spain, Mexico
Salt	16; 100%	20–5000	Fibers, fragments, films	Turkish
	12; 100%	100–5000	Fibers, fragments	USA
Mineral water	38, 100%	1–500	Fragments	Germany

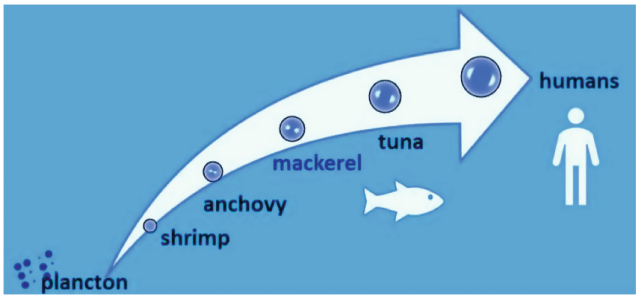

Fig. 1. Microplastics transfer through different trophic levels

2 Microplastics and Human Food Chain

The occurrence in seafood represents a potential route through which microplastics might contaminate human food. The presence of plastic debris has been detected in seafood sold for human consumption, as well as in fish and shellfish purchased from markets. This evidence raises concerns regarding the ingestion of microplastics by humans through the consumption of contaminated marine species, and the potential effects on human health [11].

Plastic particles when ingested may be toxic to organisms due to physical damage caused by small particles adsorbed to membranes and also if they cross the membrane by altering cellular functioning. In the marine environment, microplastics may act as vehicles for chemicals, including those intentionally added during their manufacturing process, as well as environmental contaminants, such as styrene, toxic metals, phthalates, bisphenol A, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) [12]. Additionally, several of the chemicals associated with microplastics may accumulate and biomagnify in marine trophic webs. This increases the risk of toxic effects especially to top predators and humans consuming species contaminated with microplastics or with chemicals released from these particles after their ingestion [13].

In addition, it has been suggested that plastic debris increase the global risk of human and animal diseases via new infection routes and the introduction of pathogens through the environmental spread of microplastics described as the "plastisphere". Microbes and other organisms that have been found on plastic debris, generally are of particular concern such as Vibrio spp., *Escherichia coli*, *Stenotrophomonas maltophilia*, *Bacillus cereus*, and *Aeromonas salmonicida*. Additionally, the "plastisphere" may also include exotic invasive species (pathogens or not) that may contribute to loss of biodiversity [5].

2.1 Implications for Human Health

Adverse effects on human health are still controversial and not well understood. Thus, several important questions remain open, such as if microplastics play a role in the

development of cancer in marine animals and, by extension, in humans; what are the long-term effects of human exposure to microplastics considering the simultaneous exposure to such particles through several routes [14].

Microplastics with size bigger than 150 μm probably will not be absorbed while microplastics smaller than 150 μm may translocate from the gut cavity to the lymph and circulatory system, causing systemic exposure. However, the absorption of these microplastics is expected to be limited ($\leq 0.3\%$). Only microplastics with size ≤ 20 μm would be able to penetrate into organs while the smallest fraction (0.1 > 10 μm) would be able to access all organs, cross cell membranes, the blood-brain barrier and the placenta [1, 15]. Therefore, it is possible that the distribution of microplastics in secondary tissues, such as liver, muscle, and brain, may occur [14]. Micro- and nanoplastic interactions with the immune system may potentially lead to immunotoxicity and consequently trigger adverse effects (i.e. immunosuppression, immune activation, and abnormal inflammatory responses) [5, 14, 15].

3 Experimental

To assess the risk of transfer of microplastics along the marine food chain, microplastics both in benthic and pelagic Tyrrhenian and Adriatic seafood will be researched. In particular, we aim to (1) evaluate differences in microplastics content between the considered fish species related to their feeding strategy; (2) assess relationships between the data related to the marine area of interest and the extent of fish contamination (SiRIMaP-PON project, 2014-2020-4.6 action). The research activities

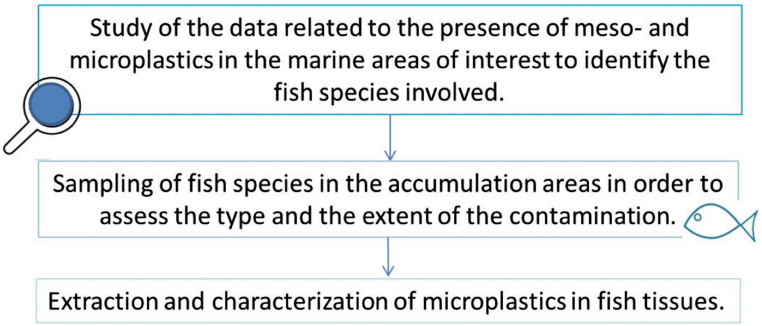

Fig. 2. Survey of fish species contaminated by plastics and microplastics

will be organized according to the flow chart reported below (Fig. 2).

Sampling and analysis are important steps in recording the presence or absence of microplastics, which need to be separated from other organic and inorganic particles materials in the sample prior to being counted, weighed and the polymer type

identified. Spectroscopy can confirm the presence of plastics and provide the polymer composition [16].

4 Discussion

The different feeding behaviors between sea organisms explain their susceptibility to microplastics. Generally, pelagic species ingest more particles, while benthic organisms mostly ingested fibers [7]. As seafood represents one pathway for human microplastic exposure, commercial fishes for human consumption represent one of the most controversial target species concerning marine litter pollution. In particular, bivalves and small fish consumed whole are more likely to expose microplastics to the human diet [17]. Taking in to account an average portions sizes of mussels (225 g, without shells) and the highest number of microplastics detected in mussels (median value 4 particles/g), the consumption of such a portion would lead to ingestion of about 900 plastic particles. In this conservative scenario, the microplastics would lead to ingestion of about 19 pg of PCBs, 170 pg of PAHs and 0.28 μg of bisphenol A. Based on these data the presence of microplastics in seafood would have a small effect on the overall exposure to additives or contaminants [1]. However, uncertainty and variability in the microplastics data represent two of the main limiting factors for an appropriate assessment of their content in the environment and in organisms, and the implications of these findings for humans who consume fish containing microplastics are not yet understood [9, 17].

5 Conclusions

Microplastics pollution in the marine environment is of concern not only because of the ecological impacts but also because may compromise food security, food safety and consequently human health. The presence of microplastics in species intended for human consumption is a global problem and we are vulnerable to microplastic exposure through the consumption of seafood and other human food items, as well as through other routes such as air. Nevertheless, information on the occurrence of microplastics in these products is scarce, the exposure levels are in general largely unknown, and the potential effects on consumers are poorly understood.

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First Observations and Monitoring of Microplastics on Oceanic and Coastal Waters off the Canary Islands (Subtropical NE Atlantic)

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1 Introduction

Broad scale sampling methods for microplastic monitoring in open ocean waters remain a challenge in oceanography. A large amount of samples is required to understand distribution, abundance and fate of microplastic particles in the environment. Despite they have been widely studied now for more than a decade, there is no standardized method that allows to obtain more data in the short term, in a quick, affordable and interoperable way.

The use of pumps as a low-cost system for circulating surface seawater together with an in-line filtration system to prevent contamination has been employed for this purpose in a few studies up to date and stated as a validated and effective technique that allows continuous sampling without interfering the regular activity of the vessel on which it is operated [1–5]. This method, encouraged by experts [6], can be adapted to a variety of monitoring microplastic opportunities from a wide array of platforms.

Bearing in mind the reports emitted by the Technical Subgroup on Marine Litter of the Marine Strategy Framework Directive (MSFD, 2008/56/EC), we have considered the use of a microplastic filtering device and a methodology based on the pump-underway system to provide the first baseline data of microplastic abundance and distribution in subsurface oceanic waters in the area. The Macaronesian Region (Subtropical Northeastern Atlantic) is a geostrategic area where, so far, there is no report of monitoring data for pelagic microplastics.

