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# Proceedings of the International Conference on Microplastic Pollution in the Mediterranean Sea

 Springer

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Editors

# Proceedings of the International Conference on Microplastic Pollution in the Mediterranean Sea

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# Sub-Basin Scale Heterogeneity in the Polymeric Composition of Floating Microplastics in the Mediterranean Sea

Giuseppe Suaria, Carlo Giacomo Avio, Francesco Regoli  
and Stefano Aliani

## 1 Introduction

Mainly owing to limited outflow of surface waters, a densely populated coastline and intensive fishing, shipping, touristic and industrial activities, substantial amounts of plastic litter are accumulating in the Mediterranean Basin which—together with the main five oceanic gyres—is now recognized as one of the greatest accumulation zones for floating plastic debris in the world [1–5].

The generic term “microplastics”, however, encompasses a wide range of different polymers, while most of the field studies mainly relied on the visual identification of particles or characterized only a restricted subset of them. Thus, detailed knowledge of the actual polymeric diversity of this emerging pollutant is lacking. Such information is urgently required to identify sources and sinks of microplastics and to better understand fate and impacts of the different polymers at sea, so that knowledge-based reduction and prevention measures can be effectively implemented.

Here, we present the results of a large-scale survey of floating microplastics (<5 mm) and meso-plastics (5–20 mm) in central-western Mediterranean waters, providing the largest polymeric characterization ever performed (n = 4050 particles). We confirm the Mediterranean Sea as severely contaminated by plastic pollution and describe for the first time the complex mixture of synthetic polymers floating on its surface, testing the hypothesis that plastic distribution and composition are not homogeneous and that geographical differences exist between sub-basins.

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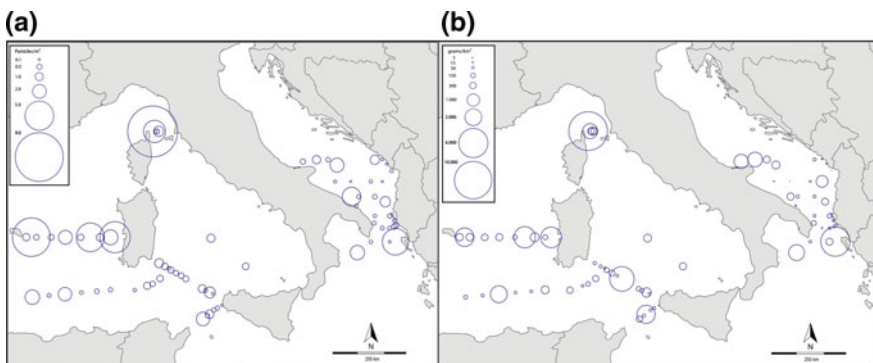
## 2 Materials and Methods

### 2.1 Sampling and Laboratory Analysis

74 offshore samples were collected during two expeditions carried out in the Mediterranean Sea on board the Italian research vessel Urania between May 9 and June 24 2013. Samples were collected using a 200  $\mu\text{m}$  Neuston net towed for  $\sim 5$  min at a speed of 1.5–2 knots. Once on board, the plankton samples were transferred to 50 mL falcon tubes and fixed with 80% EtOH. In the laboratory, all samples were examined under a dissecting stereomicroscope by two different researchers to reduce operator bias during sorting. Plastic particles were carefully hand-picked using laboratory tweezers and transferred to glass jars. All particles were then counted, weighed, dried and classified according to their shape and colour. Due to the high risk of airborne contamination, all filaments and fibres suspected of having a textile origin were not considered in density calculations. Total plastic concentrations (expressed as  $\text{g}/\text{km}^2$  and  $\text{particles}/\text{m}^2$ ) were then computed and plotted in Fig. 1.

### 2.2 FTIR Analysis

The polymeric identity of all collected particles  $>700 \mu\text{m}$  ( $n = 4050$ ) was verified through FTIR analyses. The subset was considered highly representative since it comprised 96.16% of the total weight of collected plastic. Analyses were performed using a Cary 660 FTIR spectrometer (Agilent) equipped with ATR (GladiATR Diamond Crystal Plate, Pike technologies). 128 scans per particle were performed, and  $\text{CO}_2$  interference ( $2300\text{--}2400 \text{ cm}^{-1}$ ) was removed for clarity. For each particle,



**Fig. 1** Map of the study area showing the location of all sampling stations and the distribution of total plastic concentrations expressed as **a** number of particles/ $\text{m}^2$  and **b** grams/ $\text{km}^2$ . Reprinted from [4], Sci. Rep. 6, 37551 under open copyright licence

scans were performed with a resolution of  $4 \text{ cm}^{-1}$ . Agilent Resolution Pro v5.2 was used for the output spectra, and identification of polymers was performed by comparison with a library of standard spectra. Only polymers matching reference spectra for more than 60% were accepted.

### 2.3 Statistical Analysis

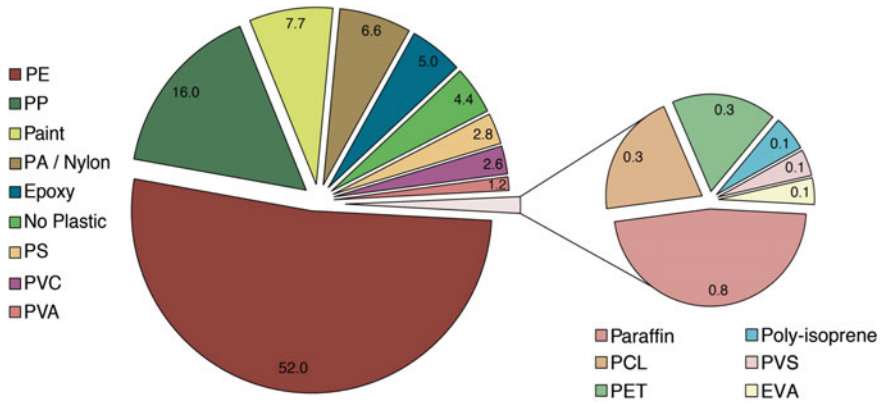
Principal component analysis (PCA) was based on a variance–covariance matrix of the relative frequencies of the seven most common polymers: PP, PE, PS, PA, PVC, nylon and paint. Mann–Whitney U test was used to verify significant differences between sub-basins. Normality and homogeneity of variance was checked, and the level of statistical significance was set at  $p < 0.05$ .

## 3 Results

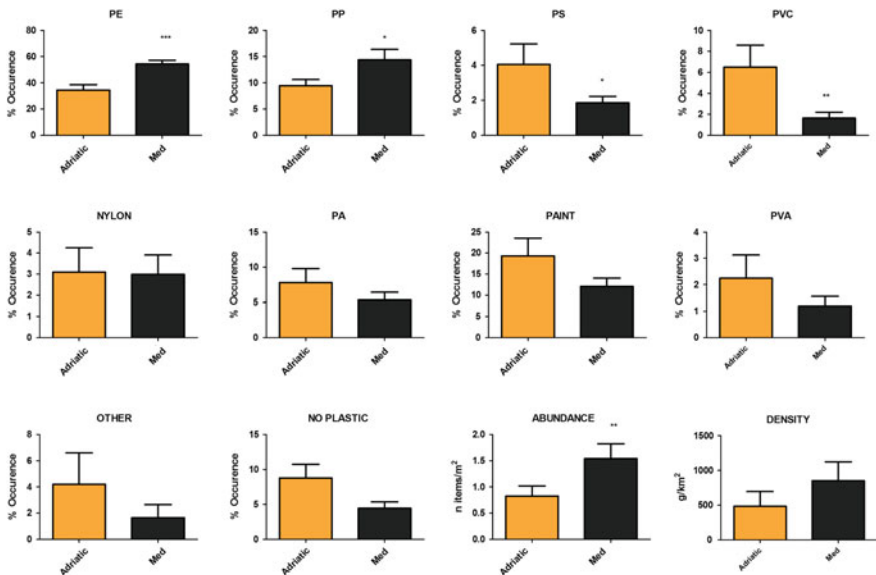
Plastic-like particles were found in all samples with a mean total concentration of  $1.25 \pm 1.62 \text{ particles/m}^2$  and  $703.16 \pm 1573.95 \text{ g/km}^2$ . Plastic concentrations showed a very high spatial heterogeneity spanning two or three orders of magnitude across the study area. The highest value ( $9.23 \text{ particles/m}^2$ ) was found in the Corsica Channel, while the lowest concentration was observed in the southern Adriatic ( $0.04 \text{ particles/m}^2$ ). Overall, plastic particles were significantly ( $p = 0.002$ ) less abundant in the Adriatic Basin ( $0.83 \pm 1.05 \text{ particles/m}^2$ ;  $485.07 \pm 1153.07 \text{ g/km}^2$ ;  $n = 30$ ) than in the western Mediterranean ( $1.54 \pm 1.87 \text{ particles/m}^2$ ;  $851.85 \pm 1803.66 \text{ g/km}^2$ ;  $n = 44$ ). Most of the 14106 collected particles were visually classified as irregularly shaped fragments (93.2%), while pellets, films and foams constituted only a small fraction of the total (2.2, 1.6 and 3.1%, respectively).

FTIR analysis revealed the presence of 16 different polymers (Fig. 2). Polyethylene (HD-PE and LD-PE) was the predominant form with an overall frequency of 52%, followed by polypropylene (PP) (16%) and synthetic paints (7.7%). Polyamides (PAs) accounted for 4.7% (excluding nylon which accounted alone for 1.9%), whereas polyvinyl chloride (PVC), polystyrene (PS) and polyvinyl alcohol (PVA) represented 2.6, 2.8 and 1.2%, respectively. Other less frequent polymers (<1%) included polyethylene terephthalate (PET), polyisoprene (synthetic rubber), poly (vinyl stearate) (PVS), ethylene-vinyl acetate (EVA) and cellulose acetate. Ten fragments of polycaprolactone, a biodegradable polymer, were found in seven different samples throughout the study area, while 201 fragments of epoxy resin (polyepoxide) were collected in the Balearic Sea. Similarly, residues of paraffin wax were exclusively found in an offshore sample in the Adriatic Sea. The molecular characterization also revealed that 4.4% of all analysed particles did not consist of plastic but were rather made of cotton, chitin, cellulose and other non-synthetic materials.

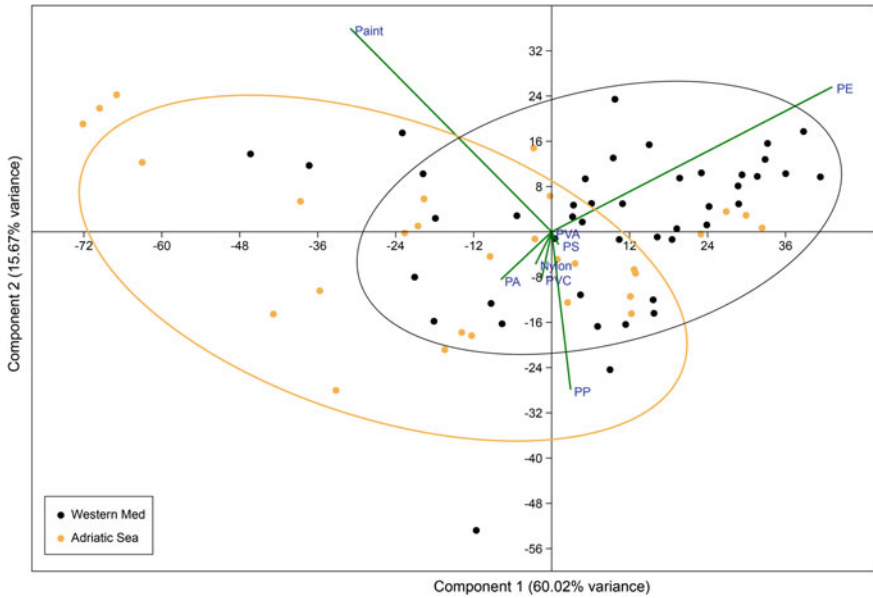
Geographical differences between Mediterranean sub-basins were found also in the relative occurrence of different polymers (Fig. 3). The composition of western Mediterranean samples was dominated by low-density polymers such as PE and



**Fig. 2** Polymeric composition of all particles >700 μm characterized through ATR FTIR analysis (n = 4050 particles). Values are expressed in percentages. Reprinted from [4], Sci. Rep. 6, 37551 under open copyright licence



**Fig. 3** Differences between Adriatic (n = 30) and western Mediterranean samples (n = 44) in the relative frequencies of the most common polymers identified through ATR FTIR (n = 4050 particles). Differences in the total abundance (items/m<sup>2</sup>) and density (g/km<sup>2</sup>) of plastic particles are also shown. Reprinted from [4], Sci. Rep. 6, 37551 under open copyright licence



**Fig. 4** PCA ordination based on the occurrence of the seven most common polymer typologies. Orange dots represent Adriatic samples ( $n = 30$ ), while western Mediterranean samples are in black ( $n = 44$ ). Distance biplot of the eigenvectors is not in scale with data points. Reprinted from [4], Sci. Rep. 6, 37551 under open copyright licence

PP. Adriatic samples instead were more heterogeneous and rather characterized by a higher presence of paint chips, PS, PVC, PVA and PAs. PCA ordination of samples produced a two-dimensional pattern, with the first two components explaining 75.7% of the total variance (Fig. 4). Despite some overlapping, most of the separation between sub-basins occurred along PC1 axis, mainly referring to PE (0.80) and paint (-0.57). On the other side, PP (-0.52) determined most of the separation along PC2.

## 4 Discussion and Conclusions

No clear accumulation pattern and a high small-scale variability in plastic abundance and composition emerged from our survey, similar to what was previously reported in the same area for floating macro-debris [4]. With this respect, the formation of permanent accumulation zones in the Mediterranean Sea is probably hampered by the highly dynamic character of the surface circulation, of which the observed heterogeneity in plastic distribution is very likely a reflection.

Our concentration values are substantially higher than most studies performed in the Mediterranean Sea. Nevertheless, a closer agreement is obtained when comparing

densities expressed in terms of mass concentrations, rather than particle counts. After removing two outliers from our data set for instance, our mean density drops to  $463.5 \text{ g/km}^2$ , which is incredibly similar to the values reported from the inner accumulation zones of all main oceanic gyres [6] and to the values of 423 and  $579.3 \text{ g/km}^2$  obtained in two other large-scale surveys of the Mediterranean Sea [3, 5].

The proportion of different polymers found in our study roughly corresponds to the global production stocks of plastic materials, with polyolefins (PE and PP) accounting for 62% of the global plastic demand [7] and for 68% of our collected particles. Being widely used in the disposable packaging industry and having lower densities than sea water, it is not surprising that polyolefins consistently account for the majority of the plastic particles floating in surface waters worldwide. Also being less susceptible to sinking, the contribution of these low-density polymers has been shown to increase with distance from land [8], hence potentially representing a proxy of the distance from pollution sources. From this perspective, the higher heterogeneity in the polymeric composition of Adriatic samples, together with a higher occurrence of high-density polymers, would indicate shorter residence times of particles at sea and a closer proximity to pollution sources, likely reflecting the distinctive hydrological features of the Adriatic Basin. Although the mechanisms through which high-density polymers can persist at the sea surface have to be clarified, the presence of paint and paraffin wax seems to suggest a high influence of ship-based pollution in the Adriatic Basin. Paint chips are typically generated during repair, maintenance and cleaning of vessel decks and hulls, and large quantities of synthetic paints have been related to intense shipping activities in Korean waters [9]. Paraffin wax, on the other hand, is transported in large quantities by cargo ships, and tank-washing residues may be legally discharged at sea beyond the 12 nautical miles, according to MARPOL regulations. Paraffin clumps were previously reported along the coasts of the North and Baltic Sea and in the stomach contents of northern fulmars [10]; nevertheless, its presence in Mediterranean waters had never been explicitly recorded before.

Polycaprolactone (PCL) instead is a synthetic polyester which is marketed as biodegradable, even though only some signs of degradation appear after 12 months in the marine environment [11]. PCL fragments were found in 9.5% of our samples, and its presence in Mediterranean offshore waters provides further evidence that some “biodegradable plastics” do not readily degrade in natural conditions, thus not representing an a priori solution for reducing marine litter.


In conclusion, our results demonstrate the pervasiveness of plastic pollution in Mediterranean waters and, confirming model predictions, provide further evidence that microplastic abundances are amongst the highest in the world. Sinks, sources, fate and residence times of different polymers at sea are the main knowledge gaps that need to be addressed, so that the global mismatch between plastic sinks and inputs can be eventually explained. The polymeric characterization of microplastics is of paramount importance for the effective identification of specific solutions and alternatives. However, irrespective of different sources and typologies, the problem of plastic pollution is a social and behavioural issue, whose causes required to be mostly sought upstream the consumption chain.

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# Floating Microplastics in the Northwestern Mediterranean Sea: Temporal and Spatial Heterogeneities

Mel Constant , Philippe Kerherve, Jennifer Sola, Anna Sanchez-Vidal, Miquel Canals and Serge Heussner

## 1 Introduction

Attention on marine litter, and their economic, social, and environmental issues, has been increasingly paid over the last few decades [1]. Litter has been found everywhere, from rivers to central open oceans, in coral reefs and polar oceans, floating at the sea surface, deposited on the seafloor, stranded on beaches or ingested by marine fauna (e.g., [2–7]).

Plastics can represent more than 70% of items described within floating marine litter [8]. Among plastic litter, an important part has a size below 5 mm (e.g., >98% [9]). These small size particles, called microplastics (MPs), can be ingested by small organisms, extending the problem to a large part of the marine trophic network.

Mediterranean ecosystems are exposed to high anthropogenic pressures such as densely populated coastlines, busy shipping routes, and strong tourism activities. In addition, the semi-enclosed morphology of the Mediterranean Sea leads to an accumulation and a density of marine litter as important as within the great accumulation zones in the oceanic gyres [10].

The Mediterranean Sea is one of the most sampled areas for floating MPs. However, only a few investigations have been conducted at small spatial and temporal scales in coastal areas. To overcome this gap, MPs (<5 mm) were

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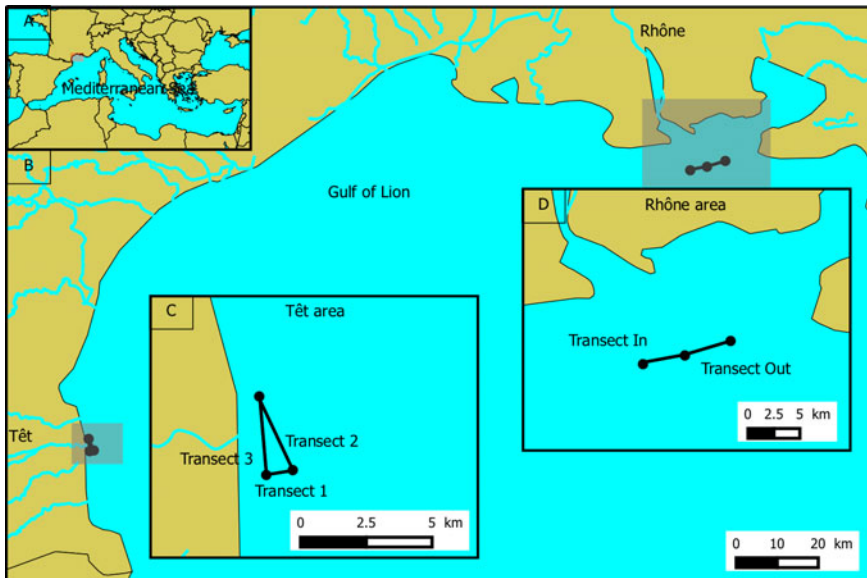
collected off the mouths of two contrasted rivers discharging into the Northwestern Mediterranean Sea: the Rhône River, the largest continental inflow to the Mediterranean Sea, and the Têt River, a small typical Mediterranean river.

## 2 Materials and Method

### 2.1 Sea Surface Sampling

Between May and November 2016, 13 samples were taken at different seasons in the Rhône area. Two transects were performed at the sea surface close to the river mouth. During the same period, a total of 18 samples were obtained in the Têt area. A triangle of three transects close to the river mouth was performed every month (Fig. 1).

Transects were carried out using a manta trawl ( $60 \times 25$  cm opening;  $333 \mu\text{m}$  mesh) towed at the top 20 cm of the sea surface at 2 knots for 30 min–1 h. Sampled water volumes were recorded with a mechanical flowmeter (Hydro-bios; model 438 110). Once on board, the trawl was rinsed, the content of the collector was emptied over a  $200 \mu\text{m}$  metal sieve and then transferred to glass bottles.