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2 Experimental

All the samples were collected during three oceanographic campaigns to the European Station for Time Series in the Ocean Canary Islands (ESTOC; 29°0'N, 15º30'W) on 24–26 March 2018 (ESTOC 1803), 6–8 December 2018 (ESTOC 1812) and 21–25 February 2019 (ESTOC1902) commissioned by the Oceanic Platform of the Canary Islands (PLOCAN) on board the oceanographic RV Ángeles Alvariño, from the Spanish Institute of Oceanography (IEO). On each of the three campaigns, two sampling modes are differentiated: samples taken on *stationary mode* (*coastal*, at the PLOCAN Test Site, and *oceanic*, at ESTOC) and samples taken *on navigation*, outlined in Fig. 1.

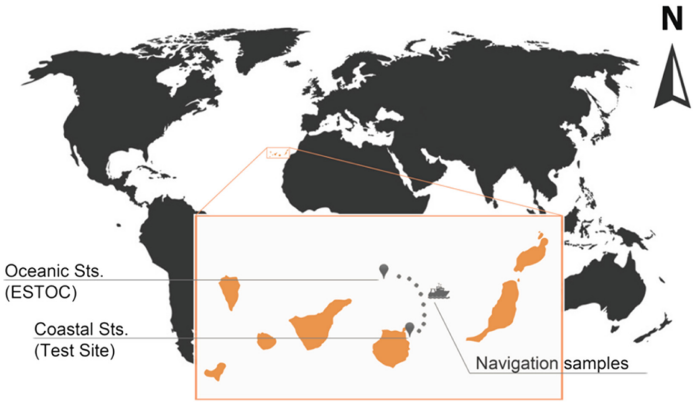

Fig. 1. Map of the sampling locations repeated on the three research cruises to ESTOC Station. Two sampling modes were performed: stationary (at the ESTOC and Test Site Stations) and on navigation (from ESTOC Station to the home port).

2.1 Materials

The microplastic filtering device employed consists of four stacked sieves (⌀ = 100 mm) with mesh sizes of 300, 200, 100 and 50 µm. The design comprises a lid -to prevent airborne contamination- and a flowmeter, to ensure equal volume sampled in the different trials performed and to allow comparison of results among other studies.

2.2 Methods

Using the microplastic filtering device, seawater samples were retrieved at the different sampling stations using the pump-underway ship-intake of the vessel, which takes the water on a constant flow from 4 m depth. The overall sampling time at each station was around 35 ± 11 min for the stationary samples: the time that took the continuous intake to retrieve 251 ± 20 L at an average flow rate of 7 L/min. For navigation samples, the same sampling procedure was followed, retrieving a total of 10472 L in

the four different transects, with a mean sampling volume of 2377 ± 895 L at an average flow rate of 5 L/min. The differences in the volumes retrieved are compensated delivering the results in abundance of microplastics per cubic meter instead of number of particles.

2.2.1 Preparation of Samples

The filtering device and all filter meshes were thoroughly cleaned with micro-filtrated water before every use. At sampling stop, the sieves were carefully withdrawn and the meshes were cleansed into their corresponding labelled container. A dress-code of non-plastic material, cleaned work surfaces and washed forearms were measured to prevent contamination from adhering dirt particles. Nonetheless, during sampling preparation, while rinsing and vacuum filtrating, open petri dishes with clean filter paper, were placed in direct proximity to the work area, providing a control of potential airborne contamination. This control filters were examined under the same microscope and protocol than the target seawater filters. After each sampling-volume was acquired, the filtering device was disconnected and each mesh was washed to a labelled screw top container with micro-filtrated water.

2.2.2 Analytical Techniques

Back in the land lab, the volume of water of each container, corresponding to a mesh size at each of the stations, was transferred to a glass beaker, cleaning it thoroughly three times with MilliQ water. The glass beaker content was vacuum filtered using a 0.7 μm Whatman glass microfibre filter (GF/F, ⌀ = 47 mm). Each filter was placed on a petri dish and dried overnight before visual inspection, that was performed under a stereomicroscope (Nikon SMZ1000, 8–80X). The smallest microplastic particles were also observed under a metallographic microscope up to 400X (NIKON LV100POL).

3 Results and Discussion

The filtering device allowed the study of the microplastic concentration on volumes up to 4000 L without clogging in transects of up to 60 miles, which is the distance from the oceanic station ESTOC and the home port. In this way, the present design was suitable for continuous sampling the stations and also while on navigation, in both cases without interfering the ships' regular activity. Microplastic particles were not found in the air contamination controls set up on board during sampling manipulation.

Total survey effort sampled 3525 L and 10472 L accounting, respectively, with the samples taken on the different stations on three consecutive oceanographic campaigns and on four navigation transects. Microplastics were found in all samples and at all stations and transects, identifying a total of 163 particles (5 mm–50 μm), ranging from 0 to 46.15 particles/m^3, with a mean value of 9.92 ± 11.22 particles/m^3 corresponding to a mean of 14.46 ± 13.23 fibres/m^3 and 5.37 ± 6.35 fragments/m^3.

Microplastic densities on the stationary and on navigation samples were significantly different, representing an underestimate of approximately 30% in the case of fibres retrieved on navigation (Fig. 2-A). There was no significant difference among the

proportion of fragments that were sampled either on oceanic or coastal waters. However, this was not the case concerning fibres. The concentration of microplastic fibres sampled in the Test Site (4 km off the coast) was significantly higher than the concentration obtained in oceanic samples: 21.39 ± 16.05 fibres/m^3, versus 11.11 ± 8.08 fibres/m^3 (Fig. 2-B).

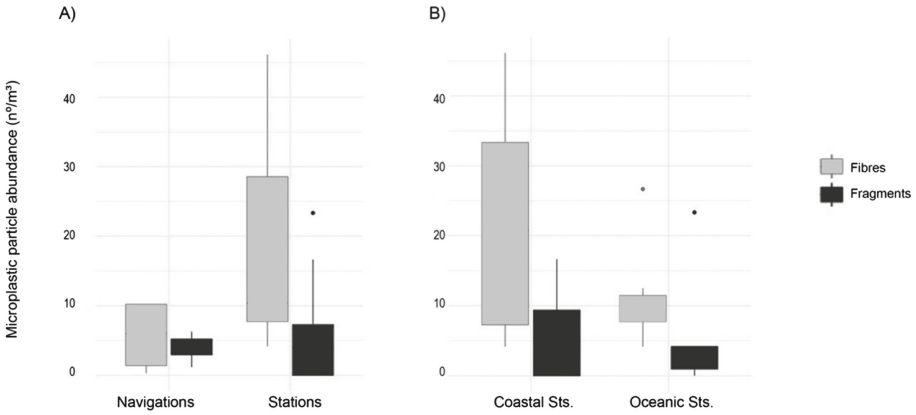

Fig. 2. Microplastic particle abundance (n°/m^3) depending on the sampling mode: (A) on navigation Vs on station; and (B) concerning station samples: coastal Vs oceanic stations.

In summary, reported microplastic abundance is higher than the available data for other areas in the NE Atlantic. Concerning coastal areas, where marine litter is generally more abundant [8], our study reinforces the fact showing concentration values significantly higher for coastal samples than for oceanic ones, a difference that was also noted by Cincinelli et al. [7].

4 Conclusions

This study reports the first baseline data on oceanic microplastic abundance on the area, placing emphasis on the necessity of a monitoring strategy. As such, the methodology employed over three consecutive oceanographic campaigns comes up as an opportunity to sample microplastic particles and report data in marine open water environments.

Microplastic particles 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. More research is needed in order to assess the monitoring method efficiency and to understand the variability dependant on the sampling mode (i.e. *navigation* vs *stationary*).

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Modelling the Global Distribution of Beaching of Marine Plastic

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1 Introduction

Marine plastic pollution is of global concern, with plastic being found in all the world's oceans [1]. Yet, we still have an incomplete understanding of the fate of plastic once it enters the ocean. While an estimated 4.8–12.7 million tons of plastic entered the ocean in 2010 [2], only 93–236 thousand tons are estimated to be floating at the ocean surface [3], which indicates the presence of large plastic sinks in the ocean. Such sinks may include ingestion by marine wildlife, sinking due to biofouling, potentially fragmentation to nanosized particles and beaching on shorelines [1].

Beaching likely has a sizeable contribution to the removal of plastic from the ocean as plastic has been found on shorelines around the world [4], with high concentrations found even on largely uninhabited islands [5]. Beaching is a complicated process, influenced by factors such as beach type, wave height, wind direction and nearshore currents [6–9], and modelling efforts of the global distribution of plastic beaching have not been done yet. However, beaching must be understood in order to determine the fate of plastic once it enters the ocean. Determining regions with high beaching would help with directing beach clean-up efforts. Furthermore, beaching likely plays an important role in the fragmentation of plastic to secondary microplastics, as elevated temperatures, the availability of oxygen and abrasive forces allow for more rapid degradation of plastic than in marine environments [10, 11]. Beaching mechanisms are therefore likely important for estimating the input of secondary microplastic into the ocean.

In this study we estimate the relative global distribution of plastic beaching and the origin of beached plastic. We do this with Lagrangian simulations using ocean circulation data [12, 13] including surface currents and Stokes drift. We use a simple parameterization of plastic beaching to indicate global hotspots for plastic beaching, the fraction of beached plastic that originates from local vs. remote sources and the likely origins of plastic.

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2 Methods

2.1 Ocean Surface Current Data Sets

We combine two surface current data sets for the period 2005–2015. The first dataset is from the HYCOM + NCODA Global 1/12° reanalysis [12], which has a temporal resolution of 3 h. HYCOM circulation patterns generally agree with observations [14, 15], but predictive skill of drifter trajectories is higher in the open ocean than on coastal shelves [16].

Since the HYCOM dataset does not include Stokes drift, we add the Stokes drift from the WaveWatch III hindcast data set [13] to the HYCOM currents. The spatial resolution is $1/4°$ and the temporal resolution is 3 h. The WaveWatch III dataset correlates well with in situ measurements of Stokes drift from drifters, with root-mean-square errors being on the order of centimetres per second [17].

2.2 Lagrangian Model Setup

In order to model the relative global distribution of beached plastic, we use Parcels (Probably A Really Computationally Efficient Lagrangian Simulator) [18] to model plastic as buoyant virtual particles that move passively with the surface ocean currents. A change in the position $\vec{x}(t)$ of a particle is calculated according to

$$\vec{x}(t+\Delta t) = \vec{x}(t) + \int_{t}^{t+\Delta t} \vec{v}(\vec{x}(\tau),\tau)d\tau + R\sqrt{\frac{2\,dt\,K_h}{r}} \tag{1}$$

where $\vec{v}(\vec{x}(t),t)$ is the surface flow velocity at the particle location $\vec{x}(t)$ at time t, $R \in [-1,\ 1]$ is a random process representing subgrid motion with a mean of zero and variance $r = 1/3$, dt is the integration timestep, and K_h is the horizontal diffusion coefficient. Equation 1 is integrated with a 4th order Runge-Kutta scheme with an integration timestep of $dt = 30$ min, where $\vec{v}(\vec{x}(t),t)$ is the sum of the HYCOM and WaveWatch III surface currents, and particle positions are saved every 48 h. We take $K_h = 10\ \mathrm{m^2\ s^{-1}}$ [19].

The source function for the microplastics is from van Sebille et al. [3], who assumed the amount of plastic waste available to enter the ocean to be proportional to the human population within 200 km of the coast, and where the input was scaled according to the amount of plastic that entered the ocean per country in 2010 [2]. Due to computational limits, very small local sources are not included as particle inputs. However, the neglected sources are only 0.9% of the total input and likely do not significantly influence our findings. Particles were released every 4 weeks for the first year of the simulation (340 830 particles in total) and advected for 10 years, from 2005 until 2015.

A particle is considered to have beached if $\vec{v}(\vec{x}(t),t) = 0$ at particle position $\vec{x}(t)$, which occurs if a particle is advected onto a land cell. However, not all relevant near-shore processes are properly resolved in the HYCOM surface current datasets, so modelled beached plastic concentrations are highly simplified estimates. The model does not allow for resuspension of particles once they have beached. Concentrations are calculated by binning the beaching locations into 1° bins and are then divided by

the lowest modelled concentration in order to determine relative beached plastic concentrations. Definitions of countries and Exclusive Economic Zones (EEZs) are according to version 2 of the Union of the ESRI Country shapefile and the Exclusive Economic Zones dataset [20].

3 Results and Discussion

3.1 Results

After 10 years of simulation, 92.7% of particles were found to beach (316 213/340 830), with plastic beaching along almost all coastlines (Fig. 1). Most particles (70.4% of total particles in the simulation) beached in Asia, with Africa having the second largest fraction of beached particles (14.0%). The highest concentrations are near regions with high population densities (e.g. eastern China, northern Egypt, Black Sea), where concentrations can be up 10^4 times higher relative to less polluted coastlines. Plastic is also found to beach in regions with low population densities, such as the south coast of Australia and islands in the South Pacific. However, the concentrations are generally relatively low. Few regions are found to be free of beached plastic, in particular the polar regions, the coast of Namibia and the north western coast of Australia.

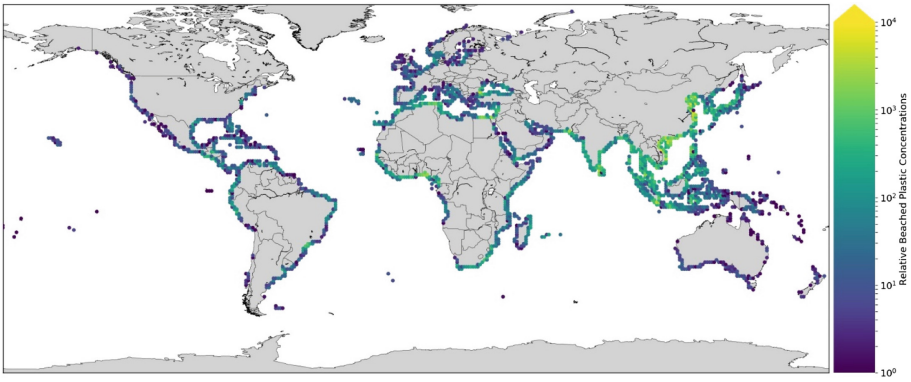

Fig. 1. Relative beaching concentrations after 10 years of simulation on a $1° \times 1°$ grid. Coastlines without markers have no beached particles in the simulation.

Beached plastic in most EEZs originates from both local and remote sources, where a particle is considered local if it originates from within the EEZ where it beaches (Fig. 2). Regions such as China and South America are dominated by local plastic, while in Canada, Australia and most island nations a large fraction of plastic comes from remote sources.

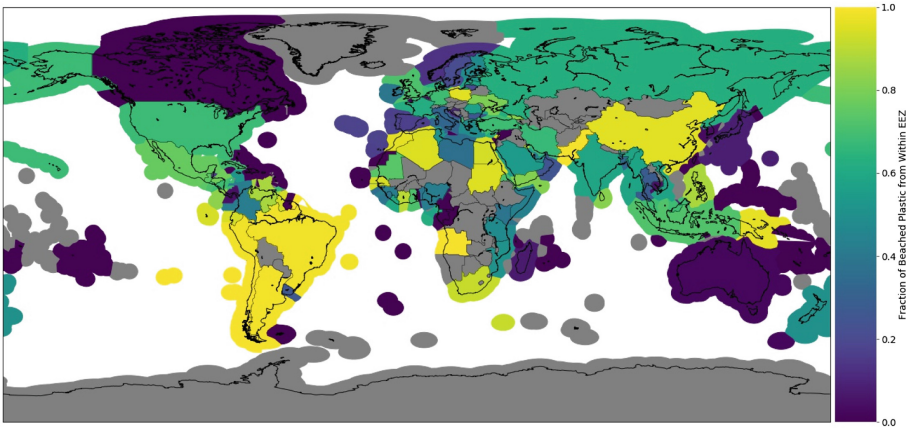

Fig. 2. The fraction of particles beached within each EEZ that originates from within the EEZ. Grey EEZs have no beached particles in the simulation.