**Fig. 1** Map of sampling sites. **A:** Mediterranean Sea; **B:** Gulf of Lion; **C:** Têt area; **D:** Rhône area. Grey rectangles represent zoom box **B**, **C** and **D**

## **2.2 *Laboratory Work***

Contamination of the samples was minimized by wearing laboratory cotton coats and using equipment made of glass or steel. Every step with an air exposure was performed under a laminar flow cabinet and beakers were covered by an aluminum foil.

### **2.2.1 Preparation of Samples**

Collected materials were transferred into beakers and biological debris was removed using hydrogen peroxide  $\text{H}_2\text{O}_2$  (modified from [11]). Two mL of  $\text{H}_2\text{O}_2$  40% were added repeatedly until most of the organic matter was digested. During the entire digestion process, beakers were heated at 50 °C on a heating plate. The resulting solutions were filtered on paper filters (Whatman). Filters were examined under a Wild Heerbrugg dissecting stereomicroscope (25 × and 50 × magnifications). MPs were separated into four categories: fibers (including filaments and fishing lines), fragments (3-D pieces of plastic, including spherules), films (2-D pieces of plastic), and foams (pieces or spherules with a spongy, soft structure).

### **2.2.2 Polymer Identification**

Forty fibers and 80 other items of each category were randomly selected and analyzed for polymer nature. Fibers were analyzed with an FTLA2000 FTIR spectrometer (ABB). Other particles were analyzed with a Frontier FTIR spectrometer (PerkinElmer). Following background scans, 100 scans per particle were performed. For each particle, scans were performed between 4000 and 700  $\text{cm}^{-1}$ . Essential FTIR trial version software was used for the output spectra and identification of polymers was performed by comparison with a self-collected spectrum database.

## **2.3 *Data Analysis***

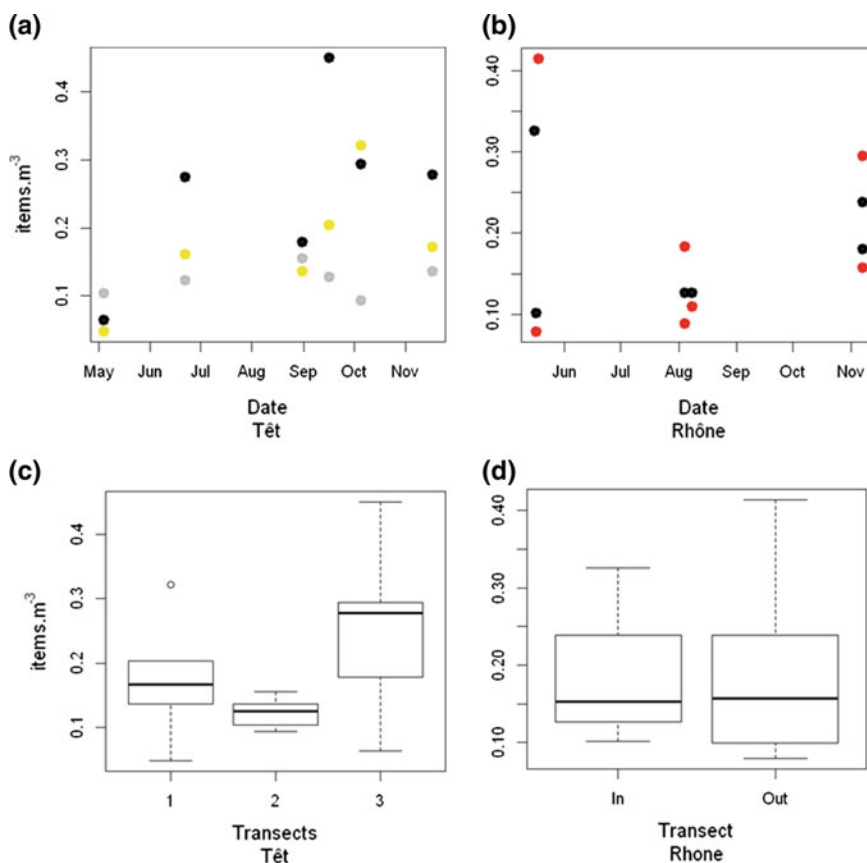
FTIR analyses allowed to estimate the number of plastic items collected. For each category, a “plastic” ratio was applied to the number of collected particles. This ratio results from the division of the number of particles which have a “plastic spectra” with the total number of particles analyzed. Finally, concentrations of MPs were estimated by dividing the adjusted numbers of plastic particles by the sampled water volumes.

### 3 Results and Discussion

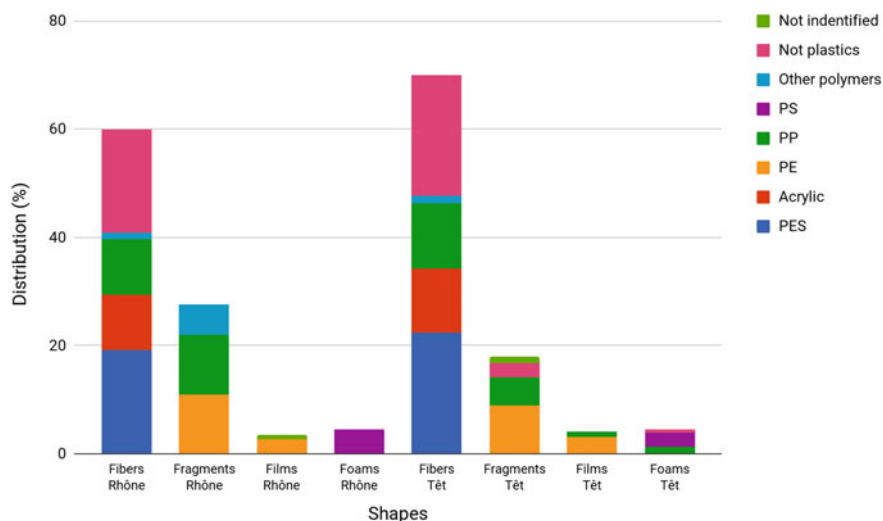
#### 3.1 Results

Adjusted concentrations ranged from 0.05 to 0.45 items.m<sup>-3</sup> for the Têt area with an average of 0.18 items.m<sup>-3</sup> (Fig. 2a) and from 0.08 to 0.41 items.m<sup>-3</sup> with an average of 0.19 items.m<sup>-3</sup> for the Rhône area (Fig. 2b), which is not significantly different (t-test; p-value = 0.96).

Concentrations inside the Têt area were most of the time higher at transect 3 (Fig. 2c), but differences between the three transects are not significant (ANOVA; p-value = 0.168). Concentrations for each transect have changed highly during the



**Fig. 2** Concentration of MPs (items.m<sup>-3</sup>) during the year 2016 **a** in the Têt area (yellow dots: transect 1; grey dots: transect 2; black dots: transect 3) and **b** in the Rhône area (black dots: transect In, red dots: transect Out); Boxplot of MPs concentration according to transects **c** in the Têt area and **d** in the Rhône area. “In”: in the Rhône plume, “out”: upstream the Rhône plume



**Fig. 3** Distribution of shape categories and corresponding polymer nature

year but combined concentrations did not significantly differ between months (ANOVA;  $p$ -value = 0.293).

Similarly, concentrations inside the Rhône area were not significantly different between the two transects (Fig. 2d;  $t$ -test;  $p$ -value = 0.91) or between the three different sampling periods (ANOVA;  $p$ -value = 0.274). Nevertheless, concentrations could occasionally change by a factor of 5 between two consecutive days at the same location (e.g., May samples), and by a factor of 3 between two consecutive trawls on the same day (e.g., August samples).

The Rhône and the Têt areas had similar categorical distributions (Fig. 3). Fibers were the most abundant shape (60–70%), followed by fragments (18–28%). Foams and films were less represented (3–5%). FTIR analysis indicates that fibers were polyester (32%), cotton (32%), PP (17%), acrylic (17%), and PA (2%). Fragments and films were mostly PE (54%) and PP (17%), while foams were essentially made of PS (67%).

### 3.2 Discussion

The Rhône River is the largest source of freshwater and sediments into the Mediterranean Sea while the Têt River is a typical small Mediterranean coastal river, with extreme low-water stages and occasional massive flood events. MPs concentrations in the downstream parts of both rivers ranged between 1 and 2 items.m<sup>-3</sup> (Constant, unpublished results), thus, about 10 times more than off

both mouths. This strong decrease in concentration has been mainly attributed to the oceanic dilution [12].

The Rhône River has an average water flow 200 times higher than the Têt River. The total amount of MPs discharged by the Rhône River, so far not precisely calculated, should be, due to the close riverine concentrations, much higher than those released by the Têt River. One could have thus expected surface concentration differences more in connection with the large differences in inputs rather than the observed similarities between both areas. These preliminary results suggest that the influence of rivers on the spatial distribution of MPs rapidly decreases with distance to the river mouths, a hypothesis supported by the fact that highest concentrations off the Têt River were observed in the transect closest to the river mouth. Alternately, the similarities in offshore MP concentrations could also indicate that the floating plastics collected within our experimental areas are not provided by straight, direct river inputs. Some mixing and dispersion processes must rapidly take place once the MPs enter the marine environment.

Concentrations estimated in this study are relatively close to previous observations from the Northwestern Mediterranean (0.1–0.3 items.m<sup>-3</sup> [13–15]) or in the costal water of the East China Sea (0.2 items.m<sup>-3</sup> [12]), but 45 times lower than concentrations observed along the Israeli coast (7 items.m<sup>-3</sup> [16]) or in the Southern California coastal waters (7.3 items.m<sup>-3</sup> [17]). Such differences may have numerous reasons ranging from hydrodynamic features to the use of different trawls and methodologies. Indeed, most of the old investigations did not check the polymeric nature of their collected particles and especially fibers, probably leading to overestimations.

No spatial or temporal trends were found but high changes at daily and kilometer scale were observed. Presence of lint composed of hundreds of fibers can partly explain the high differences observed at small scales, as well as fast-changing river inputs.

Strong spatial heterogeneities of floating MPs have been previously reported at regional scales, up to a factor of 40 [14] and 30 at subregional scales [13]. Van der Hal et al. [16] indicated a factor of 300 at subregional and seasonal scales. Collignon et al. [18] investigated fluctuations of a costal transect on a semi-monthly basis and observed differences up to a factor of 60 during the same week. Wind had been found to be a factor of heterogeneity [15]. Large hydrological structures, like central oceanic gyres, accumulate MPs [2, 10] but influence of meso and small structures, like eddies and fronts, remain unknown.

## 4 Conclusions

This study confirms that heterogeneities of floating MPs can act at small scales. These extended observations in the NW Mediterranean coastal environment underline the necessity of performing replicate sampling to get a better insight into the spatial and temporal distribution patterns of these worrying pollutants.

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# Microplastic Abundance and Polymer Types in a Mediterranean Environment

Nikoletta Digka, Catherine Tsangaris, Helen Kaberi,  
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## 1 Introduction

Microplastics have become a more and more dominant threat to marine ecosystems. The ubiquity of microplastics is one of the major problems; from the sea surface and water column to the beach and seabed sediment or even ingested by marine organisms, small plastic particles have been found. This study is focused on monitoring and assessment of microplastic pollution (plastic particles <5 mm) on the sea surface, in beach sediments, and in marine biota in the Corfu Island area (Northern Ionian Sea). Corfu is one of the main touristic destinations in Greece, and thus tourism together with maritime traffic is the main source of litter on the beaches as well as in the marine environment. This study analyzes microplastic pollution of the region by determining microplastic abundance, types, and polymer composition in three environmental compartments (sea surface, beach sediment, and marine biota).

## 2 Materials and Methods

### 2.1 Study Area and Sampling

Samples from water, sediment, and marine biota were collected from the Northern Ionian Sea. All sampling was conducted in 2014 and 2015 according to [1]. More precisely, sea surface samples were collected during April 2014 and October 2015,

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beach sediment samples were collected during July of 2014 and 2015, while fish and mussel were caught in April and July of 2015, respectively. Sea surface samples were collected by manta net transects (upper 20 cm of the water column) on 3 areas around Corfu Island (Otranto Straits, North Ionian waters, and Kerkyraikos Gulf). Sampling of beach sediment (top 3 cm of sand) was performed by hand on 2 beaches located on the W–SW side of Corfu Island (Halikounas, Issos) and 1 beach on the northern part (Acharavi). Fish (*Sardina pilchardus*, *Pagellus erythrinus*, and *Mullus barbatus*) were caught offshore North Corfu by trawling nets, while mussels (*Mytilus galloprovincialis*) were collected by hand in the port of Corfu and a mussel farm in Thesprotia. In total, 30 manta net tows of ~30 min each (total number of particles  $n = 15,217$ ) were conducted and 34 sediment samples were collected from three beaches. A total of 160 individuals were tested for fish (80) and mussels (80) separately.

## 2.2 *Detection and Quantification of Microplastics*

Sea surface samples were sieved to separate microplastics into small (<1 mm) and large microplastics (1–5 mm) and then dried for 24 h at 90 °C. Large microplastics were visually sorted, while small microplastics were separated by digestion in hydrogen peroxide [2], filtration (pore size 1.2 µm), and observation under stereoscope. In order to separate microplastics from beach sediment, samples were sieved and the retained material was counted and weighted. For small particles (<1 mm), beach sediments' samples were filtered under vacuum on fiberglass filters, after density separation with NaCl solution (1:4 v/v). Filters were dried at room temperature and observed under stereomicroscope. Tissue from fish (stomach and intestine) and mussels (digestive glands and gills) was dissected, weighted, and digested by hydrogen peroxide to enable microplastic detection [2]. Tissue samples were placed into glass beakers in 1:20 (w/v) H<sub>2</sub>O<sub>2</sub> (30% H<sub>2</sub>O<sub>2</sub>, Chem-Lab, Germany) and heated on a hot plate at 55–65 °C until H<sub>2</sub>O<sub>2</sub> was evaporated. Samples were diluted with 100 ml of purified water (Milli-Q), stirred, and filtered under vacuum on fiberglass filters (Whatmann, GE Healthcare, UK), which were placed in petri dishes and dried at room temperature. Filters were examined under a stereomicroscope (Olympus, SZE and SZX7) for items resembling microplastics. Using a digital camera, items were photographed, counted, and categorized according to maximum length, color, and shape (fragment, filament, and film). Microplastics were analyzed by the use of Fourier-transform infrared spectroscopy (FTIR). This technique was used to confirm the synthetic polymer origin of microplastics that were large enough to handle with forceps and analyzed on an Agilent Cary 630 FTIR spectrometer using a self-generated polymer library.

### **2.3 Contamination Precautions**

During all processes (sampling, tissue dissection and digestion, filtration, and stereoscopic observation), contamination by airborne fibers was minimized. During sediment sampling, metal scoops, trowels, and quadrates were used and all equipment were cleaned prior to sampling and wrapped in tinfoil or stout paper. Samples were collected and stored in stout paper bags/envelopes, metal, or glass containers. People undertaking the sampling avoided any synthetic clothing, while the position of the person sampling was downwind of the sampling area. All glassware used in the laboratory was rinsed thoroughly with purified water. For initial examination of fish gastrointestinal content, the stereomicroscope observation area was isolated using a plastic cover. Samples were covered during digestion and when not in use. A glove bag was used as working area for sample rinsing and filtration. Filters were covered with glass lids during observation under a stereomicroscope [3]. To monitor air contamination, procedural blank samples were used.

## **3 Results**

### **3.1 Microplastic Abundance**

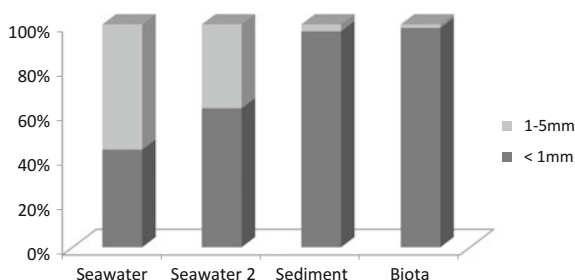
The abundance of microplastics in sea surface water ranged from 0 to 1.61 particles/m<sup>2</sup>. The average abundance of large microplastics in beach sediments ranged from 17 to 95 items/m<sup>2</sup>. The average number of small microplastics (<1 mm) detected in seawater samples and beach sediment samples was 0.18 particles/m<sup>2</sup> and 1760 particles/m<sup>2</sup>, respectively. The average number of large microplastics (1–5 mm) detected in seawater samples and beach sediment samples was 0.23 and 56.7 particles/m<sup>2</sup>, respectively (Table 1). Out of all fish and mussels tested, the percentage of individuals detected with microplastics was 41.25 and 46.25%, respectively. The average abundance of small microplastics (<1 mm) in positive fish and mussels was 1.66 particles/fish and 1.83 particles/mussel, while the average abundance of large microplastic (1–5 mm) was 0.0 particles/fish and 0 in mussels (Table 1).

### **3.2 Size Classes**

Microplastics detected in all compartments were categorized based on their size to two classes: small microplastics (<1 mm) and large microplastics (1–5 mm). Large microplastics were the most abundant size class in seawater (56.2%). However, when excluding the outliers (seawater 2), the percentage of small microplastics in

**Table 1** Number of analyzed samples, percentage of samples containing microplastics, average number of items per m<sup>2</sup> or individual in surface water, beach sediment, and biota

Environmental compartment		No. of samples	% positive samples	Size classes (mm)	Average items/m <sup>2</sup> items/ind.
Surface water		30	96.66	<1	0.18
				1–5	0.23
Sediment		34	100	<1	1760
				1–5	56.7
Biota	Fish	80	41.25	<1	1.66
				1–5	0.06
	Mussel	80	46.25	<1	1.83
				1–5	0.00

**Fig. 1** Percentages of microparticles size classes in all compartments

seawater (<1 mm) increases (62.3%). On the contrary, 96.9 and 98% of total microparticles were smaller than 1 mm in beach sediments and tissue samples from fish and mussels, respectively (Fig. 1).

Regarding floating microplastics, it appears that this size distribution changes dramatically when 6 outlier samples (>600 large particles per sample) are excluded. In this case, the two size classes are found to correlate positively with each other ( $R^2$  0.81;  $p < 0.05$ ), and the two size classes account for 72% for the small (<1 mm) and 27% for the large (1–5 mm) particles. All outlier samples were encountered in Kerkyraikos Gulf and were associated with the presence of seaweed.

### 3.3 Microplastic Types

Microplastics detected in all samples were categorized based on their shape into fragments and filaments (Table 2). Pellets, pieces of Styrofoam, and films were counted as fragments in the present analysis. Specifically, in sea surface samples both in small and large microplastic classes, the dominant type was fragments (99.7 and 100%, respectively). Filaments in both size fractions had a very small

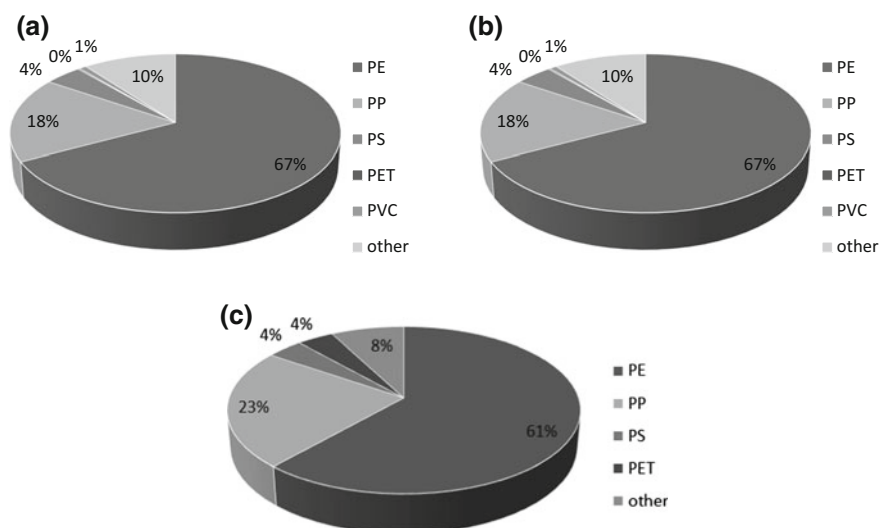
**Table 2** Distribution of microplastic type among size classes and environmental compartments

Environmental compartment	Size classes (mm)	No. of filaments	No. of fragments	% filaments	% fragments
Sea surface	<1	15	6644	0.23	99.77
	1–5	0	8472	0.00	100
Sediment	<1	719	36	95.23	4.77
	1–5	4	1656	0.24	99.76
Biota	<1	27	96	21.95	78.05
	1–5	0	2	0.00	100

contribution, while pellets were absent. In beach sediments, filaments were the most abundant microplastic type detected among the small microparticles (95.2%), while fragments dominated the large microparticles (99.7%). In marine biota, fragments are dominant in both size fractions; fragments are the 78% of the small microplastics and the 100% of large microplastics.