For most continents, the majority of beached plastic originates from within the

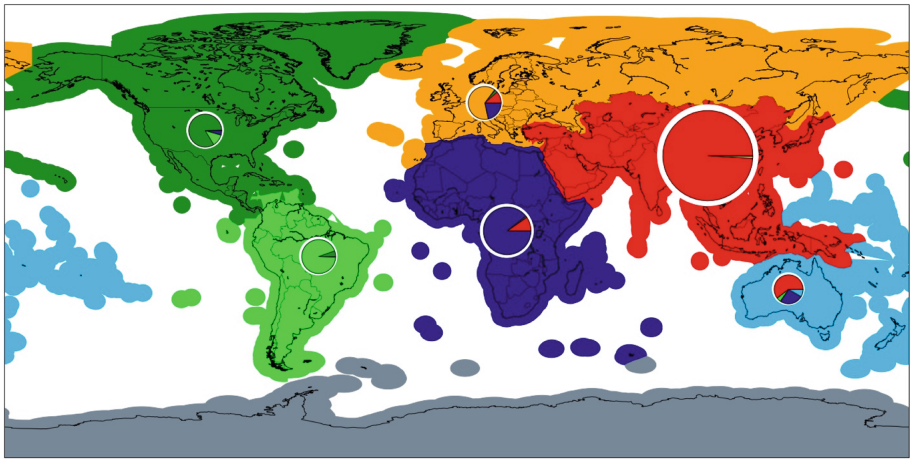

Fig. 3. The origin of beached particles in each continent. The size of the pie chart increases with an increased number of beached particles in the simulation. The fractions in the pie charts for each continent indicate the fraction of the total number of particles that beach that originate from the continent indicated by that colour.

continent (Fig. 3). Exceptions are Oceania (light blue in Fig. 3) and Europe, where 92.3% and 36.4% of plastic originates from remote continents, respectively. In

Oceania, Asian sources are the largest contributor (63.5%) while in Europe, African sources are the second largest contributor (21.6%).

3.2 Discussion

Given that 92.7% of plastic in the simulation is beached after 10 years, beaching is potentially a major sink for plastic in the ocean. However, the parametrization of beaching in this study is highly simplistic. Firstly, the flow field datasets do not have the required spatial resolution to resolve physical processes in the coastal zone which likely play a highly important role in whether plastic beaches or not. We also don't consider local topography which might affect beaching probabilities. Secondly, our model does not allow for resuspension of plastic once it has beached. Thirdly, we do not consider potential losses of plastic in the open ocean, for instance due to sinking or ecosystem uptake. Finally, we do not consider plastic properties such as size and density in the beaching estimates. Our model therefore can give an indication of how much plastic is transported towards the coast, but it likely overestimates the amount of plastic on coastlines at any given time. Improved beaching parametrizations are required to better estimate the amount of beached plastic, which will be the focus for future work.

The ratio between local and remote beached plastic is influenced by several factors. In Australia, small local inputs, a large amount of remote plastic and a comparatively long coastline result in a high relative contribution of remote sources. Another factor affecting the ratio of local to remote plastic are the local currents. For example, Japan has much larger plastic sources than Australia, but most of its plastic input is carried east by the Kuroshio current. However, the same current places Japan downstream of plastic released from China and Korea, and therefore most of the beached plastic in Japan is from remote sources.

While on the scale of EEZs plastic generally originates from both local and remote sources, on the scale of continents almost all the beached plastic originates from within the continent, especially in the cases of Asia, Africa and South America. The contribution of cross-oceanic transport to beaching appears to be relatively small, except for in Oceania. This is due to Oceania having few local sources of plastic and many islands spread out over the Pacific. However, only 0.2% of all particles in the simulation beach in Oceania, indicating that only a relatively small number of particles is transported across oceans. Smaller basins are crossed more readily, which can be seen in the large contribution of African plastic inputs to beaching in Europe. This is largely due to plastic originating from Northern Africa crossing the Mediterranean.

4 Conclusions

In this study we present the initial results of a simplistic Lagrangian model of plastic beaching on a global scale. Plastic is found to beach at almost all coastlines, with the highest concentrations found near regions of high population density. The plastic beaching in most EEZs generally originates from both local (from within the EEZ) and

remote sources, while on a continental scale the majority of beached plastic originates from within the continent. Almost all plastic beaches within 10 years, which indicates that beaching is likely a significant sink for plastic in the ocean.

Our work gives an indication of beaching hotspots on a global scale, which can be used for directing beach clean-ups. Furthermore, our work is a first step to constraining the beaching component of the marine plastic budget, while also contributing to estimating the size of secondary microplastic production on beaches. Finally, determining origins of beached plastic can assist policy makers in designing and implementing mitigation policies.

The current model setup uses a highly simplistic parameterization of beaching, which needs to be further developed to improve beaching estimates. This will require incorporating near-shore processes and local coastal topography. Furthermore, including plastic resuspension will allow more realistic estimates of how plastic is beached at any given time, but this will require experimental studies of plastic resuspension.

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Plastics and Microplastics: The OECD's Approach

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1 Introduction

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 [1].

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 [2–5].

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 related to the decrease ecosystems' energy and services, as well as the threat to human health that concern microplastics as carriers of microorganisms, even pathogens, and adsorption of POPs [6] which, in reality needs more investigations but for instance 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 [1].

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2 OECD's Approach on Plastic Materials and Microplastics

Since the mid-2010s the OECD Joint Meeting has approached the theme of plastic in the environment and its negative repercussions on ecosystems due to the existence of three concomitant factors:

(1) an ever-increasing use of the very varied applications of polymeric materials in objects of everyday life [7];
(2) the disproportionate increase, both in percentage and absolute weight, of applications for single use products (SUP – Single Use Products) with the concomitant factor of demographic and economic growth in developing countries (from Lower Middle-Income Countries – LMICs to Upper Middle-Income Countries –UMICs);
(3) together with the problem of incorrect management of collection, recycling and product placement of recycled plastics.

From the first approaches to the theme of plastic in the environment, addressing the problem of the Marine litter with the relative macroeconomic framework of this emergency up to the first proposals of economic leverage for containing plastic pollution, the Working Party on Resource Productivity and Waste was one of the most active Working Party on the subject.

The issue of the characterization of plastic pollution is now ubiquitous in the field of scientific literature and of the largest World Organizations (NOAA, UNEP, JRC, GESAMP, etc.) [8], and also the focus of the activities within the OECD - Environment and Health Program - is on the themes disseminated by scientific research, with a land based approach, of an economic, sociological, and technological nature, with a view to making the economic processes of plastics sustainable, in order to ever less impact on the environment. This happens just under the perspective of the OECD mission, through the study and implementation of regulatory actions, on a global scale to mitigate the environmental impacts and on the human health of the family of so-called plastic materials in their use on a global scale.

This work, carried out in concert between the OECD Joint Meeting and various Working Parties, has taken two main strands: the first on the sustainability of plastic materials and their commercial applications and the second on the mitigation of the environmental impacts of microplastics, starting from aquatic systems.

2.1 The Themes of the OECD Global Forum on Environment 2018

The first strand, the sustainability of plastics and its applications in consumer products, was developed and approached with The OECD Global Forum on Environment: "Plastics in a Circular Economy - Design of Sustainable Plastics from a Chemicals Perspective". This Forum was held in Copenhagen, Denmark from the 29th to 31st May, 2018, and hosted by the Danish Government [1].