### 3.4 Polymer Type

Polymer type (polyethylene: PE, polypropylene: PP, polystyrene: PS, polyethylene terephthalate: PET, polyvinyl chloride: PVC, and polyurethane: PU) of microparticles was identified by FTIR. PE was the most abundant polymer type, followed by



**Fig. 2** Average percentages (%) of polymer type distribution **a** sea surface, **b** beach sediment, and **c** biota

PP in all environmental compartments (Fig. 1). More specifically, in seawater samples, the average percentages of the polymer types of the microparticles identified were 67.2% for PE, 17.3% for PP, 4.2% for PS, 0.2% for PET, 0.8% for PVC, and 10.3% for other polymers. In beach sediment samples, the distribution of polymer types was 43.5% for PE, 38% for PP, 5.7% for PS, 6% for PET, 0.6% for PU, and 7% for other polymers. Finally, in biota, the average percentages of the polymer types identified were distributed as followed: 61.5% for PE, 23.07% for PP, 4.2% for PS, 3.8% for PET, and 7.6% for other polymers (Fig. 2).

## 4 Discussion and Conclusions

The ubiquity of microplastics in the marine environment has been previously reported [4]. This is the first study in the Northern Ionian Sea presenting an integrated picture of microplastic pollution. Agriculture, fisheries, aquaculture, maritime transport, and tourism are the main activities that influence this area and likely sources of marine pollution and eventually microplastic pollution.

Our results show that the study area is contaminated by microplastics in all studied compartments. Microplastics have been found in 96.66% of the seawater, 100% of beach sediment samples analyzed, and in 43.75% of the biota samples examined. The average abundance of large microplastics (1–5 mm) in beach sediments (56.7 particles/m<sup>2</sup>) lies between the lower levels reported in the literature, whereas that of the small microplastics (<1 mm, 1760 items/m<sup>2</sup>) is higher than the estimated global abundance [5]. Average number of microplastics in seawater (0.18 particles/m<sup>2</sup>) is comparable to values reported in previous works for the Mediterranean Sea [6, 7]. Levels of microplastics ingested by fish and mussels (1.66 particles/fish and 1.83 particles/mussel) fall in the range of values reported elsewhere [8, 9].

All studied compartments are contaminated by microplastics of both size classes (<1 mm and 1–5 mm). From the results of the present integrated study, it appears that small microplastic particles, abundant in surface seawater, tend to accumulate also on sandy beaches. Several mechanisms can be proposed such as the higher retention efficiency of the smaller particles by the sand while large-sized ones are easily backwashed through the sand pores. The abundance of large microplastics in samples from Kerkyraikos Gulf showed that large microplastics could be preferentially exported onto beaches through collision on seaweed. Regarding the marine organisms investigated (sardines, common pandora, red mullets, and mussels), it is clear that they ingest mostly small microplastics which can be related to their feeding preferences.

A clear dominance of fragments was found in all environmental compartments, with the exception of beach sediment samples where filaments were most abundant in the small microplastics. Our results show that microplastic pollution in Corfu Island is mainly due to low-density polymers (PE, PP, PS), in agreement with previous works for the Mediterranean Sea and worldwide [7, 8]. Furthermore, the

proportion of PE found on the sea surface (67%) and ingested in biota (61%) reflects the global production and use of this material [11]. Microplastics characterized as PET, which is a high-density polymer, were almost absent in the sea surface samples, and its contribution was little in beach sediment and biota samples.

It has been shown that microplastics carry chemical compounds which may penetrate into cells and be biochemically active [10]. The presence of microplastics raises concerns of the potential presence of chemically hazardous compounds in sea surface, beach sediments, and marine biota of the study area.

In conclusion, this study provided an assessment of the microplastic pollution of the three environmental compartments examined in Corfu Island area. All compartments were detected with microplastics. Fragments were the most common microplastic type found, and the most frequent polymer type was PE. In biota, the most abundant microplastics are the small-sized microplastics (<1 mm). The present work describes a holistic image of the pollution caused by microplastics in the study area and can be useful for an integrated microplastic monitoring.

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# TARA Mediterranean Expedition: Assessing the Impact of Microplastics on Mediterranean Ecosystem

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## 1 Introduction

The accumulation of plastic debris on the surface of the oceans is widely recognized as a newly emerging problem for worldwide marine systems. Yet, too little is known about the fate of this plastic and its role in ecosystem dynamics to predict the inevitable impacts on the marine biodiversity and on human health. Under the effect of circular oceanic currents, plastic accumulate in the world's oceans, with five main accumulation zones in the subtropical gyres but also even the most remote polar seas areas are submerged with plastics debris [1–3].

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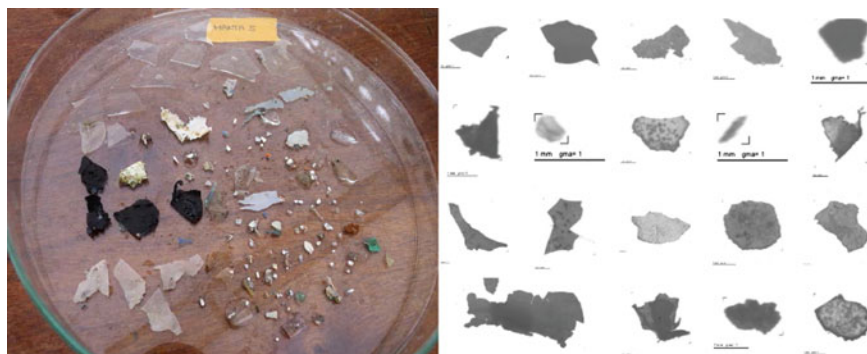


In Mediterranean Sea, recent results on the impact of microplastics showed that plastic concentration in the Basin is similar to that of the inner accumulation zones of the subtropical ocean gyres [4–6]. The Mediterranean Sea is the largest and deepest enclosed sea on Earth and one of the main world’s shipping routes with 30% of the world sea traffic [7]. The Basin is an essential economic source for the neighboring countries where intense fishing and touristic activities taking place. The consequence is a strong demographic pressure around the coastal areas with 466 million inhabitants and by 2050 they expected to become approximately 600 million [8]. Mediterranean Sea has no significant surface outflow and is subject to permanent waste inputs occurring on the continental shelf, by the river outflow, overland flow and by winds that flush wastes toward offshore waters [9]. Plastic composing more than 80% of observed marine litter and constitute the most important part of floating marine litter, comprising sometimes up to 100% items [10]. Due to the important variability of the circulation system in Mediterranean Sea [11], we have little or no information on trends of microplastics, and it is not clear whether this small debris is transported in the same manner as larger items. Additionally, we have little information on the Eastern part of Mediterranean Sea.

To address this critical knowledge gap, TARA Mediterranean the first large-scale expedition with cross-disciplinary approach crossed the entire Mediterranean Sea in 2014. The aim was to better understand the ecosystem-level impacts of surface plastics in the Mediterranean Sea. The spatial distribution of floating plastic fragments (0.3–50 mm) was assessed concomitantly with the ecosystem structure of plankton in contact with plastic fragments. Acquisition of environmental descriptors—T, S, turbidity, pigments, ocean color was also achieved in order to describe the physical structure of the surface water masses during the sampling period.

## 2 Methods

Surface floating microplastics were collected in the Mediterranean Sea in the framework of the TARA Mediterranean Expedition from May to November 2014. Sampling designed to the study of plastic and plankton distribution was performed during day and night at different locations across the Basin. Surface samples were collected with a 330  $\mu\text{m}$  Manta net towed at an average speed of 2.5 knots during 60 min at the top 10 cm of the sea surface (neustonic layer). The physic-chemical samples were collected using a SBE thermosalinograph and a hyperspectral ACs. Ocean color satellite images were supplied by ACRI-ST and the Mercator circulation model was used to determine the zones of interest for the sampling.



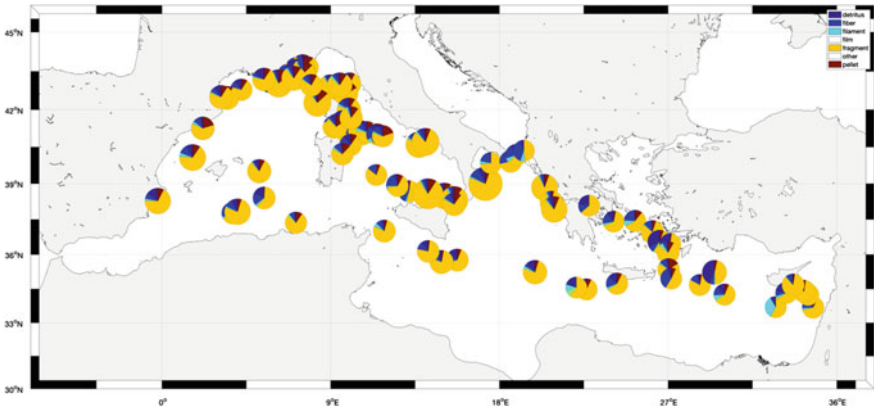
**Fig. 1** Plastic fragments separated from organic material containing zooplankton and imaged by ZoosCan. Bars = 1 mm

## 2.1 Sample Processing

The plastics and plankton were sorted from 124 samples using a dissecting microscope and analyzed at the Villefranche Oceanography Laboratory (LOV-Université Pierre et Marie Curie, France). Plastic particles were removed from preserved organic material containing zooplankton and organic tissues. Microplastics and zooplankton were enumerated, identified, and digitally imaged with a Zooscan digital scanner with a resolution of 2400 dpi [12], (Fig. 1). Image post-processing was performed with the Zooprocess plankton Identifier software that enumerates and gives a large set of morphological parameters for each object such as the ferret length (approximately equivalent to particle length), circularity, and two-dimensional surface area (mm). Moreover, the taxonomic identification of TARA neustonic samples collected was analyzed by different taxonomist expert of the different Mediterranean basins belonging to the consortium. Particle characteristics were determined and the distribution and concentration of floating microplastics were compared to the abundance of the neustonic zooplankton. Plastics samples extracted were then transferred to the LERPAC Ifremer station in La Seine sur Mer (France) to be weighted. Plastic were separated in three size class (<5, >5, >20 mm) counted and the total dry weight was recorded.

## 3 Results and Discussion

The first global biogeography map of the 98 dominant zooplankton groups and six categories of plastic particles in the basin were established. Results showed that plastic debris were present in all Manta tows. The vast majority of the plastic items analyzed contained mainly fragments (Fig. 2 ) but different typology described as foam, filaments, film, row pellets and fibers were also present. Fibers are likely



**Fig. 2** Plastic sampling during the Tara Mediterranean Expedition (2014) with different categories of floating plastic debris

underestimated because they are also classified among aggregates. To confirm the plastic nature of the material, collected samples are analyzed in LMBA laboratory (Université Bretagne Sud, France) using the Fourier transform infrared spectrometer technics. The average surface debris concentration over the entire survey was  $2.6 \times 10^5$  particles  $\text{km}^2$  with values varying from  $2 \times 10^3$  particles  $\text{km}^2$  in the Eastern basin, to more than  $2 \times 10^6$  particles  $\text{km}^2$  in the Western basin. Coastal zones of Naples, Corsica, and Marseille were clearly identified as areas of particularly high plastic concentration. The high ratio of plastic abundance to zooplankton abundance may suggest a potential impact of microdebris on various taxa and their incorporation into the neustonic food web, with consequences on the pelagic biota in the most polluted areas.

The next step is to link the basin-wide distribution of microplastics to surface circulation models in order identify hotspots of plastic accumulation and predict the pathways and fate of plastic debris in the Mediterranean Sea. Ecological implications of microplastics in the Mediterranean are the subject of work in progress conducted by the 20 partner institutions of TARA-Med Consortium.

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# Statistical Methodology for Identifying Microplastic Samples Collected During TARA Mediterranean Campaign

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## 1 Introduction

The annual global plastic production rose from 1.7 million tons in 1950 to 322 million tons in 2015 [1]. This is caused by the increase of consumption of this costless and light material in different field such as packaging consisting mainly in polyethylene and polypropylene. Despite the national laws and local management for recycling, a large part of these plastics is introduced into the natural environment and ended after water and wind transportation in the oceans [2, 3]. In fact, from 4.8 to 12.7 million tons of plastics would be released every year in the oceans [4]. This plastics' pollution stands on 88% of the ocean surfaces [5]. Different expeditions are running around the world to sample microplastics and analyse them since 2010. Two major expeditions were led in the Mediterranean Sea: Expedition MED which is conducted every year [6] and Tara Mediterranean Expedition in 2014. During the sampling campaign, a high number of plastic particles were collected. Microplastics must be analysed in order to determine their chemical nature. Nevertheless, the different technics developed to chemically characterise microplastics by micro- or macro-spectroscopy are time-consuming [7] and stand as a barrier to work on all samples from large libraries.

There are three ways to deal with this important number of plastics to characterise it: semi-automated spectroscopy methods [7], a physical method to separate microparticles according to their categories or a statistical method. The third

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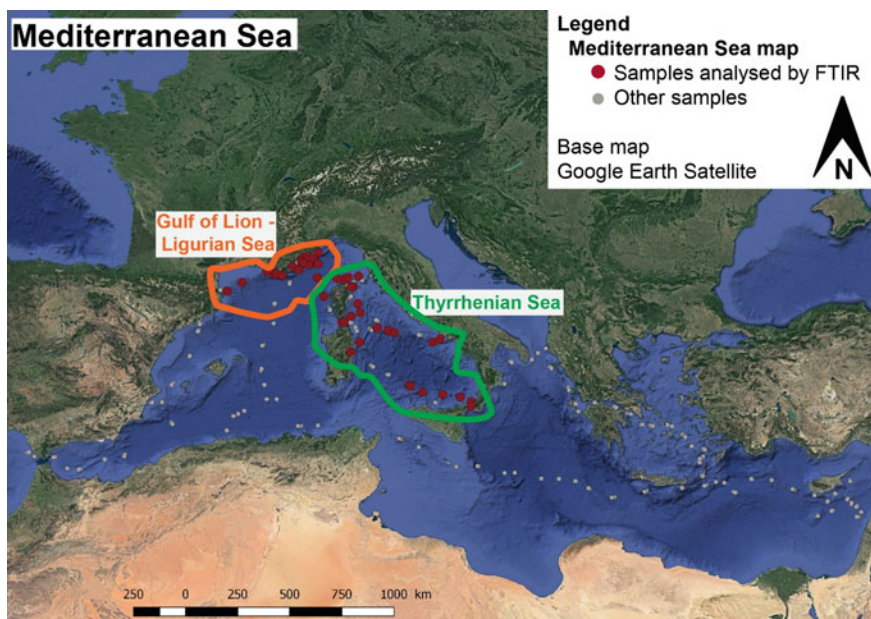
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way was not so much investigated according to the literature but, it could be an interesting way to obtain an overview on each campaign with lowest incertitude.

## 2 Experimental

### 2.1 Materials

Analysed microplastics have been sampled during the TARA Mediterranean cruise (June to November 2014) using a Manta trawl net with 333  $\mu\text{m}$  mesh size [6] (Fig. 1). The total library counts 75,000 microplastics collected from sampling sites across the Mediterranean basin, each comprising between 11 and 8000 samples and size ranging from 5 mm to 32  $\mu\text{m}$ . Seven basins were determined according to the Mediterranean geographical areas and to the collecting effort done during the campaign. For this first study, we focused on samples from the Western Mediterranean basin. Samples from 77 sites are available in the IRDL laboratory. The first analysis work focused on the Gulf of Lion-Ligurian Sea basin (22 sites). Laboratory work will continue with the Tyrrhenian Sea basin (26 sites) (Fig. 1). Around 25,000 microplastics were collected in these two basins.



**Fig. 1** Sampling effort during the Tara Mediterranean campaign (2014) and samples analysed by Fourier transform infrared macro-spectroscopy (FTIR) in the IRDL laboratory

## 2.2 *Methods*

### 2.2.1 Preparation of Samples

Samples were first counted in the LERPAC Ifremer station in La Seine sur Mer (France). After being analysed in Villefranche Oceanography Laboratory (UPMC: Université Pierre et Marie Curie; France) by Zooscan method [6], samples were given to the IRDL laboratory for chemical characterisation. At this stage of the work, microplastics were already separated from plankton. Moreover, particles collected in the same site were consolidated in one vial.

The contamination risks were avoided as far as possible during the sample preparation stage by cleaning the different material and by working in controlled conditions. The first step consists in wet sieving to separate microplastics following their size range. A column with ten different mesh sizes ranges from 5 mm to 32  $\mu\text{m}$  is used. The particles could then be sort out and isolate. This microplastic isolation permits to create a database giving a specific code for every particle. This code will be useful during random draw step fulfilled in the statistical method.

### 2.2.2 Analytical Techniques

A Fourier transform infrared spectrometer with a diamond attenuated total reflectance (ATR) was used. All spectra were obtained in the 4000–600  $\text{cm}^{-1}$  region with absorbance mode and 16 scans.

Statistical method was developed in cooperation with LMBA laboratory (Université Bretagne Sud, France). The aim is to analyse a representative stake of the global population from microplastics with a size range upper than 315  $\mu\text{m}$ . The 315  $\mu\text{m}$  size was chosen after a limit of detection test to determine the lowest size detected by the FTIR device. A total detection was obtained for the five replicates until 315  $\mu\text{m}$  plastic fragments. Moreover, this was the microplastics size range initially targeted by the Manta trawl mesh size.

The statistical method is based on two steps of random draw: first, the number of microplastics which need to be analysed by site is estimated. Then, the microplastics that will be characterised are determined by a second random draw using their codes given during the isolation process.

We first totally analysed by FTIR macro-spectroscopy samples from five sites with different density population (from 15 to 767 particles collected) to test the best parameters and the limit of the method comparing with the results obtained on the initial population on these five sites.

Then, 1056 microplastics out of 4130 from the Gulf of Lion-Ligurian Sea basin were selected with the random draw method in the aim to analyse their chemical nature.

### 3 Results and Discussion

The analysis of the first results done on the five sites which the chemical nature of the microplastics was fully determined, highlights the best combination of parameters for the statistical programme. It appeared that analysis of one-fifth microplastics randomly drawn from the initial population permits to be statistically significant. Furthermore, more the iteration number is high, more the samples randomly drawn are representative. The equilibrium of the proportions (mesh size and chemical nature) between the initial population characteristics and the randomly drawn one was obtained for 1000 iterations.

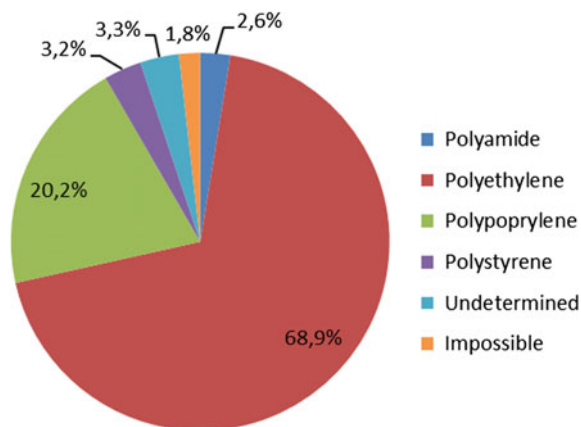
The preliminary results for the 1056 particles randomly drawn in the first basin (Gulf of Lion-Ligurian Sea) show that plastics analysed by FTIR consist mainly in polyolefins. In fact, we found 68.9% of polyethylene (PE) and 20.2% of polypropylene (PP) (Fig. 2). This occurrence could be explained by the fact that these polymers are mainly used for packaging which are single-used. Moreover, their light density comprising from 0.85 to 0.97 g mL<sup>-1</sup> [8] allow them to lay on the ocean surface.

We also found 2.6% of polyamide (PA) (Fig. 2). This synthetic fibre could come from textile fibres or from fishing lines [6]. PA is set up on the Mediterranean Sea surface because of the water density in this region. In fact, PA density ranges from 1.02 to 1.05 g mL<sup>-1</sup> [8] while the Mediterranean Sea density is around 1.026 g mL<sup>-1</sup> [6].

3.8% of polystyrene (PS) were characterised by FTIR macro-spectroscopy (Fig. 2); these particles could be transported by the wind on the surface of the ocean.

These results are consistent with the results obtained in other analyses of plastics collected in ocean surface [6, 8, 9].

**Fig. 2** Chemical nature of microplastics collected in the basin Gulf of Lion—Ligurian Sea (results based on the 1056 particles analysed after random draw using the statistics programme)





## 4 Conclusions

The statistical method is a new approach in the microplastics topic to deal with important number of particles to chemically characterise. Preparation step which could be time-consuming is needed to isolate particles. Nevertheless, using this approach permits to significantly decrease time spent in the laboratory during FTIR macro-spectroscopy while being representative. This method is an opportunity to help scientists to manage the important number of plastics collected during expeditions by getting a global overview on the different campaigns.

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# Toward 3D Modeling the Plastic Marine Debris in the Mediterranean

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## 1 Introduction

Ocean currents and spatiotemporal distribution of plastic inputs play first fiddle in the transport and fate of plastic marine debris in the Mediterranean [1–4]. Although the amount of research focused on other contributors has swelled in recent years, we are still far from clearly prioritizing the processes of (1) beaching and washing off; (2) vertical buoyant movement of plastics; (3) sedimentation and detachment from seabed; (4) fragmentation; (5) uptake and excretion by biota; etc. Hence, working at the Mediterranean basin- and sub-basin scales, we have started with a simple 2D stochastic model increasing the complexity in a gradual way, and thoroughly analyzing the contribution of each process.

At the outset, we implemented a 2D Markov chain model for the Adriatic Sea postulating that beaching is the main sink of the floating plastic debris [5]. Using the Lagrangian oil spill model MEDSLIK-II [6] forced by the Adriatic Forecasting System ocean current simulations [7] and ECMWF wind analyses, we computed a plastics' half-life of 43.7 days, which defined the Adriatic as a highly dissipative basin. Covering the period over 2009–2015, a predominant influence of the Western Adriatic Coastal Current and South Adriatic Gyre on dispersal of plastics from the realistically distributed Adriatic inputs was shown.

In the present work, some preliminary results are delivered regarding a new 2D Lagrangian tracking model implemented in the framework probabilistic oceanog-

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raphy. Our approach is based on (1) directly particle tracking from terrestrial and maritime inputs over January 2013–May 2017 (hereafter, 2013–2017); (2) ocean currents and the Stokes drift components provided by the Copernicus Marine Environment Monitoring Service (CMEMS [8]); a few parametric stochastic algorithms for (3) beaching; and (4) sedimentation of the plastics.

## 2 Modeling

### 2.1 *Plastic Inputs into the Mediterranean*

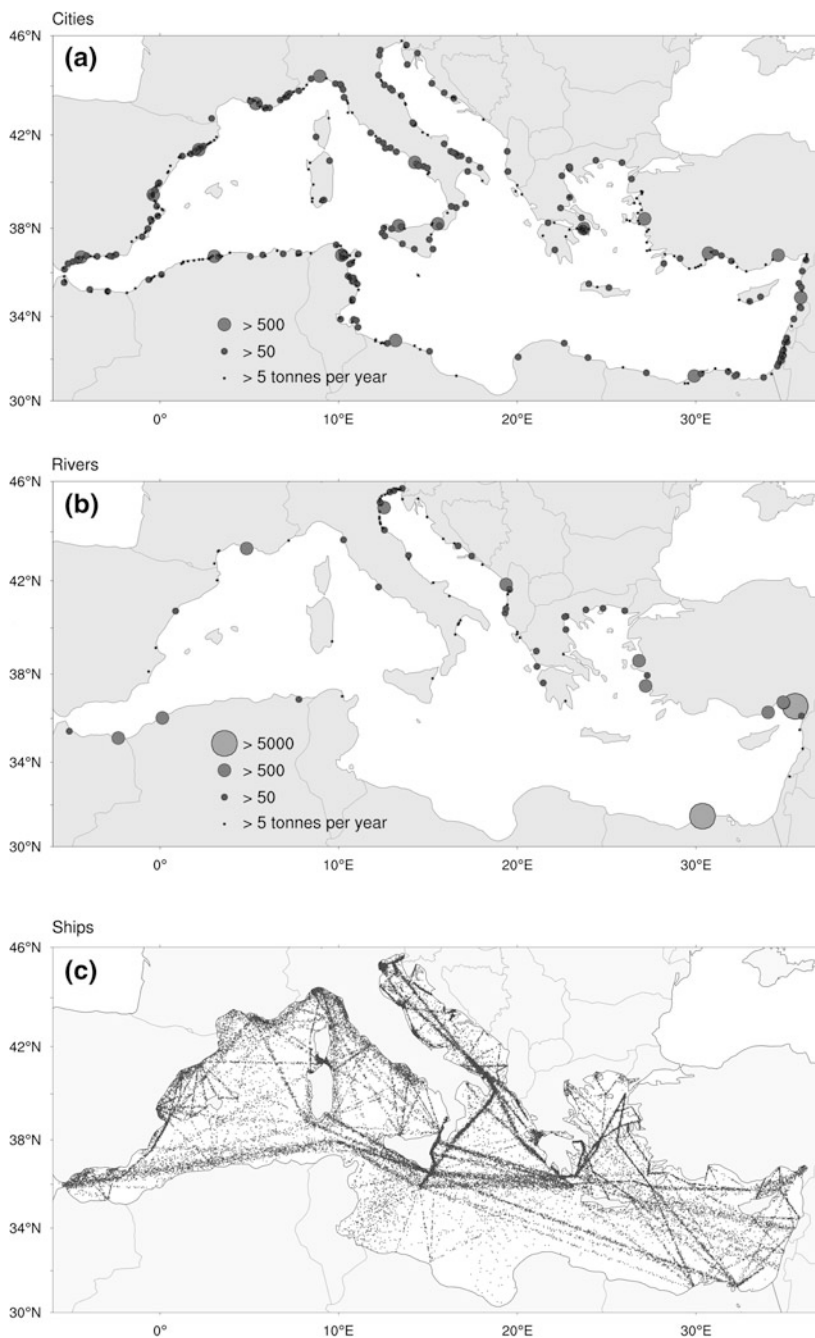
To our knowledge, the values of total plastic inputs into the Mediterranean Basin have not been updated as compared with [9, 10]. Hence, assuming a stable annual input of 100,000 tons of plastics into the Mediterranean, we apply the so-called cities/rivers/shipping lanes ratio of 50/30/20%, which is slightly shifted in relation to the values of 40/40/20% reported for the global ocean [11]. Then, a global map of mismanaged plastic waste [9] is used to normalize the inputs from different Mediterranean countries. Finally (Fig. 1), we take into consideration: (1) 110 riverine inputs, which are proportional to the average annual rivers' runoffs [12–14]; (2) 495 largest coastal cities, which are proportional to the human populations [15] (we include the cities inside a 10 km coastal belt, which contained more than 20,000 inhabitants in 2013); and 332 most congested shipping lanes from an annually averaged traffic density map provided by Automatic Identification System [16]. The latter are preprocessed by means of an original methodology [17].

The top 10 plastic litter inputs into the Mediterranean Sea are listed according to their relative contributions in Table 1.

Likewise for the Adriatic Sea [5], maritime inputs dominate in the Mediterranean. As shown in Table 1, the Nile River (6.8%) is a greatest contributor among the rivers, while Alexandria is a greatest one (2.2%) among the cities. In general, the ten main sources are responsible almost for 50% of total marine plastic inputs.

### 2.2 *Particle Tracking*

A Lagrangian module releases 6000 virtual floating particles per day since January 1, 2013. Being driven by sea surface currents and waves, each particle is eventually subjected to the beaching or sinking to the bottom, which makes the system of particles dissipative. Compared with our previous work [5], a significant improvement is made by including the Stokes drift directly obtained from the wave-current coupled model instead of the approximate formulations. Random walk technique is applied to simulated horizontal diffusion. A few parametric



**Fig. 1** Plastic inputs originated from the **a** human coastal zone population, **b** riverine plastic inputs, **c** maritime plastic inputs

**Table 1** Top 10 marine litter inputs into the Mediterranean in relative units (% of total mass)

Descending rate	Input	Country	Input contribution (% of total mass)
1	Shipping lanes	International	20.0
2	Nile River	Egypt	6.8
3	Ceyhan River	Turkey	5.1
4	Seyhan River	Turkey	3.5
5	Buyuk Menderes River	Turkey	2.4
6	Alexandria City	Egypt	2.2
7	Barcelona City	Spain	1.8
8	Izmir City	Turkey	1.6
9	Rhone River	France	1.5
10	Po River	Italy	1.4
	Total over the top 10		46.1

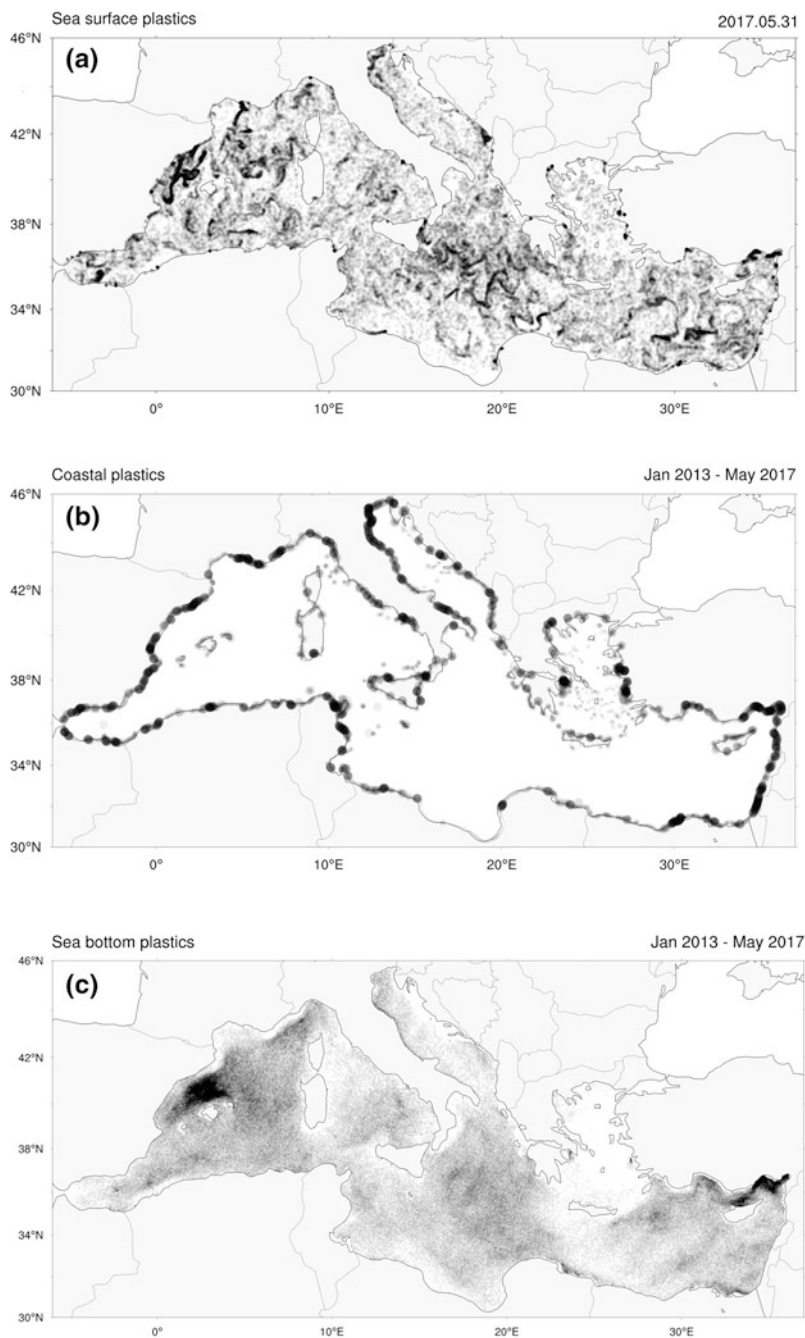
probability functions are introduced to describe the processes of beaching/washing off and sedimentation of the plastics. Since wind- and wave-driven currents are directly expressed in the operational oceanography datasets used, no additional wind drag term is applied to the motion of particles. No particle influxes from the Gibraltar and Dardanelles into the Mediterranean are imposed.

### 2.3 Oceanographic Data

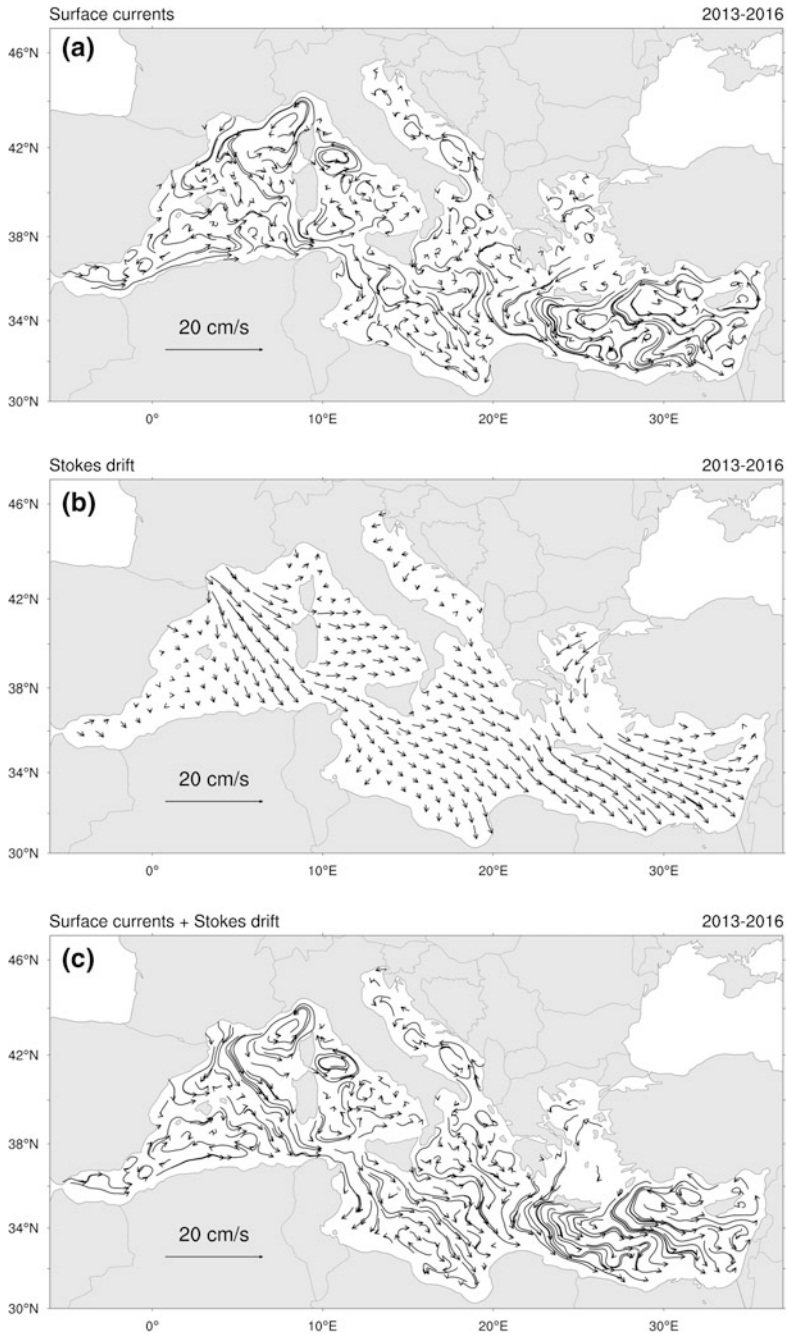
The ocean currents and Stokes drift components are provided by the Mediterranean Forecasting System (MFS [18]) coupled to the WaveWatch-III model [19] in the frameworks of the Copernicus Marine Environment Monitoring Service (CMEMS). The data are available at a  $1/16^\circ \times 1/16^\circ$  horizontal resolution and 72 unevenly spaced, vertical levels. The analyses are produced by a data assimilation system that uses satellite and in situ data.

## 3 Results and Discussion

The calculations (Fig. 2) show that the spin-up period of 90 days is enough to populate the Mediterranean basin by virtual particles, which is completely consistent with [1]. Long-term integration of the Lagrangian transport equations has shown that the system of virtual particles possesses a multiple timescale regime in the Mediterranean.



**Fig. 2** Virtual particles' snapshots on May 31, 2017 at the **a** sea surface, on the **b** coastlines, at the **c** bottom



**Fig. 3** Averaged over 2013–2016: **a** sea surface currents, **b** Stokes drift, **c** sum of surface currents and Stokes drift components

At a scale of 6–8 days, the virtual particles released from the semi-enclosed and/or stagnant environment (e.g., from the Gulfs of Genoa, Izmir, Saronic, Naples, Tunis, etc.) will be quickly washed ashore. If we only consider the beaching as a sink, the mean particle half-life is equal to 100 days. If we complement the sedimentation, the mean half-life shrinks up to 80 days.

### ***3.1 Virtual Particle Distributions***

As the preliminary outputs, geographical locations of the virtual particles snapped at the end of calculations May 31, 2017 are presented in this work.

In Fig. 2, each virtual particle is depicted by a semitransparent circle. Consequently, dark areas at the maps show overlapping the particles or their elevated abundance. Daily sea surface snapshots seem to be very variable (Fig. 2a). They tend to demonstrate the spatial scales of underlying dynamics. While coastal (Fig. 2b) and bottom (Fig. 2c) snapshots clearly indicate the main areas of plastic accumulation: the Catalan Sea (W Mediterranean) and the Cilician Sub-basin (NE Levantine).

### ***3.2 Influence of the Stokes Drift***

Stokes drift contributes to the surface dynamics, which deviating the particle pathways due to the nonlinearities of the wave orbital velocities. Even the 2013–2016 averaged maps clearly indicate the Stokes drift associated differences (Fig. 3). When considering the transport of the plastic, a pronounced deflecting the particles is found in the North Western Mediterranean, where they are taken away from the Gulf of Lion and delivered to the center of the Western Mediterranean.

## **4 Conclusions**

In the present work, we have built the new 2D Lagrangian tracking model with stochastic representation of beaching the plastic and its occurrence at the bottom. Virtual floating particles are released everyday from the realistic Mediterranean inputs and tracked over 2013–2017, encompassing an overall particle ensemble of  $\sim 10^7$  members. Copernicus Marine Environment Monitoring Service is used as a source of high-resolution datasets on the Mediterranean currents and Stokes drift components.

For approximately 90 days, the Mediterranean Sea has been entirely populated by virtual particles. Multiple timescale regime comprises fast-scale (half-life of ca. 6–8 days) and slow-scale ( $\sim 100$  days) beaching, and intermediate timescale



arriving at the bottom. As a result, the mean particle half-life in the Mediterranean is equal to  $\sim 80$  days.

Preliminary results show (1) the pronounced mesoscale dynamics at the sea surface; the elevated abundance of the virtual particles on the coastlines and at the bottom of the Cilician Basin (NE Levantine) and the Catalan Sea sub-basin.

To properly address the plastics' transport in the Western Mediterranean, the Stokes drift components are taken into consideration.

The next step will be done by re-gridding the virtual particle coordinates and rendering the concentration patterns at the sea surface, on the coastlines, and at the bottom. After that, the impact matrices will be calculated to quantify the source-receptor relationships among the Mediterranean sub-basins.

We believe that our approach will provide an appropriate beginning platform for later inclusion of vertical transport of the plastic, which supports the applicability to the real sea.

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This study has been conducted using the Copernicus Marine Service Products.

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# Assessment of Microplastics Marine Pollution from an Environmental NGO's Point of View: The First Study About the Widespread Presence of Plastic Pellets Along the Italian Coast

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## 1 Introduction

Raw materials used for the fabrication of plastic products, namely pellets or nurdles are an important source of microplastics dispersed in the marine environment. They can reach the environment for accidental loss during transportation or as result of an improper handling. In particular plastic pellets, when handled irresponsibly, reach drains, rivers and the sea, with several consequences. Pellets in the oceans were high starting from 1970s, the first scientific paper reporting this new phenomena was published in 1972 [1]. Highest concentrations of pellets on the beach were often found close to plastic producing/processing plants.

The European plastic industry produces around 58 million tonnes of raw plastic material a year. There are estimated to be 57,270 companies in the plastic production industry within the EU and 46% of these companies are classified as small-medium enterprises [2]. Annual European plastic pellet production in 2014 was estimated at 59 million tones [3].