The idea behind the Global Forum was - and currently implemented within the WPRPW – that of making the supply chain of plastic products sustainable starting from the design of polymeric compounds. The need to improve the sustainability of plastics through chemical re-design [2] and make them more circular as possible when it is feasible to know and select the chemical substances present in mixtures of plastic

materials (polymers and their blends). Exactly these chemicals are those that have certain consequences for the environment and human health, relating the entire life cycle of plastic products up to disposal methods. The "greening design" of plastic materials or the production of plastic items so called "benign by design" [3] are the key step for the realization of the circular economy for plastics. Advancement in the circular plastic economy will allow: better management of plastic waste, recovery of various types of polymers and their reuse in closed chains, with gradually improved environmental management of the products. The releases in the environment of plastics, starting from the infamous disposable products (SUP) will decrease with a clear benefit for human health and the environment, given that widespread losses in the natural environment were creating damage that could not be ignored, instead giving a real signal of reduction of the marine litter starting from land [4]. Very briefly [5] the results of the global forum on what can make the cycle of plastic sustainable in their use are: choice of design goals oriented to the life cycle starting from the selection of raw materials, production and manufacturing, product's use, disposal/recovery plan and options. In addition, for each independent phase of the product's life, it will be necessary make an assessment also giving a benchmark against products that provide the same service realizing a continual improvement of the evaluation, optimization, and design of plastic products.

The existing tools that are available to evaluate the above considerations are risk assessment and life cycle analysis, their outcomes and their continuous implementation make it possible to improve sustainability [3].

The final objectives of this way towards sustainability are: decrease in depletion of natural resources, increased recovery of materials with material recycling policies and re-use of products or their components (Circular Economy perspective) [2], encouraged by a new product and process design concept, with a gradual assessment in the supply chain. The last is to achieve an increasingly reduced impact in the management of plastic waste, with the elimination of emissions into the environment and a drastic reduction in marine litter in the first place [4].

2.2 The Work on Microplastics of the OECD Working Party on Resource Productivity and Waste (WPRPW) and of the OECD Working Party on Biodiversity, Water and Ecosystems (WBWE)

Starting from the work of the past years and that of OECD Global Forum on Environment held in 2018 and considering the growing concerns about the adverse environmental side effects of plastics related to the leakage of plastics in the natural environment, and particularly in oceans and freshwater, the two OECD Working Parties - Working Party on Resource Productivity and Waste (WPRPW) and Working Party on Biodiversity, Water and Ecosystems (WBWE) - are developing possible solutions, with a policy and technological approach, from their different perspectives, in containing and reducing microplastics pollution.

3 Discussion and Results About Works of the Two OECD WPs

Without claiming to frame the source, the fate, and the type of transport of microplastics in an exhaustive and definitive way, it is sufficient to remember that these are now a widespread problem even if they have arisen with the production of plastic materials in the last 50 years [7]. Plastic debris are now common in a ubiquitous way: they are more present in coastal areas and even more in estuarine areas. They represent damage to ecosystems because the plastic materials released into the environment imply a number of impacts on the quality of marine and coastal environments [8].

This type of pollution has been proposed as a geological indicator for the current geological era of the Anthropocene [9]. Starting from the ingestion of macro-plastics by marine wildlife, up to the global sustainability of the fishery system [10], up to the risk of human health, from the direct ingestion of plastic materials from marine foods and seafood contaminated by microplastics, the alarm is now ubiquitous and well publicized, even if there is still no conclusive scientific evidence about chemical bioaccumulation in the food chain. For this reason, microplastic pollution is the tip of the iceberg of the concern of plastics. At a global scale microplastics have been recognized as the theme to work on to mitigate the environmental impacts already acknowledged by scientific research.

Microplastics represent a great part of the total marine litter that flows into the oceans by seaside and rivers (approx 15% of mismanaged plastics waste by weight); and a substantial part of the total amount of marine litter present in the oceans, and their size implies greater ease of ingestion by all trophic levels not only of the marine environment [11], in fact there is also alarm for human ingestion of microplastics both orally (food, water) and by inhalation.

The problem of microplastics must be faced with prevention and reduction actions, up to their complete reduction, because for their removal from ecosystems there are no practical technological solutions, as is already the case for macro-plastics (optimization of waste management, clean up action, and so on). Targeted and specific removal actions can be put into action because microplastics (both primary [12] and secondary, both intentionally and unintentionally released [13]) derive from a limited number of applications in product categories which in turn have precise routes of release and transport in the environment [14]. Here below the synoptic framework of the different origins of microplastics in the sea (Table 1).

It is believed that most of the microplastics produced and microplastics resulting from product wear come from five key product categories: personal care products, plastic pellets, synthetic textiles, vehicle tyres, paints, and they find their way into the environment along a limited number of pathways; perhaps more than many words, Horton's [15] Graphical Abstract is enough for this purpose (Fig. 1).

Depending on the transport pathways described above, the mitigation approaches could be based on solutions related at source reduction, for certain way of contamination as it happens for microbeads, pellets, paintings, etc. Starting from the last few years strict prohibitions, restrictions and rules apply to use e transport such types of plastic applications (i.e. different Countries all around the world apply Microbeads Ban laws [16]).

Table 1. Synoptic framework of the different origins of microplastics in the sea

Application and primary use	Eunomia (2016) [14]	IUCN (2017) [13]
Tyres	28.4%	28.0%
Pellets	24.2%	0.3%
Textiles	20.0%	35.0%
City dust	–	24.0%
Building paint	13.7%	–
Road paint	8.4%	7.0%
Cosmetics	3.7%	2.0%
Marine paint	1.7%	3.7%

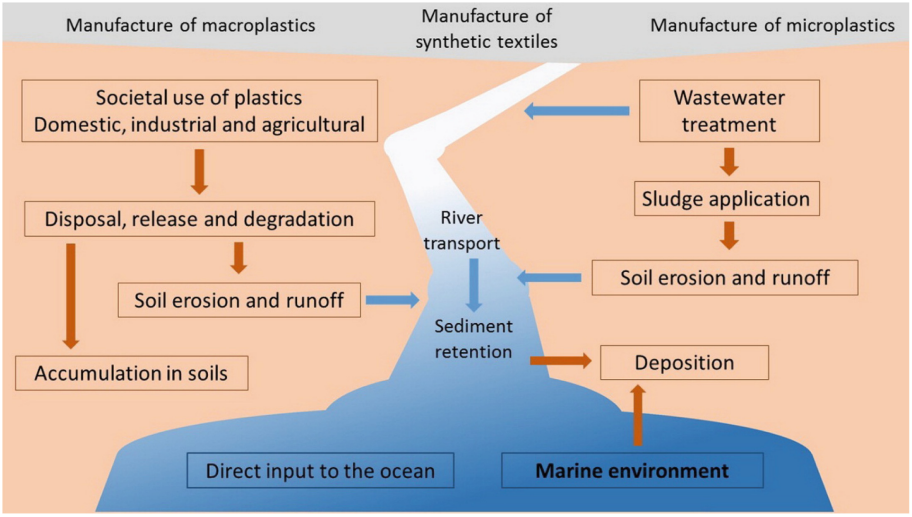

Fig. 1. Key transport pathways between terrestrial, freshwater, and marine environments. (free download from: http://dx.doi.org/10.1016/j.scitotenv.2017.01.190. With the author's permission [15])

Of course, the unintended releases still need to be resolved not only for these types of microplastics, but also those due to clothing products and those that release pieces that are mainly due to applications in fabrics of synthetic source and to the release of tyre pieces from vehicles [13].

Microplastics derived from the abrasion of vehicle tires and construction and road paints, as well as various forms of city dust are the classic example of diffuse microplastic contamination, of an unintended nature.

These types of microplastics are probably transported directly onto the ground or into the water through surface runoff during rainy events and represent the methods of depositing this contamination both on the ground and at sea [16], through the drainage water network, up to the rivers, true and proper highways to the oceans. Wind transport

can also play a role. Some studies suggests that the annual addition of microplastics to soil may be of a similar magnitude to the microplastics that make their way into the oceans [17].

4 Conclusion

Given the small size and the widespread dispersion of microplastics as can be easily understood, the option to remove them from the environment through cleaning, filtering or dredging is generally considered not feasible [8, 12, 15, 17]. Instead, the approaches that focus on reducing the flow of microplastics in the environment are recognized as the most effective [18]. At least three approaches are available:

(a) **source-reduction**: starting from minimising (micro)-plastics applications for instance: this is already happening with *the bans* [16];
(b) **waste prevention** through product design measures [18, 19], such as increased *product durability* and the adoption of *technologies to reduce* [20] the generation of *microplastics*;
(c) **end-of-pipe solutions**: that is, identifying the point at which to apply a technology to contain the release into the environment by filtering and removing the microplastics. This can be done by *upgrading wastewater treatment plants* or in the case of microfibres derived from fabrics, this could lead to *greater use of filters in washing machines*. The effectiveness and relative cost of these different approaches will vary and depend on the type, source and routes of the microplastics in the local context [15, 17].

The future work of the OECD will address these three issues, always evaluating the implications also of an economic nature, with a view to environmental and social sustainability, and using all the results and scientific evidence available to support and implement congruent local and global policies also evaluating all the sociological aspects to implement and direct them in the right direction.

Glossary

GESAMP – Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection

JRC – Joint Research Center EU Science Hub

LMICs – Lower Middle-Income Countries

NOAA – National Oceanic Atmospheric Administration

OECD – Organisation for Economic Co-operation and Development

OECD Joint Meeting – Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology

POPs – Persistent Organic Pollutants

SUP – Single Use Plastics

UMICs – Upper Middle-Income Countries

UNEP – Environmental Program of the United Nations

WPs – Working Parties

WPRPW – OECD Working Party on Resource Productivity and Waste

WPBWE – OECD Working Party on Biodiversity, Water and Ecosystems

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Solid-Liquid-Liquid Microextraction (μSLLE) for Determining Persistent Pollutants at Marine Microplastics

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1 Introduction

Persistent organic pollutants (POPs) enter to the ocean through the air-ocean interface, or from coastal inputs, especially in the mouths of rivers and gorges [1]. Their stability and persistence, on top of their pervasiveness from man-made pollution, means that POPs can be found in almost all matrices in the environment, especially at hydrophobic matrices as microplastic [2, 3]. Microplastics with POPs over their surface, can be transported over large distances [4]. They are found in all oceanic areas over the whole world, including remote polar regions such as the Arctic Ocean. Microplastics enter by ingestion to the food chain with the subsequent bio-accumulation and bio-magnification of POPs associated [5, 6].

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 [7–10].

Miniaturised extraction systems enable us to analyse samples quicker and cheaper in both financial and environmental terms [11]. These techniques are highly referenced for analysing liquid samples, but not for solid samples, and even less so for analysing pollutants from microplastics [12]. A miniaturised solid-liquid-liquid extraction technique (μSLLE) 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 drying the extract. The pollutants are analysed using single quadrupole gas chromatography with mass spectrometry (GC-MS).

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2 Experimental

2.1 Materials

A VWR®, model SZB250 routine stereo-microscope was used for visually identifying the microplastics samples.

A Cole-Parmer® model 08895-22 ultrasound bath and a VWR® vortex shaker were used for the extraction procedure. A Selecta® rotaterm orbital shaker was used to study the adsorption rate of POPs on microplastics in laboratory, moreover of a Jouan® BR 3.11centrifuge.

A SPE online C8 brand Supelco®, and pump model 230 brand Agilent Technologies®.

2.1.1 Reagents

The determination of POPs were performed with a gas chromatography with mass spectrometry (GC-MS), model 7820A and 5977 MSD with an HP-5MS Ultra Inert 19091S-433UI column of the brand of the Agilent Technologies®, following its multi-residual analysis methodology [13]. 1 μl n-hexane is injected with IS in the following conditions of oven temperature: 60 °C (2 min), 20 °C/min, 175 °C, 5 °C/min, 250 °C, 10 °C/min, 325 °C (5 min). The transfer line set at 280 °C.

Were analysed 15 organochlorinated pesticides (OCPs), 8 polychlorinated biphenyls (PCBs) and 6 polycyclic aromatic hydrocarbons (PAHs).

The reagents used to determine the POPs were: Triton X-100 (Fisher scientific™), methanol (Merk), n-hexane SupraSolv (Merck) and Milli-Q water (Millipore). The internal standards (IS) used were: Chrysene D12, Acenapthene D10, Penanthrene D10, Perylene D12 (Supelco), at a concentration of 2.5 $ng \cdot mL^{-1}$ and Telodrin for analysing OCPs from Dr. Ehrenstorfer GmbH®, at a concentration of 25 $ng \cdot mL^{-1}$.

2.2 Methods

2.2.1 Preparation of Samples

HDPE granules (high density polyethylene) that were previously cleaned with methanol and ultrapure water in the sound bath were used to ensure that there was no POP on the surface. These were contaminated with a known concentration and it was leave repose 24 h until the study start.

2.2.2 Micelar Solid-Liquid-Liquid Extraction (Micelar u-SLLE)

This methodology developed in this study is based on a solid-liquid extraction followed of a liquid-liquid extraction.

Solid phase extraction consists of contacting the gram of pellets with a volume of surfactant (Triton X-100) [14]. The pellets with the extractant are subjected to an ultrasound bath for 4 min and kept in contact for a further 24 h at rest.

After 24 h, the 3% Triton is diluted to 0.5% and passed through an online SPE C8 with a flow of 1 ml/min. After the adsorption process, 100 mL of Milli-Q water is passed through the SPE on line for cleaning and 1 min of air for subsequent drying. The desorption of analytes is carried out using 5 mL of methanol.

The pellets that have already passed through the system are now contacted for 4 min in the ultrasonic bath with 5 mL of methanol, which is also allowed to stand for 24 h.

After 48 h, the first 5 mL of methanol are mixed with the second 5 mL. And 150 µL of n-hexane is added to the methanol extract with the internal standards necessary for its subsequent study with the gas-mass chromatograph and they are mixed well. To remove methanol and n-hexane by liquid-liquid extraction, add 8.5 mL of MilliQ water, centrifuge at 3500 rpm for 5 min at 0 °C (to avoid evaporation of n-hexane).

Subsequently, at least 40 µl of n-hexane are extracted with the analytes of interest from the mixture and introduced into the GC-MS for further analysis.

3 Results and Discussion

3.1 Results

Different optimizations were developed until obtaining the best result, in this way the final methodology was as shown in Fig. 1.