Pathways of loss to the environment of this material are poorly understood but pre-production microplastic pellet loss has been identified as one of the largest source of microplastic pollution affect EU water.

Plastic pellets are ingested by a range of coastal, marine and freshwater species. For example they have been found in the stomach contents of several seabird species including the Atlantic puffin (*Fratercula arctica*; IUCN-listed as

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“Vulnerable”), Northern fulmar (*Fulmaris glacialis*), Common gull (*Larus canus*), Cory’s shearwater (*Calonectris borealis*) and various tern species [4–8].

Moreover once emitted to the environment, plastic pellets attracts hydrophobic contaminants and, like any microplastic they have the ability to both adsorb toxins and transfer them to the tissues of species. Pellets have been used to measure concentrations of persistent organic pollutants in coastal waters and environmental samples of lost pellets have, in one study, been observed to carry levels of polychlorinated biphenyl (PCB) 1 million times more than those observed in the surrounding seawater [9].

Legambiente, as part of the European Coalition to End Plastic Pellet Loss, is working for defining the problem and solutions in Italy. Legambiente is the only Italian representative joining the coalition, that include twelve non-governmental organizations (Fauna & Flora International, Fidra, Plastic Soup Foundation, S.O.S. Mal de Seine, Norges Naturvernforbundet, Seas At Risk, Surfrider Europe, Plastic Change, Legambiente, North Sea Foundation, Marine Conservation Society and Zero Waste Europe), representing six European Union Member States (United Kingdom, The Netherlands, Belgium, France, Denmark and Italy) and a further non-EU country (Norway).

According to the last briefing note written by the Coalition, the emission of plastic pellets is not a necessary stage of any plastic production process, notwithstanding this fact plastic pellets have been found on the coastlines of all North Sea coastal countries (United Kingdom, Norway, Finland, Sweden, Denmark, Germany, The Netherlands, Belgium, France, Republic of Ireland, Portugal) and in most Mediterranean countries (Spain, Italy, Croatia, Albania and Turkey). Pellets have also been found in freshwater, inland water bodies including estuaries, rivers and lakes [10].

According to this, at the European level the primary source of data of pellet pollution in the environment is citizen science/non-governmental organization collected data on the density or presence/absence of pellets on European beaches, rivers and lakes.

In 2016 Legambiente began a collaboration with ENEA, the Italian National Agency for New Technologies, Energy and Sustainable Economic Development, in order to study the presence of microplastic in the Italian lakes and marine environment, with in-depth analysis of the identification of the polymeric matrices of the waste observed in the sea and on the beach. This information is crucial to be able to assess the possibility of recycling and reusing this waste.

## 2 Experimental

In Italy, Legambiente Onlus, according with the European Coalition to End Plastic Pellet Loss, conducted the monitoring of presence/absence of pellets classifying them as few (from 1 to 30 pellet), many (from 30 to 100) or hot spots. The results of

Rifiuti			
Rifiuti	Vetro – Plastica – Carta – RSU – Rif. da mancata depurazione (ad es: cotton fioc, assorbenti)		
Altri rifiuti:			
PLASTIC PELLET/ NURDLES	NO	POCHI (1-30)	NOTE:
	TANTI (30-100)	HOT SPOT	

**Fig. 1** Technical sheet

pellet pollution monitoring comes mostly from the activities of Goletta Verde and Goletta dei Laghi campaigns, but also involving people in citizen science advisories.

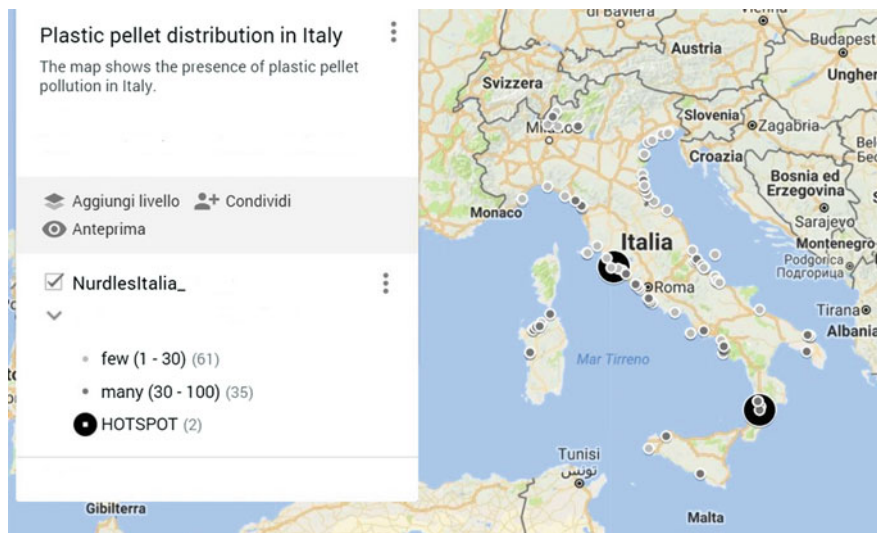
During the Goletta Verde and Goletta dei Laghi campaigns technician team travel around marine and lacustrine coasts, monitoring over 300 beach, sampling water and observing surrounding conditions, signing it on a recording sheet. In this sheet is included the waste presence and, in particular, the nurdles pollution (Fig. 1). The image show a detail of the sampling sheet for recording information about waste founded on sampling site, pellets are included. Levels of pollution is estimated as no found, few pellet founded (from 1 to 30 pellet), many (from 30 to 100) and hot spots.

Thanks to the Scottish ONG Fidra, Legambiente produced a short and visual guide in Italian and English language in order to explain the pellets issue and asking for warnings, geographically identified.

Besides the presence/absence monitoring activities conducted by Legambiente, the research also envisaged a work of study and analysis in the laboratory, conducted by Enea. The main objective was to identify the polymer matrices of the nurdles and to collect data on degradation processes once dispersed in the marine environment. Concurrently ENEA conducted an analysis on 467 pellets collected on some Lazio's beaches. Fourier transform infrared spectroscopy (FT-IR) measurements were carried out with a IRAffinity-1 FT-IR Shimadzu apparatus. The spectrum peaks (range 4000–600  $\text{cm}^{-1}$ ) were compared with spectra database and instrument libraries. In the IR analysis of photodegraded polyolefin, three regions of the middle infrared spectra were considered: the range of the O-H stretching modes, the range of the C=O stretching modes and the range of the rocking of the C=C bonds. The range of the C=O stretches is particularly interesting, since oxidation leads to the appearance of a broad band with several maxima between 1700 and 1780  $\text{cm}^{-1}$ .

### 3 Results and Discussion

Since 2016 a total of more than 120 finds have been reported from Legambiente across Italian beaches of these surveys, plastic pellets were recorded at a third of the sites and a number of potential pellet hotspots are presented.



**Fig. 2** The distribution of plastic pellet pollution in Italian marine coasts and lacustrine shores. The presence of pellets is represented in different tags depending on the number of objects founded: few (from 1 to 30 pellet), many (from 30 to 100) or hot spots

**Table 1** Composition of pellets collected

Polymer	d	%	UE Demand	Polymer	d	%	UE Demand
PE <sup>a</sup>	0.89–0.98	36.2	14.3 (30%)	PVC	1.26–1.55	3.6	5 (10%)
PP	0.83–0.92	28.7	9.3 (19%)	PES	1.24–2.3	1.7	
PS <sup>b</sup>	1.02–1.15	12.3	3.8 (7%)	ABS		1.1	
PET	0.96–1.40	12.1	3.8 (6%)	Other	–	4.3	

PE—Polyethylene, PP—Polypropylene, PS—Polystyrene, PET—Polyethylene terephthalate, PVC—Polyvinyl chloride, PES—Polyester, ABS—Acrylonitrile butadiene styrene, Other—not identified. UE demand (Mtons).

d—density ( $g/cm^3$ ), density of plastic can be modified by additives and degradation occurring by UV and oxygen

<sup>a</sup>HDPE and LDPE

<sup>b</sup>Excluding expanded polystyrene micro-spherule EPS

Pellets were also found within inland fresh water lakes in Northern Italy. Major hot-spots include, for example, the mouth of the river Angitola in Calabria region and the coastline of Tuscany (Fig. 2).

In total, 467 pellet collected were analysed by the FT-IR spectroscopy. The chemical composition and percentage of particles are showed in Table 1, 7

polymeric materials were identified and according to Hidalgo-Ruz et al. [11], polyethylene, polypropylene, polystyrene and polyethylene terephthalate are the polymers most commonly present within the collected pellets. At the same time these polymers are the best-selling polymers. The nurdles, having an average weight of 22.1 mg (range: 15.3–35.1 mg; n = 65). Polyvinylchloride (PVC) pellets are little present probably because the high density ( $\approx 1.4 \text{ g/cm}^3$ ) limits the transport by sea.

## 4 Conclusions

Microplastics marine pollution is a global threat and Italy is not immune to it. The potential solutions need to take into consideration the changing global attitude towards plastics; moreover, legislative and preventative measures must also be considered in order to reduce the dangerous impact of microplastics pollution on the entire ecosystem. We expect governments take the lead in applying these measures to private and public businesses, especially those who are dealing with plastic production and transformation.

Legambiente and Enea at MicroMed 2017 conference presented the first time a preliminary study about the problematic presence of nurdles all over Italian coastal locations; a research conducted with the help of citizens and volunteers' reports and collaboration with an important Scientific research institute. The first results show the presence of plastic pellets very widespread along the sea coasts and lakes in Italy. The polymer matrices identified are the most common, polypropylene, polyethylene, polystyrene and also the most easily recyclable. But to reduce this type of pollution the first solution to be implemented is prevention. So, the work will be implemented with the comparison between the presence of nurdles on the beaches and the possible land based pollution sources. The main objective is to study and to evaluate the best solutions, to prevent their dispersion in the environment. At the same time, we will develop an awareness and policy action to raise awareness and knowledge of the problem.

Understanding problems, on a local and global scale, is the first step to raising awareness among stakeholders and policy makers, which can then contribute to solutions and citizens taking direct action, just as Legambiente has done over the last several years. This issue truly requires full-scale engagement, it's necessary to develop new thinking about the way we prevent, design, produce, consume and dispose of plastics. To do this, a strong network between countries needs to drive and foster awareness campaigns and joint policies, as well as pave the way for the decisive transition towards a circular global economy.

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# Microplastics from Wastewater Treatment Plants—Preliminary Data

Ricardo Gouveia, Joana Antunes, Paula Sobral and Leonor Amaral

## 1 Introduction

Wastewater effluent is a known source of various types of microplastics and expected to be a pathway to enter in the environment [1, 2]. High concentration of microplastics in wastewater treatment plants (WWTP) influent ( $10^4$ – $10^5$  microplastic/ $m^3$ ) and incomplete removal during the treatment process (70–100%) are the main causes for microplastic pollution in the receiving water [3]. Microbeads from cosmetic products and polymer fibres from clothes are most common present in WWTP. Few studies were done to quantify microplastics in wastewater.

The objective of this study was to investigate the abundance and type of microplastic in two municipal WWTP effluents serving different communities—one bigger station treating mixed domestic and industrial wastewater averaging 18,000  $m^3$ /day and a smaller station with treating mostly domestic wastewater averaging 7250  $m^3$ /day. This study also intends to contribute to a standardized methodology to analyse microplastics in wastewater, from sampling to identification, measuring and handling processes in the laboratory. Wastewater samples are complex, normally in handling microplastics with less dimensions ( $>1$  mm), and these facts may lead to incorrect estimation if there is an inappropriate methodology.

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## **2 Experimental**

### **2.1 Sampling**

Wastewater samples were collected in two different WWTP. The first WWTP, further named as WWTP A, treats mostly domestic wastewater, and the second one named as WWTP B treats a mix of domestic and industrial wastewater. Both unload on the same river. Samples are divided in 24 volumes corresponding to the hours of the day to make up a sum of 1 litter/day (affluent) and 2 litters/day (treated effluent). Samples were taken from the entrance of the WWTP, without undergoing treatment, and at the exit of the WWTP, after all processes are completed. The samples were collected automatically with an equipment specific to that end. They were contained in a plastic recipient and sent to the laboratory.

### **2.2 Methods**

#### **2.2.1 Preparation of Samples**

In the laboratory, wastewater samples were treated with a NaCl saturated solution to remove a part of the organic matter present in the wastewater and filtered through GFC/C filters ( $\sim 1 \mu\text{m}$ ) in a laminar flow chamber. Contamination by airborne microplastics was prevented at all times during manipulation and observation under the stereoscopic microscope (Leica<sup>®</sup>). The samples were left to decant for a minimum of two days to enable a more rapid filtration and easy observation. The last millilitres of the sample, which had a higher concentration of sludge, namely on the samples of the entrance, were diluted in water and added a saline solution to promote the separation between the sludge and the plastic components.

#### **2.2.2 Analytical Techniques**

Particles were sorted by type (fragments, beads, fibres, colour and shape), counted, measured and analysed with FTIR to sort out their natural or non-natural origin.

## **3 Results and Discussion**

So far, we have been able to detect large quantities of microparticles of various sizes especially floating in the treated effluent but also embedded in the particles that make up the sludge fraction of the incoming wastewater; we could also confirm that the

smaller microplastics invisible to the naked eye appeared on the top fraction of the sample, and the bigger microplastics appeared on the bottom layer in the sludge.

### 3.1 Results

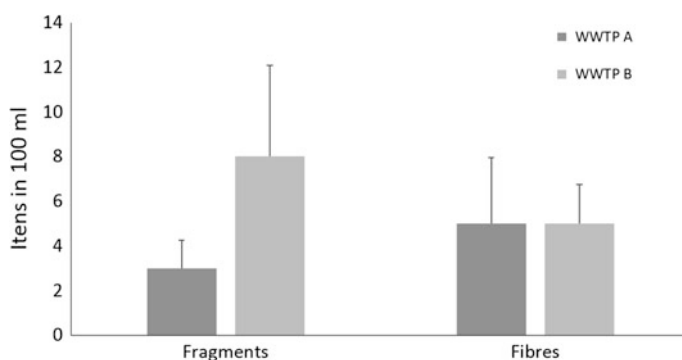
A total of 6065 microplastics were observed in 18 samples (WWTP A  $n = 10$  and WWTP B  $n = 8$ ). Fragments (FP) and fibres were the microplastics type collected. 90% of the microplastics analysed were fragments and were collected from treated effluent (404 items in average per 100 mL). Fibres presented a higher percentage in the affluent (88%), comparing to treated effluent (12%). Table 1 resumes preliminary data.

Comparing WWTPs, the highest accumulation of fragments was registered in WWTP B (Fig. 1), regarding the affluent. Fibres' accumulation was similar in the both WWTP.

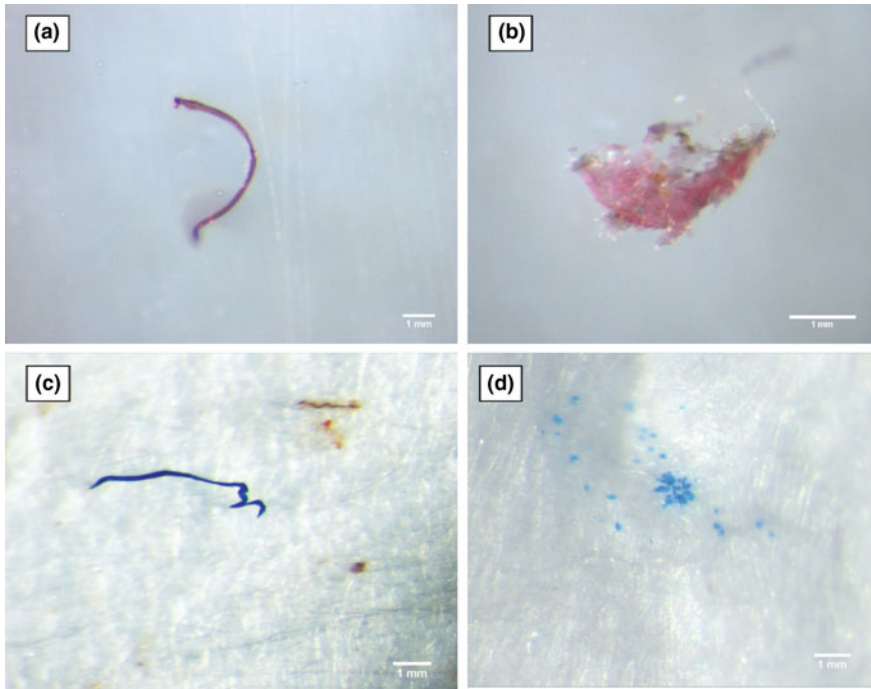
Significantly differences were observed in the treated effluent (Table 1). WWTP A registered a higher number of fragments ( $783 \pm 1069$  items average per 100 ml) in this effluent (Table 1).

**Table 1** Microplastics accumulation (average  $\pm$  sd) in the affluent ( $n = 8$ ) and in the treated effluent ( $n = 10$ ), regarding type of microplastics (fragments and fibres) for two different WWTPs

In 100 ml	Affluent		Treated effluent	
	WWTP A	WWTP B	WWTP A	WWTP B
Fragments	$3 \pm 1$	$8 \pm 4$	$783 \pm 1069$	$26 \pm 44$
Fibres	$5 \pm 3$	$5 \pm 5$	$0 \pm 0$	$2 \pm 1$
Total MP	$8 \pm 3$	$11 \pm 3$	$783 \pm 1069$	$27 \pm 44$



**Fig. 1** Microplastics accumulation (fragments and fibres) in 100 ml of wastewater collected in the affluent (average  $\pm$  sd) ( $n = 8$ )



**Fig. 2** Microplastics present in affluent (a and b) and treated effluent (c and d) of a wastewater sample collected in Portugal

Microplastics analysed had sizes between  $89 \mu\text{m}$  and  $3000 \mu\text{m}$ . In average, there were  $81 \pm 4$  and  $481 \pm 887$  microplastics per filter (100 ml), regarding the affluent and the final effluent, respectively. Examples of microplastics found in affluent (a, b) and treated effluent (c, d) are shown in Fig. 2.

### 3.2 Discussion

This study provides a perception into the impact of wastewater treatment plant discharges on the environment. Fibres and fragments occurred in a high number, as similar occurred with [4] study. As expected, fibres' accumulation was higher in affluent comparing to treated effluent. In the treated effluent, fragments' accumulation was higher. This result was not expected and probably occurred due to fragmentation from bigger microplastics into reduced dimensions or probably due to a pontual contamination in the WWTP interior. Results of microplastics studies are difficult to compare due to variation in sampling methodologies and units used in reporting results [5].

## 4 Conclusions

This study suggests that although low concentrations of microplastics are detected in final wastewater effluent, WWTPs still have the potential to act as a pathway to release microplastics given large volumes of effluent discharged to the aquatic environment. A long-term monitoring is recommended in future studies to further characterize microplastics in wastewater.

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# Challenging the Microplastic Extraction from Sandy Sediments

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## 1 Introduction

As plastic particles are one of the most commonly waste found on beaches [1], this pollution requires the use of innovative extraction methodologies especially for smallest size ranges of plastic particles, as microplastics. In fact, plastic extraction based on sodium chloride solution (NaCl), the most commonly used process through the flotation method to extract plastics from sand [2], does not allow recovering the denser of them [3–5]. The use of solutions denser than NaCl ones as zinc chloride [4, 6], sodium polytungstate [7, 8] or sodium iodide [3, 9] ensures a very efficient extraction. However, using these dense salts is very expensive limiting thereby the spread of these extraction methods in the scientific community. To lower the extraction costs, protocols based on two separation steps, microplastic concentration and extraction by flotation in dense, solution have been designed. Two main methods have been developed: the elutriation column [3, 10, 11] and the AIO system [5]. For these two methodologies, there is a significant need to better determine their limits and to optimize the control of the process. This need is particularly important due to the fact that these methods begin to spread in the scientific community [12, 13]. Based on the example of two extraction technics, the sodium iodide (NaI) density separation and the elutriation system, the present communication aims first (i) to question the representativeness of the microplastic extracted from sand, and then (ii) to show how the microplastic extraction costs can be lowered.

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## 2 Experimental

### 2.1 Extraction Representativeness

The quantitative occurrence of the “big six” as determined from various solutions used is determined after reviewing the scientific literature on microplastics extraction from sand. The data of the European plastic production were gathered and compared with plastic densities [14, 15].