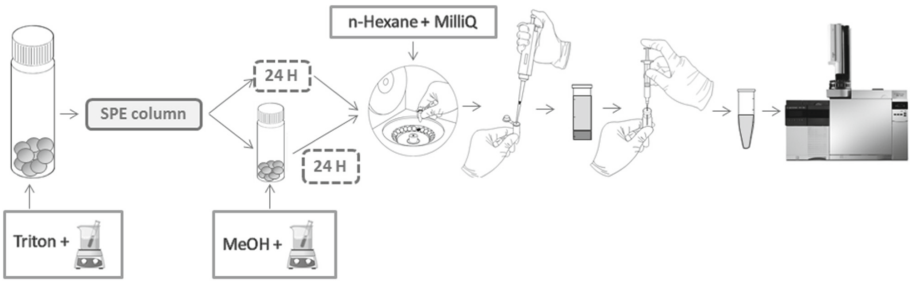

Fig. 1. Micelar solid-liquid-liquid extraction

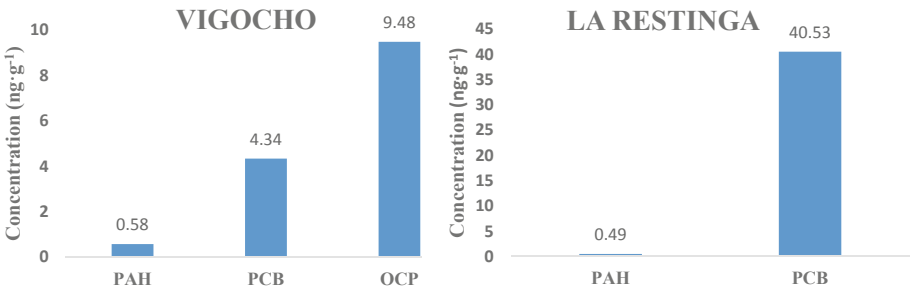

Fig. 2. Concentration evaluated with the micelar µSLLE methodology in pellets collected in two beaches; Vigocho (left) and La Restinga (right) in $ng \cdot g^{-1}$.

4 Conclusions

The current study has advantages over traditional techniques, miniaturized techniques are performed in a shorter time, more economically and cleaner for the environment. With only a few simple steps and in less than 72 h, semi-quantitative results of contamination quality are achieved.

The Canary Islands is an area affected by the amount of plastic waste that arrives each year [7, 15, 16], and there is scientific evidence that these plastics carry with them a quantity of persistent chemical pollutants [7]. The methodology presented in this study was applied to samples of pellets collected in Fuerteventura and Gran Canaria (Canary Islands, Spain. Fig. 2) and the results were similar, finding differences in the most tourist beaches.

This μSLLE methodology allows for a quick determination of up to 27 analytes using a single quadrupole GC-MS, making it a feasible economic technique for many routine laboratories that do not have more sophisticated and expensive equipment like triple quadrupole GC-MS-MS.

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Erosion Behaviour of Different Microplastic Particles

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1 Introduction

Microplastic (MP) has already been reported in terrestrial [1, 2], marine [3, 4] and limnic environments [5, 6] as well as in the atmosphere [7, 8], with the research focus recently shifting from marine to limnic compartments [9]. Although there are more and more studies on MP concentrations in rivers, the predominant transport mechanisms of MP have been investigated only sporadically [6, 10–12]. For this reason, it has not yet been possible to make reliable statements about hotspots, transport routes, sources and sinks of MPs in rivers [2, 13].

Recent studies assume that a large proportion of the MP input into the oceans occurs via rivers [14]. However, a part of the MP introduced into the rivers also deposited there and gets either buried or remobilised at increasing flow rates, for example during floods or strong water conditions [15]. The extent to which this process, known as erosion, depends on the MP particle properties and the natural sediment bed has not yet been researched [16].

It is generally assumed that MPs in surface waters behave similarly to sediments [17]. However, due to strongly varying particle properties and insufficient knowledge of the prevailing transport mechanisms, there is no scientific confirmation of this hypothesis [18]. On the contrary, Waldschläger and Schüttrumpf [11] were able to prove that the settling and rising velocities of MPs could only be calculated insufficiently with the equations from sediment transport. It should therefore be examined whether MP differs from sediment in other transport mechanisms, like erosion, in order to increase the accuracy of numerical simulations. If MP behaves like sediment in water, conventional sediment transport models could be used in future. However, if the particles behave differently, the original models must be adopted.

Sediment transport is divided into two different categories: Bed load and suspended load. Bed load is the transport of material by sliding, rolling or saltating in a ballistic track directly above the ground. It starts when the critical shear stress at the riverbed is reached. Suspended load, on the other hand, is the process by which particles are transported through turbulence without contact with the river bed, i.e. in suspension [19]. For the consideration of erosion, the bed load is therefore more important.

With non-uniform sediments, the so-called ‘hiding-exposure effect’ occurs, which means that larger grains shield the smaller particles and thus protect them from erosion. Larger grains, on the other hand, are more likely to be moved because of their position,

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as they lie freely on the smaller grains of the bed. However, smaller grains on the bed of larger grains have a lower critical shear stress than the bed material [19]. This process could be particularly important for MPs on riverbeds, as the particles have significantly different diameters than the natural sediment.

2 Experimental Investigations

The erosion potential of MPs in rivers can be determined by the critical shear stress, which depends on the particle size, shape and density as well as the hydrodynamic conditions and sediment properties (size distribution, organic components). Normally the shear stress of particles is determined experimentally in a simulated benthic environment [16, 20].

The annular flume of the Institute of Hydraulic Engineering and Water Resources Management (IWW) at RWTH Aachen University is particularly suitable for this purpose. It can simulate an infinitely long stationary flow without using any pumps [21], which allows to investigate the erosion behaviour independently of turbulence caused unintentionally by the pumps.

The annular flume has an average diameter of 3.25 m and the flume a width of 0.25 m. The flume can be filled up to a water height of 0.5 m. The supporting components of the apparatus are welded and bolted steel square tubes (blue) between which 8 mm thick float glass panes allow observation of the experiments and contactless measurements (cf. Fig. 1).

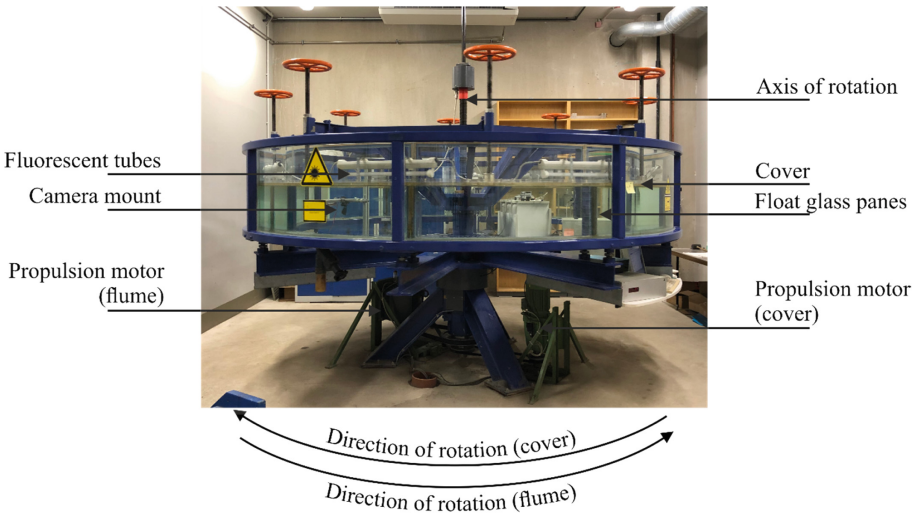

Fig. 1. Annular Flume of the Institute of Hydraulic Engineering and Water Resources Management at RWTH Aachen University

The cover of the channel can be lowered to the water surface and is equipped with waterproof fluorescent tubes which ensure adequate lighting.

Various approaches are possible for the flow generation of a circular flume. Both the rotation of the flume itself, the drive through the cover or the movement of the inner and outer wall are options. In the case of the IWW annular flume, the cover and the flume are driven counter-rotating, which minimises the secondary flows in the water column [21]. The resulting movement can be compared to a Couette flow and a maximum shear stress of 0.9 N/m^2 can be achieved.

2.1 Materials

Based on investigations of MP compositions in rivers and the production volumes of the individual polymers, the particles to be studied were selected. They consist of the polymer types PA, PS, PET and PVC, including shapes like pellets, spheres, fibres and fragments. The particle sizes are between 0.5 and 8 mm and 14 different particles were analysed (cf. Table 1).

As representative sediments for river beds medium sand, coarse sand and fine

Table 1. Investigated MP particles and their particle properties

Polymer	Abbreviation	Density [kg/m^3]	Shape	Size [mm]
Polystyrene	PS	1008	Fragment	1 to 2
Polystyrene	PS	1021	Sphere	4.83
Polystyrene	PS	1008	Pellet cylindric	3 × 3 × 2
Polyamide	PA	1107	Fibre	Diameter: 0.5 mm Length: 10 mm
Polyamide	PA	1107	Fibre	Diameter: 0.5 mm Length: 10 mm
Polyamide	PA	1140	Pellet cubic	1 × 1 × 1
Polyamide	PA	1140	Pellet cylindric	1 × 1 × 1
Polyethylene terephthalate	PET	1368	Fragment	1 to 2
Polyethylene terephthalate	PET	1368	Fibre	Diameter: 1 mm Length: 10 mm
Polyethylene terephthalate	PET	1368	Pellet cylindric	3 × 2.5 × 2.5
Polyethylene terephthalate	PET	1350	Pellet cylindric	3 × 2 × 2
Polyvinyl chloride	PVC	1307	Fragment	1 to 2
Polyvinyl chloride	PVC	1307	Pellet lenticular	4 × 4 × 2
Polyvinyl chloride	PVC	1307	Pellet lenticular	8 × 8 × 2

gravel were selected as seen in Table 2 and Fig. 2.

Table 2. Selected sediment beds with sediment division according to DIN 4022

Sediment	Grain size per definition [mm]	Grain size in experiment [mm]
Medium sand	0.2–0.6	0.3–0.6
Coarse sand	0.6–2.0	0.71–1.25
Fine gravel	2.0–6.3	2–4

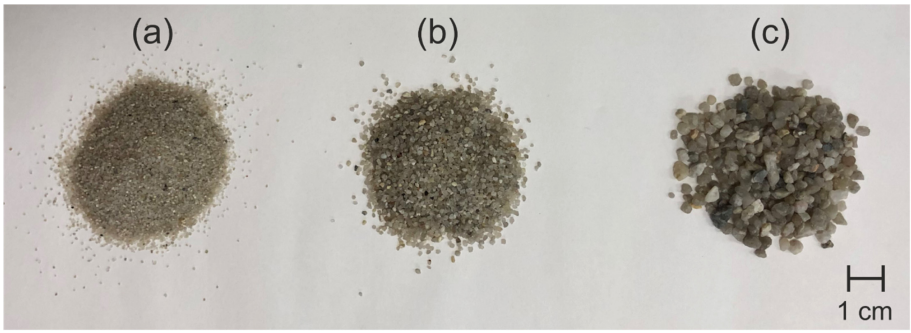

Fig. 2. Sediment used in the experiments: **(a)** medium sand 0.3–0.6 mm, **(b)** coarse sand 0.71–1.25 mm, **(c)** fine gravel 2–4 mm

2.2 Methods

In the course of the experiments, the different sediments are deposited into the annular flume and individual MP particles are placed on top of the sediment bed. The shear stress is then slowly increased to determine the critical shear stress of the individual particles.

On the basis of previous research [21], the shear stress occurring on the ground is known for two specific water heights (175 mm and 325 mm), so that the shear stress can be specifically increased by 0.0001 N/m^2 each second. In all tests, a water height of 325 mm was selected. In order to prove the reproducibility of the experiments, ten runs are carried out per particle. In addition, the multiple runs are intended to demonstrate effects of the different particle shapes on erosion behaviour.

At first, experiments without any sediment are carried out to represent a smooth bed, i.e. within canals. Afterwards, the uniform sediments are installed in order to be able to consider the erosion behaviour in dependence of the sediments. After the erosion of MP on uniform sediments with different grain sizes has been examined, the sediments are mixed in a ratio of 1:1:1 in order to observe the erosion behaviour on a mixed bed. All sediment beds are installed in a regular thickness of about 2 cm.

A GoPro Hero5 is installed inside the annular flume, so that it rotates with the channel and therefore is directed to the same point of the sediment bed. Thus, the introduced MP particles can be observed continuously. The aim is to observe the onset of erosion and to assign the simultaneous bottom shear stress. For the experiments a camera settling of 1080p (90/90 fps) is used.

3 Results and Discussion

At this point only preliminary results can be presented, as the experiments are still in progress. The experiments without sediment as well as with medium sand and coarse sand have been carried out so far.

Figure 3 shows the critical shear stress of the MP on no sediment, medium sand and coarse sand. Some particle/sediment combinations lack the critical shear stresses in

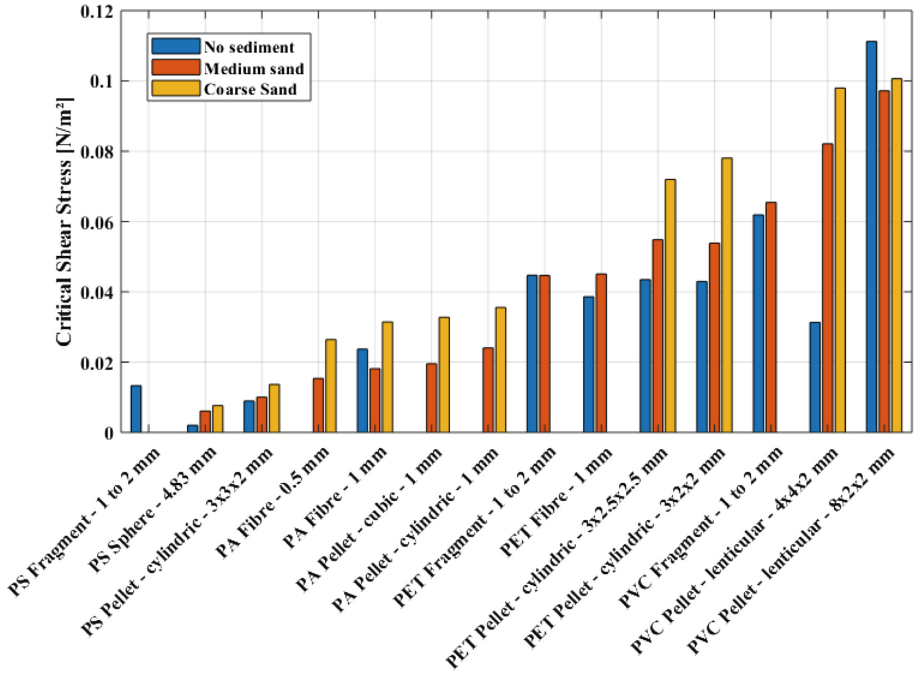

Fig. 3. Preliminary results for the critical shear stresses of different MP particles on different sediment types

the graph, because it was not possible to distinguish between sediment and MP in the camera images and therefore the critical shear stress could not be determined yet.

A comparison of the onset of erosion on the two sediments showed that all MP particles on the coarser sediment (0.71–1.25 mm) have a greater critical shear stress than on the finer sediment (0.3–0.6 mm). In addition, a comparison of particles with the same density and shape (PVC pellets lenticular) but different diameters (4 and 8 mm) shows that the larger particles are eroded later than the smaller ones. As expected, the PS spheres require less shear stress than cylindrical PS pellets because the spheres start rolling more easily due to their shape. When looking at the fibres, it seems that the thinner the fibre, the easier it erodes. Most particles erode on the smooth bed earlier than on the sediment beds, with the exception of PA fibres and the bigger, lenticular PVC pellets. It also becomes obvious that the density of the particles plays a decisive role in the onset of erosion, as the density rises from PS via PA and PVC to PET, while PVC and PET are almost the same.

4 Conclusions

In physical model experiments, the erosion behaviour of different microplastic particles is investigated. So far, it has been shown that a coarser sediment bed leads to larger critical shear stress of MP particles. In addition, clear differences between the individual particle shapes could be demonstrated. A more detailed analysis of the results is currently pending due to the ongoing experiments on fine gravel and a mixed sediment.

The on-going experiments are expected to produce more results, which will also be compared with Shields Diagrams of natural sediments and a final conclusion can be made if microplastics behave like sediment concerning their erosion behaviour.

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