### 2.2 Evaluation of the Extraction Cost

Here, the calculation is based on the hypothesis that 90% of the sand is removed. Then plastic extraction needs to be refined with sodium iodide solution ( $d = 1.80 \text{ g/mL}$ ). The price of the NaI salt is 215 euros for 500 g (Sigma Aldrich, 17-05-2016). A mass of 30 g of NaI with 20 g of water allows creating a 28 mL solution. The volume of NaI solution required for extraction is almost twice the sand volume and the calculation takes into account the salt loss during the recycling phase. From these considerations, the extraction cost per kilogram has been calculated for three different protocols:

- an extraction only based on sodium iodide;
- an elutriation and a refined with sodium iodide;
- an elutriation and a refined with sodium iodide with a recycling step.

The density of each initial NaI solution is  $1.80 \text{ g/mL}$ . For the first experiment, the use of a pure NaI solution is performed by dissolving 30 g of NaI in 20 mL of water and boiling it at  $130 \text{ }^\circ\text{C}$  for 1 h. After weighing the sample, a new cycle is operated by adding water to obtain a 60% (w/w) NaI solution and so forth until the ten cycles are achieved.

In the second experiment, the impact of marine sediments on the recycling is tested. The sediment used has been sampled from the foreshore of the Kernevel beach (GPS coordinates: 47.7173,  $-3.3666$ ; Lorient, France) and dried in an oven (Memmert) at  $60 \text{ }^\circ\text{C}$  during three weeks. For the first cycle, a volume of sand of 15 mL is added to the NaI solutions (28 mL). Then, sand and solution are manually stirred during 10 min. This step promotes eventual NaCl and organic matter dissolution, impurities that may have a significant impact on the NaI solution density of the next cycle. The solution is then filtered through a filter funnel (diameter: 120 mm, porosity 4) then the solution is evaporated during 1 h, the mass is measured and the sand used is thrown away. A second cycle is then operated. Due to NaI salt loss during the recycling cycle, the volume of sand added is, from the second cycle, 0.5 times the NaI solution volume.

In the third experiment, the sediment is rinsed with 100 mL of distilled water during the filtration step. As a consequence, the total volume of the solution reached ranges between 100 and 130 mL and 3 h are necessary to allow full evaporation.

### 3 Results and Discussion

#### 3.1 Extraction Representativeness

The different dense solutions used to extract microplastics have not the same ability to recover the denser particles (Table 1). For example, PVC and PET are recovered in only 10–20% for studies using NaCl solution separation in opposite to 75–100% for publications using NaI or ZnCl<sub>2</sub>. On the contrary to NaCl solutions, the use of dense NaI or ZnCl<sub>2</sub> solutions is generally known to yield a fair representation of the plastic distribution present in the sediment.

If PVC extracted either by zinc chloride or sodium iodide, with a maximum specific gravity of about 1.5, is the densest among the common polymers, some artificial polymers as fluoropolymers (1.7–2.28), epoxide resins (1.85–2), melamine (1.45–2) and phenolic resins (1.17–2) or silicone (1.1–2.33) can be denser than PVC. To recover the densest microplastics particles, the use of dense solutions as zinc chloride and sodium iodide could help to study the sediment “plasticomes” [16]. The term “plasticome” proposed here can be defined as all the plastic particles present in an environmental compartment (air, water, soil, organisms), in a limited area and for a given period of time.

**Table 1** Presence yield of the “big six” (poly(ethylene) [PE, 0.87–0.97], Poly(styrene) [PS, 0.01–1.1], poly(vinyl chloride) [PVC, 1.17–1.56], poly(ethylene terephthalate) [PET, 1.34–1.39], poly(propylene) [PP, 0.9–0.91]) as a function of the extraction protocol in different plastic pollution studies

Extraction technic	Number of study	PE (%)	PS (%)	PVC (%)	PET (%)	PP (%)	References
Trawling on water surface	7	100	57	14	14	100	[19–25]
Density separation with NaCl	9	78	67	11	22	89	[2, 26–32]
Density separation with ZnCl <sub>2</sub> or NaI	4	100	100	100	75	100	[9, 33–35]

If light plastics (PE, PS and PP) are commonly recovered, the results are more variable for medium dense plastics (PVC and PET). In fact, this kind of plastics are not buoyant in water or NaCl saturated water solutions and are not usually recovered in sediment without the help of dense solution such as ZnCl<sub>2</sub> or NaI [17]



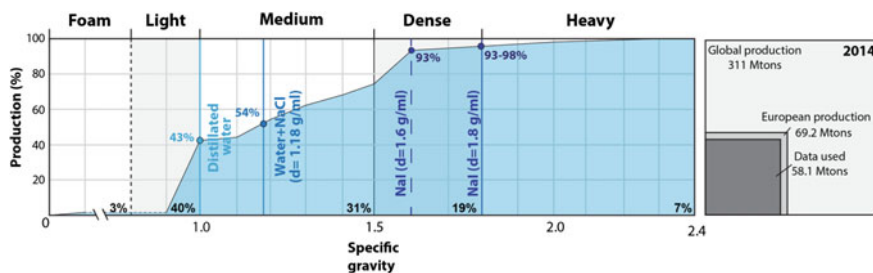


Fig. 1 Specific gravity and mass production of different plastics

The European plastic production represents about 20% of the global production of plastics. Foam and light plastics, which have the ability to float, feature about 43% of the plastic production and are largely dominated by PE, PP and a part of PS. The density reached a saturated solution of NaCl allows extracting 54% of plastics, but medium dense plastics like PVC and PET have a low likelihood to be recovered. The use of NaI solutions with a density of 1.8 g/mL can extract these two plastics and about 93–98% of all the plastics (Fig. 1) [17].

The use of the European plastic production to determine the representability of the plastic extraction can be questionable. Firstly, the European production represents only 20% of the world production in 2014 [15]. Thus, the European production may not mimic the world plastic's chemical distribution. Secondly, there is probably a drift from the production to the use of the plastics, from the use to the loss and from the loss to the deposit environment. Consequently, percent of different plastics in the environment likely differ from the production's statistical distribution. Concentrating or diluting effects due to local human activities or physical aquatic processes such as stream flow, tide action or littoral drift may drastically alter the plastic's fates. It is possible that these environmental processes has the capacity to "sort" microplastics as a function of their density, size and shape along the shore just as in the case of the sand particles [18]. It means that, locally, the possibility that sediments can be highly contaminated with dense and heavy microplastics (>1.5) cannot be ruled out. Another bias is that the European polymer production presented here only takes account of the plastics in their primary forms. However, other products, like fiberglass, can be added to the plastics in the aim to modify their physical properties and, in this case, a change in density of the plastics can be observed. This change can be very important with dense inorganic fillers such as silicates, barium sulfate and titanium dioxide and, consequently, could increase the proportion of dense and heavy plastics in sediments.

### 3.2 Extraction Cost

The loss of NaI salt depends on the experiment. For the first experiment, the solution is just evaporated and the final mass of NaI salt did not show a significant

decrease. In the second experiment, the NaI solution is in contact with sand and the salt mass decreases rapidly along recycling. In the third experiment with a rinse phase, the salt losses are low from a cycle to another. In opposite, whatever the experimental protocol, the mean density of NaI solutions remains relatively constant along the cycles.

Based on the recycling protocol developed in this work, it is possible to recover 95% of the NaI salt after each cycle. The main consequence is that, coupled with the elutriation column, the extraction cost by sodium iodide becomes similar to that by sodium chloride. Finally, as a result of the high extraction efficiency and the low extraction cost (11.1 €/kg), the methodology based on the use of sodium iodide is a major breakthrough to extract dense plastics such as poly(ethylene terephthalate) or poly(vinylchloride) with no unaffordable costs.

## 4 Conclusions

The use of  $d = 1.80$  g/mL sodium iodide solutions allows extracting about 93–98% of plastics produced in Europe during the year 2014. However, a small part of these dense plastics in primary forms has a density higher than 1.80 g/mL and the presence of fillers, like fiberglass, in plastics may decrease the extraction efficiency since they increase the particle's intrinsic density.

The results have shown that the sodium iodide solutions can be efficiently recycled with a loss of about 4.8% of the initial salt mass, without any density decrease. As a consequence, this extractive process, associated to an elutriation system, becomes competitive as compared to sodium chloride and is far better than NaCl solution in terms of extraction yield for medium dense to heavy microplastics. Hence using NaI dense solutions is a fair cost effective option to efficiently extract microplastics from sediments and thus may provide a more accurate overview of the “plasticome”.

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# Are Our Synthetic Fabrics Released into the Marine Environment? Evidences on Microplastics Pollution in Wastewater Coming from Our Laundry

Raquel Villalba, Àngels Rovira and Laura Gelabert

## 1 Introduction

Microplastic particles of synthetic clothes coming from laundry wastewater have been encountered in marine sediments, ecosystems and runoff and sewage waters. The “*Accumulation of Microplastic on Shorelines Worldwide: Sources and sinks*” states that “*the source of the microplastic fibers in the sewage treatment plants is most likely to be from washing machine wastewater as the mixture of fibers found in synthetic textiles is similar to the mixture of microplastic fibers found in beaches at disposal sites and in the wastewater of sewage treatment plants.*” On average, more than 1900 fibers of microplastics can be released by a synthetic garment during one wash [1].

MERMAIDS project [2] has promoted the mitigation of impact caused by micro- and nanoplastic particles resulting from laundry wastewater on European seas’ ecosystems, by demonstrating and implementing innovative technologies and additives for laundry processes and textile finishing treatments. Existing technologies and products have been analyzed to determine the main factors involved in the fibers release during laundry process to further improve them. Mitigation measures have been proposed for each step in the manufacture of synthetic fabrics and also from a detergency approach.

The project results are illustrated in the *Handbook for zero microplastics from textiles and laundry*, *Good practice guidelines for consumers*, and a set of *Policy recommendations* to support policy makers in their future decisions on the review

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of the EU regulatory framework that could directly or indirectly address microplastics release coming from wastewater of laundry activities.

A reduction of 24% of the total microplastic release in laundry wastewater has been demonstrated for standard fabrics by means of the improvements achieved. However, additional research is needed to better define the many different parameters involved to propose further mitigation measures.

## **2 Experimental**

The preliminary actions of the project aimed to gather all the current and available knowledge and main findings concerning the impact of microplastics. A literature review was made by means of several research databases, and a survey on households' laundry habits was launched in October 2014. Then, a standardized method to quantify the microplastics released by washing processes was developed involving simulations of both industrial and domestic washing processes and a counting method based on scanning electron microscopy (SEM).

Afterward, the scientific activities were focused on the main factors involved in the problem: types of fabric, textile auxiliaries, washing conditions, and types of detergents.

Firstly, the different fabric characteristics and textile processes, which could enable the microplastics formation, were determined along with the types of textile auxiliaries with the potential to impede the loss of micro/nanofibers in the domestic washing.

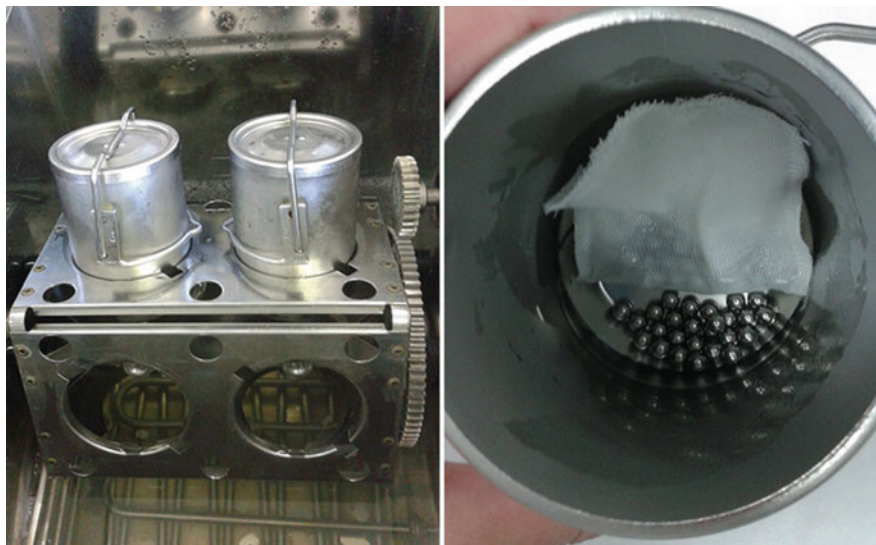
The results reveal that these products have the advantage to create a protective layer on the fabric.

Secondly, the influence of different types of laundry products for washing machine on the release of fibers was evaluated by means of a market study of common detergent products used in Europe and of several laundry tests. The main conclusions are: powder detergents, higher pH of the washing liquor, and the usage of powder oxidizing agents favor the fiber release; instead, softener or special detergents (for delicate and synthetic fabrics) reduce the release (Fig. 1).

The core activities of the project were the implementation actions, based on the development of innovative textile and detergent additives that could reduce the amount of fibers released.

### ***2.1 Laboratory-Scale Trials and Pilot Scale Trials Related to Textiles***

The textile manufacturing process involves many stages at which the textile material is susceptible of producing fibers. Also, the mechanical action at each stage



**Fig. 1** Lintest equipment that simulates the washing processes

**Table 1** Factors to be taken into account to evaluate the microplastic release propensity

Fiber influence	Yarn influence	Fabric influence
✓ Fiber diameter	✓ Spinning type	✓ Fabric composition
✓ Fiber length	✓ Yarn structure	✓ Fabric structure
✓ Fiber transversal section	(continuous or staple fibers)	✓ Fabric weight
✓ Fiber curling	✓ Yarn number of plies	✓ Dyeing process
✓ Fiber tensile strength	✓ Yarn	✓ Mechanical finishing
	✓ Yarn twist	✓ Chemical finishing

can be responsible for damaging the fibers and makes them more prone to liberate fibers. The fiber, yarn, fabric characteristics, and production processes are important factors to be taken into account to evaluate their microplastic release propensity. These factors are presented in the next Table 1.

The influence of the main fiber, yarn, and fabric characteristics was studied during the project, performing standardized tests. The influence of washing on each fabric type was analyzed as well. The main conclusions obtained from these tests are expressed in Table 2.

Several textile additives were selected in order to prevent the pill formation and to minimize the fiber loss during washing. For initial samples, a complete characterization was carried out to determine which final treatment is suitable for solving or minimizing the problem of the fiber release.

The characterization of fabrics was done before and after the selected treatments.

**Table 2** Summary of textile performance tests in MERMAIDS

Characteristic of the fiber, yarn or fabric	Methodology used	Test results
Fiber length	Leitat methodology	The shorter the fibers the higher probability to migrate to the yarn surface increasing their hairiness, their pilling and as a consequence their release during laundry
Yarn twist	ISO 17202 & DIN EN ISO 2061	The yarn resistance and elasticity increase with the twist. More compact yarns are achieved with higher twist values
Linear density	Yarn count—DIN EN ISO 1973	The number of fibers released will increase with the yarn count due to a larger amount of fibers per cross-section
Fabric density	DIN EN 14971	A higher number of yarns per unit length will result in a tighter structure with lower probability to fiber release
Textile auxiliaries	Not applicable	They provide physical protection of fibers against abrasion/reduction of coefficient of friction (fiber-fiber, fiber-detergent) during laundry
Process conditions	Not applicable	The optimization of the process conditions depends on the textile treatment selected

Main differences of the fabrics before/after treatment were identified. This study was performed initially on woven polyester fabric. This fabric was selected to be the worst quality textile in terms of pilling formation. Several auxiliary products, based on different polymers (acrylic, polysiloxane, polyurethane, chitosan), were studied. Polysiloxane-based product showed the lowest amount of microplastics released in comparison with the fabrics treated with the other finishing compounds and the untreated fabric. In the case of the acrylic resin, a significant improvement on the pilling index (measured by Martindale test) was showed.

Two selected finishing treatments, the silicone emulsion and the acrylic resin, were applied at pilot scale (three meters of standard polyester woven fabric), with the contribution of a textile company that performs finishing, printing, and dyeing processes in outsourcing.

## 2.2 Laboratory-Scale Trials Related to Detergents

During the washing cycle, several factors are involved in the cleaning effectiveness which means that they may produce modifications in the fabrics surface and, consequently, they may be involved in the microplastics release. These factors are *Chemical action*, *Mechanical action*, *Time*, *Temperature*, and *Water hardness*.

Taking into account all these factors, several laboratory assays (at laboratory-scale and pilot experiences) were carried out in order to identify the main



causes of the fibers' release during the washing cycle from a detergency approach. Different types of laundry products, as well as several washing conditions, were tested, and certainly, some differences were found depending on the laundry products used and the washing conditions. The main conclusions obtained after the experimental part are the following:

- ☑ Powder detergents produce the release of more fibers than the liquid ones.
- ☑ Light-duty detergents decrease the amount of microplastics released during the washing cycle.
- ☑ The washing temperature has a big influence in the release of fibers; at higher temperatures, more microplastics are released to water than at low temperatures.
- ☑ Some additives for detergents are capable to reduce the friction between the fibers and, consequently, decreasing the number of microfibrils released from the fabrics.

The use of softeners also produces a reduction in the amount of microplastics released, thanks to their capacity for reducing the friction between fibers.

### 3 Results and Discussion

Microplastic release in wastewater from laundry was quantified and compared at project trials, at different washing conditions, and by washing non-treated and treated fabrics (100% polyester).

With the data obtained, long-term environmental benefits were assessed. The parameters selected were:

- Laundry load (kg fabrics washed by laundry).
- Laundry frequency (n° laundries per year).
- Percentage of non-treated synthetic fabrics by laundry.
- Washing machine conditions (T, centrifuge,...).
- Percentage use of softeners and/or special care detergents.
- Percentage treatments applied in the manufacturing of synthetic textiles.

The results were expressed as tonnes of microplastics released from laundry activities by year in Europe. The variation of the microplastic release in the different scenarios assessed has determined the potential impact of the project at European scale.

Results of this investigation pointed a potential improvement of the fibers' release in around 24% improvement (at real scale trials) where the MERMAIDS solutions were implemented.

## 4 Conclusions

The experimental actions of the project demonstrated that both textile and detergent sectors are clearly involved in the release of the fibers during the laundry processes.

The analyses performed on textiles allow us to confirm the influence of the fiber, yarn, and fabric characteristics in the amount of microplastics released during laundry. The findings point that each stage of their manufacturing process has to be taken into account for the establishment of policy recommendations in the textile industry, with the aim of participate in the mitigation of this emerging pollutant.

Regarding detergents, on the basis of the results obtained in the experimental assays, we can confirm the influence of the detergency in the amount of microplastics released during a washing cycle. The fact that the factors involved in laundry are related to the emission of microplastic fibers to water is an important point to be considered for the establishment of policy recommendations in detergency and laundry field, with the aim of contributing to the mitigation of this emerging pollutant.

The project results were not strong enough to determine quantitative data in the relationship between all the factors involved in the production of synthetic textiles and the microplastics release during their laundry. However, qualitative and partial quantitative data were obtained and many tendencies were outlined, related to both textile and laundry industries.

Further research in both industries is highly encouraged and also in the field of the wastewater treatment plants. The work performed in MERMAIDS and the subsequent "*Handbook for zero microplastics from textiles and laundry*" are offered as starting point.

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# Analytical Approach for the Detection of Micro-sized Fibers from Textile Laundry

Jasmin Haap and Edith Classen

## 1 Introduction

Contamination of the aquatic ecosystem by microplastic is a growing worldwide problem. Sewage from textile laundry has been identified as a potentially important source of synthetic fragments in the environment; field studies registered synthetic textile fibers in ocean and freshwater samples as well as in sediments [1–3].

Nowadays, synthetic polymers like polyester, polyamide, or polypropylene dominate the fiber industry and are preferably used in textiles due to their durability and low price [4]. In textile cleaning processes, the garments are exposed to different strains, e.g., mechanical strain, detergents, and temperature which may cause damages to the fibers and the textile surface. These can lead to the loss of particles and fibers with different sizes and shapes [5]. The washing machine filters and wastewater treatment plants have limited retention capacity, and the fibers are often too small to be filtered out. Thus, the microplastic particles and fibers enter the freshwater systems and accumulate [6].

Different research groups have analyzed the fiber release for different textile types in domestic laundry [7–9]. However, there is still a gap of knowledge regarding the extent of fiber discharge and the main influence factors. Furthermore, there is no information about the discharge of fibers in industrial laundries [5].

Detailed investigations on the release of micro-sized fibers from textile laundry processes require precise and reproducible analytic methods to detect the microplastic particles in sewage. Up to date, there is no accepted standard for the characterization and quantitation of microplastic [5].

Reported procedures for microplastic fiber detection in sewage used different sample handling and analytical methods. Most commonly, the detection was based on filtration of the effluent combined with visual analysis of the particles,

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e.g., microscopy [7, 8]. This workflow is time consuming and only allows an estimation and extrapolation of the fiber content per wash. Commonly applied particle detection systems like light scattering or laser diffraction are limited due to the nonspherical shape of the fibers or the turbidity of the sewage medium [10].

This German research project AiF 19219 aims to develop an analytic method to characterize and quantify the particles and fibers from fabrics in laundry sewage. To do so, we use the dynamic image analysis as a new analytical approach in the field of microplastic. This optical detection system allows a fast and nondestructive measurement of fibers and particles in a broad size range (10–3500  $\mu\text{m}$ ). Furthermore, it enables the statistical analysis regarding various fiber characteristics like size, elongation, straightness and gives quantitative information about the number of fibers per wash and the size distribution. This study addresses the possibilities as well as the challenges in analyzing micro-sized fibers and particles in complex medium like wastewater.

## 2 Experimental

### 2.1 Materials

Polyamide flock fibers (PA 6.6, 0.3 and 0.5 mm cut lengths, 1.7 dtex) obtained from Borchert + Moller GmbH were used as reference fibers for the examinations.

### 2.2 Methods

#### 2.2.1 Preparation of Samples

For scanning electron microscopy (SEM) examination, a small amount of dry fibers was put on a SEM sample carrier with conductive glue and coated with gold in a sputter coater (Balzers SCD 004).

For dynamic image analysis and microscopy measurements, the PA-fibers were suspended in a detergent solution according to ISO 15797:2002 (5 g L<sup>-1</sup>, without optical brightener) and stirred with a magnetic stir plate.

A 500  $\mu\text{L}$  aliquot of the 500 mL fiber suspension was pipetted into a chamber of a multiwell culture plate and analyzed using a microscope.

#### 2.2.2 Analytical Techniques

##### *Dynamic image analysis*

In this study, the dynamic image analysis apparatus QICPIC (Sympatec GmbH) with Windox 5 software was used. The detection system was equipped with a

0.2 mm flow cell and was connected to a peristaltic pump. The liquid was pumped with a flow velocity of 150 mL/min from a drainage beaker into the flow cell. To prevent fiber sedimentation, the mixture was magnetically stirred repeatedly for 5 s with intervals of 30 s. The frame rate of the detection system was 85 frames per second.

### ***Scanning Electron Microscopy (SEM)***

Pictures were taken using a scanning electron microscope (Jeol JSM-5610-LV). The accelerating voltage was set to 2.5 kV.

### ***Microscopy analysis***

For visual analysis of the fiber amount, a Keyence digital microscope with  $\times 50$  magnification was used. The microscope was equipped with a VHXS15 profile measurement unit.

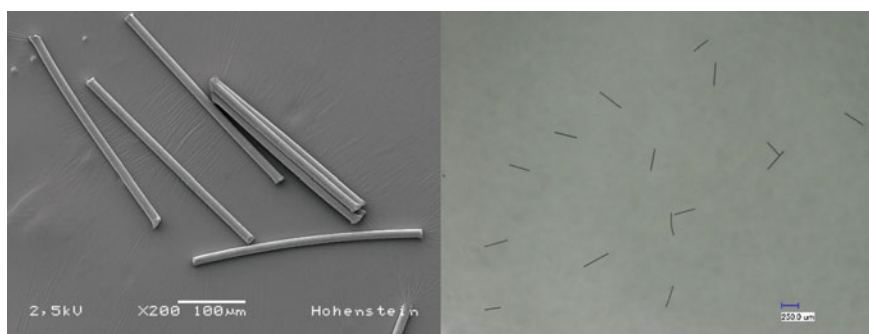
## **3 Results and Discussion**

### ***3.1 Results***

#### **3.1.1 Examination of the Sample Material**

In Fig. 1, microscopy images of the 0.3 mm fibers are shown. For the SEM image, a dry fiber sample was used. For microscopy, the fibers were suspended in a standard powder detergent without optical brightener.

The microscopy experiments indicated that the particles have similar sizes.



**Fig. 1** Polyamide flock fibers (PA 6.6) with a length of 0.3 mm, left: Scanning electron microscopy (SEM) image of the dry fibers, right: Microscopy image,  $\times 50$  magnification of the flock fibers suspended in detergent solution

### 3.1.2 Size and Shape Analysis—Quality Measurements

When developing and implementing an analytic method for new applications, validation of the measuring results is an indispensable step. The repeatability of the size and shape measurements by dynamic image analysis was evaluated using flock fibers with a length of 0.3 and 0.5 mm. Four replicate measurements of each sample were conducted in detergent solution. The average particle size and shape results are summarized in Tables 1 and 2.

The size and shape parameters are in good accordance with all four measurements for the 0.3 mm sample. For the 0.5 mm sample, there are small deviations in the detected diameter; however, for the remaining parameters there is a good result agreement.

The four replicates of the 0.3 mm fiber sample were summarized in the size distribution diagram Fig. 2. The ordinates show the cumulative distribution function  $Q_0$  (gray line with triangles) and the density distribution (black line with dots), and the abscissa represents the particle size. Figure 2 shows a narrow distribution at about 300  $\mu\text{m}$ , indicating a small standard derivation of the particle sizes.

### 3.1.3 Quantitative Measurements

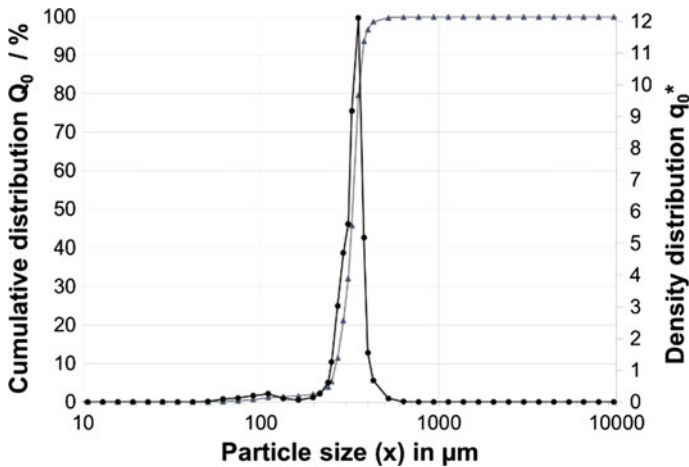
In addition to size and shape characterization, quantitative measurements are a further factor of interest in microplastic detection. In this study, first experiments determining the total amount of fibers in detergent solution were conducted by dynamic image analysis. However, the experiments showed analytical challenges

**Table 1** Size and shape results of 0.3 mm fiber suspension in detergent measured four times by dynamic image analysis

Measurement	Length ( $\mu\text{m}$ )	Diameter ( $\mu\text{m}$ )	Straightness	Sphericity	Elongation
1	328 $\pm$ 45	18 $\pm$ 2	0.99 $\pm$ 0.01	0.38 $\pm$ 0.03	0.05 $\pm$ 0.01
2	327 $\pm$ 46	17 $\pm$ 2	0.99 $\pm$ 0.01	0.38 $\pm$ 0.03	0.05 $\pm$ 0.01
3	329 $\pm$ 46	17 $\pm$ 2	0.99 $\pm$ 0.01	0.38 $\pm$ 0.03	0.05 $\pm$ 0.01
4	328 $\pm$ 45	17 $\pm$ 2	0.99 $\pm$ 0.01	0.38 $\pm$ 0.03	0.05 $\pm$ 0.01

**Table 2** Size and shape results of 0.5 mm fiber suspension in detergent measured four times by dynamic image analysis

Measurement	Length ( $\mu\text{m}$ )	Diameter ( $\mu\text{m}$ )	Straightness	Sphericity	Elongation
1	498 $\pm$ 48	15 $\pm$ 1	0.99 $\pm$ 0.01	0.29 $\pm$ 0.02	0.03 $\pm$ 0.01
2	501 $\pm$ 46	15 $\pm$ 1	0.99 $\pm$ 0.01	0.29 $\pm$ 0.02	0.03 $\pm$ 0.01
3	498 $\pm$ 56	17 $\pm$ 2	0.99 $\pm$ 0.01	0.30 $\pm$ 0.03	0.03 $\pm$ 0.01
4	498 $\pm$ 52	17 $\pm$ 2	0.99 $\pm$ 0.01	0.30 $\pm$ 0.03	0.04 $\pm$ 0.01



**Fig. 2** Particle size distribution of 0.3 mm PA-flock fibers in detergent solution. The diagram shows a cumulative distribution of four replicate measurements combined to one curve

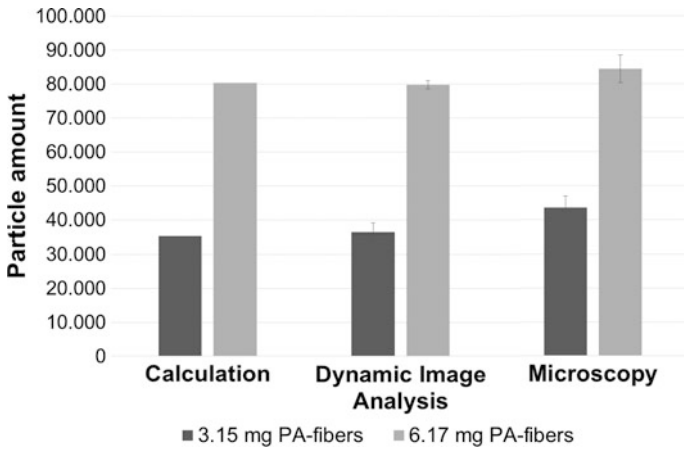
associated with the quantitative measurements as the particle amount cannot be measured directly. One reason is the limited detection range of the camera, capturing only a section of the entire particle flow. Additionally, particles detected on image edges, micro-sized detergent ingredients and inconsistent liquid flow increase the error of particle counting. For this reason, the detected value has to be corrected by calculation factors. This requires a detailed characterization of the sample and detection system in preliminary studies.

Figure 3 shows a data comparison of different quantitative methods. First, the amount of fibers was calculated based on the results of the dynamic images analysis in Table 1. Secondly, the figure includes the edited results from the dynamic image analysis. As a last quantitative detection method, aliquots of the fiber suspension were visually analyzed using a microscope. The counted amount was extrapolated to number of fibers in the entire solution.

Particle amount results obtained for a 3.15 mg fiber solution (0.3 mm cut length) with the dynamic image analysis are in good accordance with the calculation and are in similar range as the microscopy measurements. Doubling the fiber concentration to 6.17 mg leads to the double fiber quantity for all three methods.

### 3.2 Discussion

This study highlights the analysis of fibers in detergent solution with a new analytical approach. First, the sample material was characterized using microscopy. The measurements indicated highly size-uniform particles. Dynamic image analysis measurements confirmed a narrow size distribution of the 0.3 mm sample fibers in



**Fig. 3** Results of the quantitative fiber determination for suspended fibers (0.3 mm cut length) in detergent solution. Particle amount obtained by three methods: calculation, dynamic image analysis and microscopy for two fiber concentrations 3.15 and 6.17 mg. The results of the dynamic image analysis measurements were not measured directly, however edited using evaluated correction factors. The microscopy aliquots were extrapolated to the entire sample volume

detergent solution. Furthermore, the evaluation of the size and shape parameters length, diameter, straightness, sphericity and elongation by dynamic image analysis were found to have a small standard derivation. Four replicate measurements showed reproducible results for the 0.3 and 0.5 mm samples, indicating good method repeatability.

For quantitative evaluation visual counting with an optical microscope is one of the most common reported techniques for quantifying textile fibers in sewage; however, it is time consuming and prone to human error. In this study, the conventional microscopy was compared to dynamic image analysis as well as a calculation. The method comparison confirmed a good correlation for all quantitative results.

## 4 Conclusions

The dynamic image analysis has been introduced as useful and powerful analytic tool for particles and fiber analysis in detergent solution. First experiments were performed with shortcut fibers in the size range of 0.3–0.5 mm, and the particle characterization measurements showed reproducible results.

Besides a precise measurement, the dynamic image analysis has technical advantages like the fast and nondestructive measurement. Furthermore, no additional sample preparation is needed for sewage samples and the measurement can be performed in the sample medium directly. The statistical evaluation is supported by sophisticated algorithms and the integrated software.



Up to date, only flock fiber–detergent suspensions have been analyzed in a small size range. Further experiments are needed to investigate mixtures of particles with different sizes and to determine whether this analytical system can be applied to real sewage samples from textile washing machines.

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# Study on Microplastics Release from Fishing Nets

Alessio Montarsolo, Raffaella Mossotti, Alessia Patrucco, Marina Zoccola, Rosalinda Caringella, Pier Davide Pozzo and Claudio Tonin

## 1 Introduction

Recent estimates indicate that 10% of total plastic waste ends up in the oceans and have reached even remote areas such as polar region. Depending on the chemical composition of plastic polymers, these wastes can accumulate on the seabed (about 70%) or remain suspended in the water column. Due to the ocean currents convergence zones, the so-called garbage patches are forming causing strong impacts on marine ecosystems. In particular, they cause profound alterations to the habitats and represent a risk for marine organisms that may be entangled or ingest them. In general, the plastic materials, although they remain in the marine environment for hundreds of years, can be subjected to fragmentation and degradation processes that lead to the formation of microplastics (defined as fragments having one dimension lower than 5 mm). The sources of microplastics are various and they can originate from the fragmentation of macroplastics by abrasive action of the waves and the degradation by UV radiation, or they may derive from microgranules of plastics intentionally added in industrial products. In addition to that, these fragments can be a vehicle for persistent organic pollutants and they can be ingested by aquatic organisms, especially filter feeders. About 1.15 million tonnes per year of plastic debris that are released in seas and oceans are represented by lost or deliberately abandoned fishing nets. In recent decades, the problem has been further aggravated by the increase in fishing operations and the introduction of particularly resistant equipment because of non-biodegradable synthetic materials. The term “ghost

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fishing” is used when abandoned nets and fishing gear, in general, continue to catch marine vertebrates and invertebrates that may become tangled in them. Commonly known as Abandoned, Lost, or otherwise Discarded Fishing Gear (ALDFG) [1], and often nearly invisible in dim light, ALDFG may either get snagged on the rocky bottom (as in the “tegnùe” habitats of the north Adriatic) or drift in the open sea for long periods due to its low biodegradability. ALDFG may be lost due to technical problems during fishing or in adverse weather conditions. As losing fishing gear is a financial loss for operators, they will certainly try to recover it. However, as time and labor spent retrieving gear depend on its value, retrieval may often be impractical and operators may prefer to carry on fishing rather than take chances and try to recover their lost gear.

Unfortunately, once fishing gear has lost its financial value, it may deliberately be abandoned by careless and irresponsible fishermen who believe dumping it in the sea is a handy way of disposing of it. Eventually, abrasive environmental conditions cause fishing nets to break up into progressively smaller fragments. The lighter plastic and polystyrene parts continue to float and may be washed ashore or disintegrate due to the effects of sunlight, leading to the formation of microplastics. These particles are absorbed by plankton and filter-feeding organisms and enter marine food chain. They may also release hazardous chemicals such as phthalates, known to interfere with the reproductive activities of many sea organisms. The degradation potential of plastic litter items remains a critical issue for marine litter research. In literature, some study about simulated degradation in laboratory are available [2]. In the present study, fishing nets recovered from the Venetian lagoon were studied in order to determine the release of microplastics due to their degradation. To simulate the environmental conditions, an internal method was set up in the laboratory merging the conditions reported in standard methods UNI EN ISO 105-E02, UNI EN ISO 105-E03 (used in textile field to assess fastness to sea water), and UNI EN ISO 105-C06 (fastness to washing). That was done to determine the microplastics release from this abandoned material, which represents an environmental problem.

## 2 Experimental

Five different fishing nets recovered from Venetian lagoon were available for the analyses. They were at first characterized by Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), and scanning electron microscopy (SEM) to determine chemical composition and surface morphology. Moreover, textile characteristics were visually observed. Washings were performed using a solution simulating marine water, and a Linitest laboratory equipment was employed to reproduce the mechanical stresses the net are subjected to. After washing, water effluents were filtered on polyvinylidene fluoride (PVDF) filters (inserted in a XX4304700 Millipore in-line filter holder) with a porosity of 5  $\mu\text{m}$  by means of a peristaltic pump. After filtration, the filter was cut into eight circular

sections and for the readings, four of them were chosen. Twenty fields at 250X for each section were observed with a total number of eighty fields in the whole filter area. The counting method was adapted from an analytical method used for the analysis of asbestos fibers (Italian Law 6-9-1994).

## **2.1 Materials**

PVDF filters of 5  $\mu\text{m}$  porosity were purchased from Merck-Millipore, Italy. All the reagents used to prepare the saline solution were purchased from Sigma Aldrich, Italy.

## **2.2 Methods**

### **2.2.1 Preparation of Samples**

The nets were washed in a beaker with hot water (Milli-Q). For each test, about 1 g of net was cut. The salinity of the seawater was recreated according to UNI EN ISO 105-E02:1998 (distilled water solution with 30 g/L of sodium chloride). The bath ratio (quantity of water for each gram of net) was 1:50. The value of the bath ratio was borrowed by UNI EN ISO 105-E03:1998. The washing process was performed in Linitest according to UNI EN ISO 105-C06:1999. This standard method is useful to simulate domestic and commercial washings in order to assess the colorfastness. Inside the metallic container, 10 stainless steel balls were added in order to simulate the mechanical stress. For each washing condition (20 and 15  $^{\circ}\text{C}$ , 1 h), the experiments were carried out on selected samples in three replicates. In the case of experiments at 15  $^{\circ}\text{C}$ , ice was added in the thermostatic bath in order to maintain the temperature.

## **3 Results and Discussion**

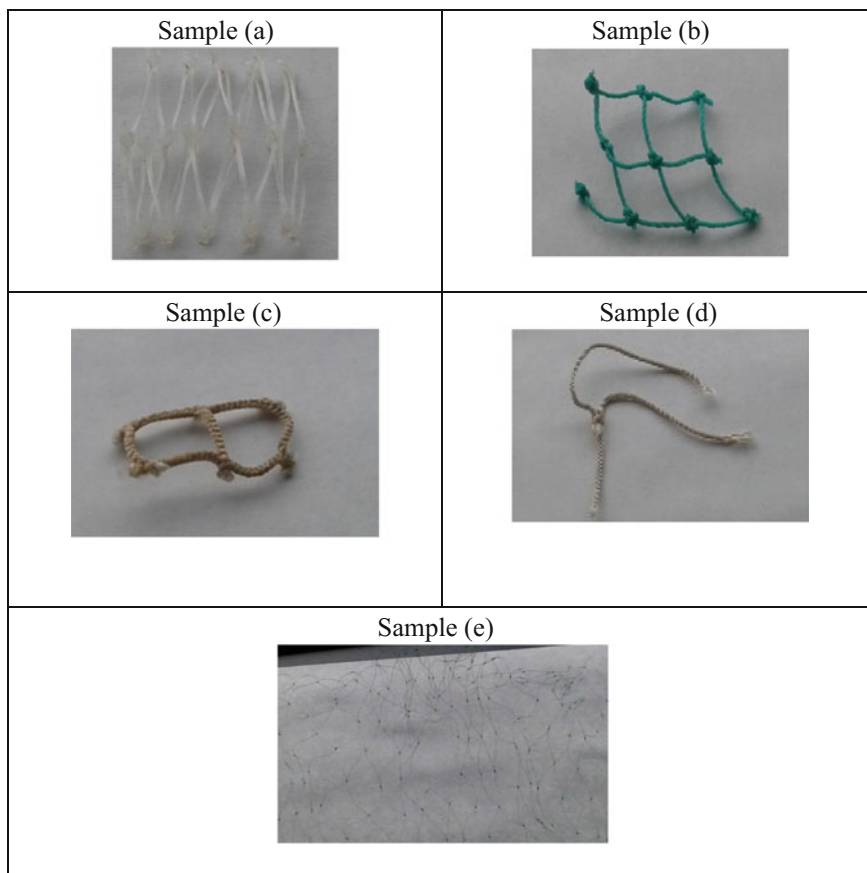
### **3.1 Characterization of the Nets**

Photographs of the analyzed nets are shown in Fig. 1.

Results of morphological analysis on abandoned nets are reported in Table 1.

In this contribution, we reported the results of characterization and microplastics release from C and D nets, which showed to be constituted by textile filaments.

The net C is constituted by textile filaments with a circular cross section and a diameter of 28  $\mu\text{m}$  (Fig. 2).



**Fig. 1** Photographs of the analyzed nets

**Table 1** Morphological analysis of abandoned nets

Sample	Type of filament and morphology	Average diameter (mm)
A	Non-textile	1.12
B	Non-textile	0.29
C	Textile filaments (circular cross section)	0.028
D	Textile filaments (circular cross section)	0.031
E	Non-textile	0.16

The sample was analyzed by FT-IR ATR. The net shows the characteristic signals of polyamide, related to the presence of  $-CH_2$  and amide functional groups (Fig. 3). DSC analysis showed a melting point at 215 °C attributable to polyamide 6 (Fig. 3).

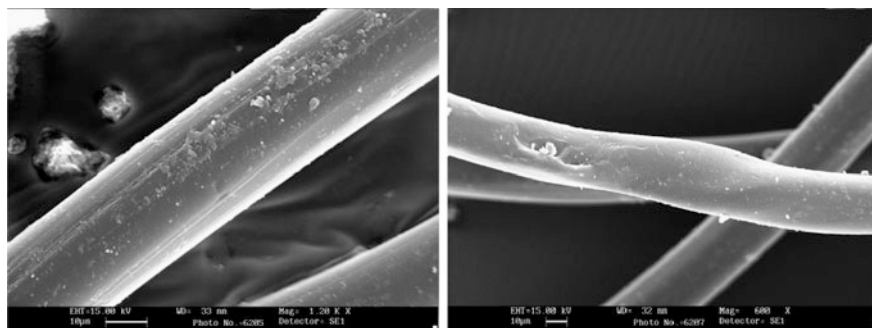


Fig. 2 SEM images of sample C

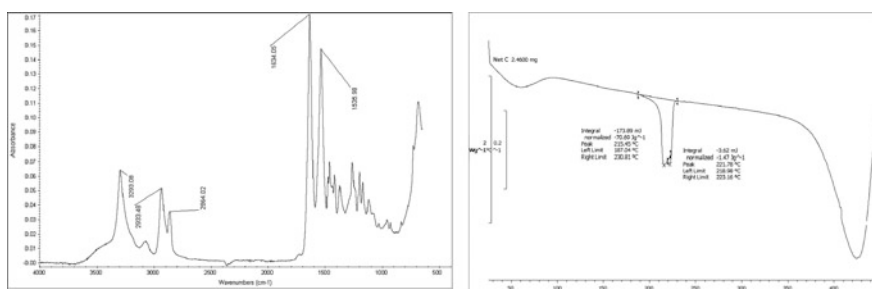


Fig. 3 FT-IR ATR and DSC analysis of net C

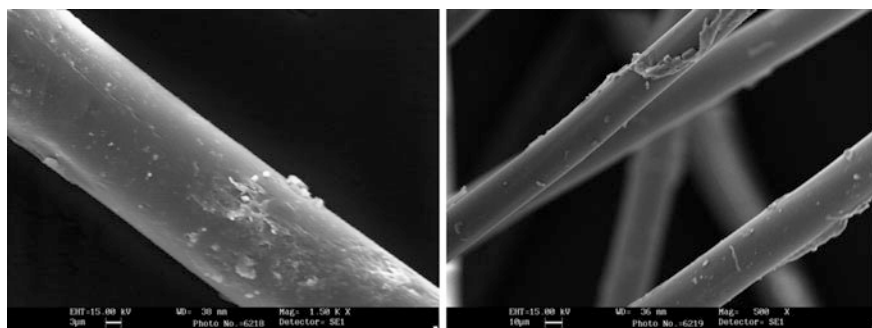
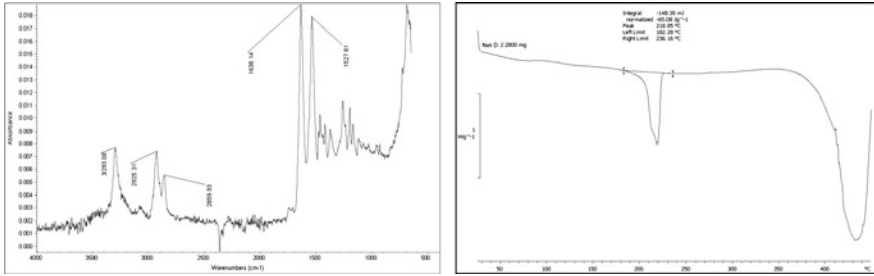


Fig. 4 SEM images of sample D

The D net is made of textile filaments with a circular cross section and a diameter of 31  $\mu\text{m}$  (Fig. 4).

The FT-IR spectrum of net D is shown in Fig. 5, and the characteristic signals of polyamide, related to the presence of  $-\text{CH}_2$  and amide functional groups are visible.



**Fig. 5** FT-IR ATR and DSC analysis of net D

Melting point around 219 °C DSC suggested that Net D is constituted by polyamide 6 (Fig. 5).

### 3.2 *Laboratory Simulation of Marine Environment Effect on Fishing Nets*

In order to quantify the microplastics release coming from the abandoned fishing net in the Venetian Lagoon, experiments with the Linitest equipment were performed as described in the Methods section. As said before, we reported here the tests performed on two of the five samples because they were constituted by textile fibers.

After the washing processes, the resulting solutions were then filtrated on PVDF membranes in order to collect microplastics released by the nets. The quantification by SEM was performed with this internal procedure:

cut the filter into 8 circular sectors

analysis of 20 fields (250X) for each circular sector from the center of the filter to the circumference

counting 1 for each microplastic entirely contained in the field or crossing the whole field, 0.5 for each microplastic ending in the field analyzed

elaboration of results on the basis of the whole filter area and express them on the basis of the quantity of material washed (weight in grams).

The microplastics from nets C and D can be mainly identified by SEM analysis on the basis of the shape of the original fibers since they are actually fiber snippets. Figure 6 shows images of the microplastics released from the nets C.

Figure 7 shows the results of the SEM analysis on the filters (three replicates for every condition). The average values are related to the quantity of material washed.

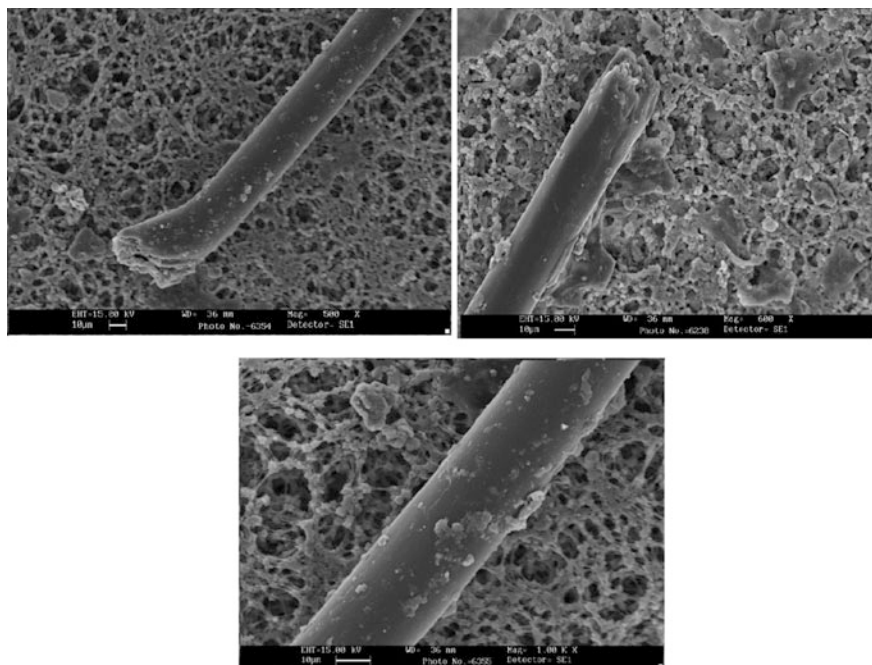


Fig. 6 SEM images of microplastics released by net C collected on PVDF filter

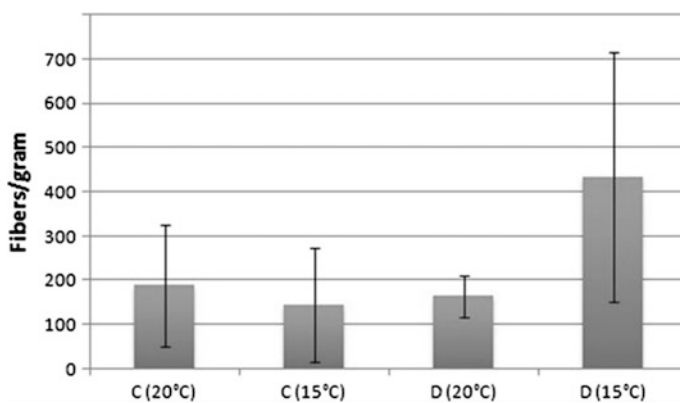


Fig. 7 Results of SEM analysis of filters



## 4 Conclusions

In this work, five samples of fishing nets abandoned in the Venetian Lagoon were analyzed to characterize them by the chemical and morphological point of view. Then, two of these nets were selected because constituted by textile filament yarns to simulate in laboratory a further consumption due to marine environment and the possible release of microplastics fragments. The nets were washed merging different textile standard method for quality control. Then, the washing effluents were collected and filtered on filters of defined microporosity. The filters were analyzed with SEM, and a statistical counting was performed based on an internal method. Results were very variable changing the temperature conditions, but for this variability, it was not possible to highlight a general behavior or tendency in the microplastics release. However, these results demonstrated the environmental problem represented by abandoned fishing gear because they release microplastics fragments that can enter directly in the marine food chain. Further, studies have to be conducted to obtain a higher number of data to establish connections between marine environment stress on the nets and the release of microplastics.

**Acknowledgements** The authors would like to acknowledge the team of Life Ghost (LIFE12 BIO/IT/000556) European Project for supplying nets samples.

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# A Research on Microplastic Presence in Outdoor Air

Meral Yurtsever, Ahmet Tunahan Kaya and Senem Çiftçi Bayraktar

## 1 Introduction

Microplastics (MPs) are among major micropollutants (<5 mm) which can be found in water sources [1] and air in substantial quantities and which still are not covered by standard sorting and analysis procedures [2]. Even though researchers often come across larger “MP fragments and films” in water sources under review, it is understood that the rather more common microplastics in the wastewater treatment plants (WWTPs) [3–5] and the atmosphere [6] are of the “microplastic fiber” variety.

Microplastic fibers (MFs) from a number of sources can settle on the ground, only to be lifted into the air thanks to wind and air flows; remain suspended in the air for some time [7–10]; and find its way to the lungs of people through the respiratory system, just like air pollutant emissions. In 1998 while microplastics as a generic term are unknown yet, a group of cancer researchers at the end of the studies on 114 human lung tissues explained that bioresistant cellulosic and plastic fibers are candidate agents contributing to the risk of lung cancer [11]. This is a quite remarkable work. Even though nowadays the term microplastics (MPs) is heard of by ever wider audiences, their effects on lungs, the wider respiratory system, and human health in general, as well as their association with cancer are far from well-understood.

The objective of the present study is to make an assessment of the volume of microplastic fibers finding their way to soil, compared to those freely floating in the air due to their lightweight and micronano-size.

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## 2 Materials and Methods

### 2.1 Preparation of Samples

The aim of this study is to determine the amount of microplastic fibers (MFs) in the air of a random selected crowded area. For this purpose, the amount of MP in the samples taken through half an hour of vacuum suction ( $0.3 \text{ m}^3/\text{min}$ ) at the intercity terminal located on the entrance to the university campus in Sakarya Province, as well as of MP fallouts in the soil samples taken from the same location, was assessed. The samples were taken in the period December 2016–May 2017.

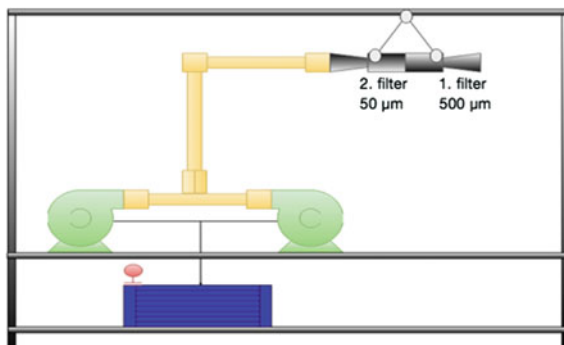
With a view to investigating the impact of weather conditions at the time of sampling, on the amount of MPs in the air, the variables such as temperature, wind, humidity applicable during sampling are recorded in Table 1.

Air vacuum filter used to observation of MPs in the air is schematized in Fig. 1. The 500 and 50  $\mu\text{m}$  clean steel filters were used in the studies. Samples taken through vacuuming were immediately sealed and brought to the laboratory, to be examined under microscope after washing–separating procedures. They were then classified according to the results of the examination.

**Table 1** Weather conditions at different sampling periods

Dates	Temperature (°C)	Wind direction (°)	Wind speed (m/s)	Relative humidity (%)	Conditions
22/12/2016	2	331	2	74	After snowfall
31/01/2017	4	122	2	74	Foggy
23/02/2017	18	238	4	74	Partly cloudy
22/03/2017	14	48	5	74	Sunny
21/04/2017	11	348	1	74	Partly cloudy
17/05/2017	17	37	3	74	Sunny

**Fig. 1** Air vacuum filter



Performed separating processes on the receiving air and soil samples can be listed briefly as follows:

- Hydrogen peroxide ( $H_2O_2$ ) treatment (wet peroxide oxidation) to remove organic impurities;
- MP flotation with zinc chloride ( $ZnCl_2$ );
- Centrifuge;
- Filtration using steel filters (500 and 50  $\mu m$  pore size steel filters for air samples; 20  $\mu m$  pore size Whatman black-band filter paper for soil samples).

## 2.2 Microscopic Examination

The MPs found on the filter (50  $\mu m$ ) used to take the air samples with a flow rate of 0.3  $m^3/min$  were examined under the microscope. During the process, counts were taken at seven distinct areas, to provide input for overall MP figure calculations. The soil samples from the locations where air samples were taken at the campus, on the other hand, were processed through dissolution in water, treatment with  $H_2O_2$ , flotation with  $ZnCl_2$ , and sorting, followed by the review of the remainder on a 20  $\mu m$  filter. Image acquisition and digitalization were performed (4X magnification) with a digital camera (DP20; Olympus, Tokyo, Japan) coupled to a light microscope (BX51; Olympus).

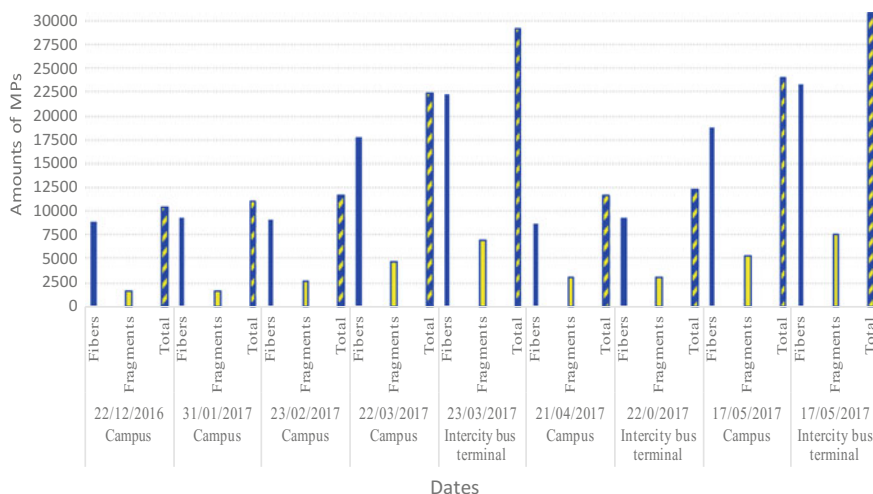
## 3 Results and Discussion

### 3.1 Results

Presence of MPs in the air samples was examined in detail according to their colors and shapes and tried to determine their numbers (See Fig. 2 and Table 2).

The average, STD, range, and median values of the total MP amount obtained from the counting with microscope are given in Table 2. The MPs remaining on the steel filter are shown as an example in Fig. 3.

The results of the review carried out over 1 g of the soil samples are presented in Table 3, showing MP particle count per gram. The MPs in triple sampling containers placed on the ground, with a view to determining the number of MPs settling through atmospheric fallout during a sampling period of 30 min, were also counted and noted. The containers kept outdoors for 30 min were observed to have an average of 8 MPs (dark blue 2 fibers-1 fragment, black 1 fiber-1 fragment, 1 blue fiber, 2 brown fragments, and other particles) in various sizes, settling through gravitation.



**Fig. 2** Number of microplastics in outdoor air for different times (in 30 min sampling period)

The samples were examined under light microscope, followed by the use of ATR FT-IR (attenuated total reflection Fourier transform infrared) (Opus 7.5, Lumos, Bruker, Germany) to establish the polymer types of the plastics. However, as the MPs observed were very small in size and were mostly composed of “fibers,” the less-than-concrete results of the procedure employing steel filter are disregarded in this study.

The samples taken in the period December 2016–May 2017 revealed different amounts of MP fibers in each month, not to mention a number of MP particles, while dark blue, white, transparent, and brown were the most frequently observed MP colors. The factor leading to the variation in MP volumes from one month to the next is the change in weather. For instance, samples taken after rainfall revealed substantial falls in microplastic counts.

### 3.2 Discussion

The volume of MP in circulation in the atmosphere can vary substantially along the axes of time, space, and seasonal conditions. That is why it is difficult to specify a clear figure for MP volumes a location is expected to have; however, it is possible to claim that atmosphere certainly contains some amount of MP, albeit in varying quantities. That is why microplastics in the air should be taken into consideration among Particulate Matters (PMs; as coarse, fine, or ultra-fine particles) which are considered major air pollutants, alongside the emissions of  $SO_x$ ,  $NO_x$ , and  $CO_x$  which had been under close supervision for quite some time and thus should be studied in depth in terms of their impact on human health.

**Table 2** Amounts of MPs (during 30 min air sampling periods)

MPs		Colors	Average	Range	STD	MED
22/12/ 2016 Campus	Fibers	Dark blue and similar colors	5046	4025–6843	1239	4528
		White and similar colors	3810	2214–6440	1383	3522
	Fragments	Transparent and similar colors	1380	906–1811	245	1409
		Brown and similar colors	259	0–805	446	0
	Total		10495	7044–14894	2724	9057
31/01/ 2017 Campus	Fibers	Dark blue and similar colors	5103	3623–6440	1466	5032
		White and similar colors	4212	2616–4830	1706	4025
	Fragments	Transparent and similar colors	1394	1208–2214	351	1610
		Brown and similar colors	302	403–1208	279	302
	Total		11012	8654–13686	3516	11472
23/02/ 2017 Campus	Fibers	Dark blue and similar colors	5161	6642–15296	977	5132
		White and similar colors	3867	5635–10667	865	3925
	Fragments	Transparent and similar colors	1768	1610–4428	348	1811
		Brown and similar colors	791	403–2013	263	805
	Total		11587	14894–32202	1769	11673
22/03/ 2017 Campus	Fibers	Dark blue and similar colors	10106	10063–13283	3630	8856
		White and similar colors	7619	8252–11271	2257	6038
	Fragments	Transparent and similar colors	3235	4428–6139	1094	3421
		Brown and similar colors	1394	1208–2113	605	1610
	Total		22355	26768–30995	7125	19925

(continued)

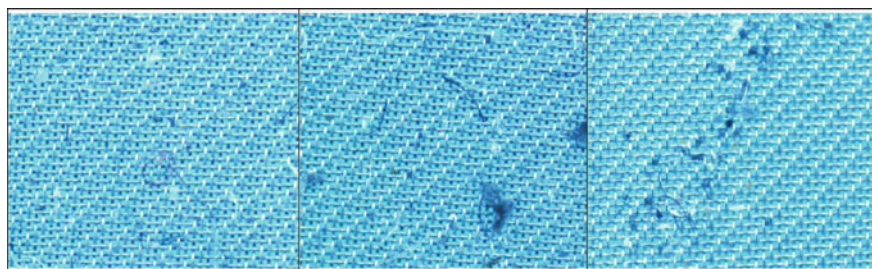
**Table 2** (continued)

MPs		Colors	Average	Range	STD	MED
23/03/ 2017 Intercity bus terminal	Fibers	Dark blue and similar colors	12335	4227–6642	1155	12881
		White and similar colors	9891	2214–4428	996	10063
	Fragments	Transparent and similar colors	5190	1610–2616	667	5032
		Brown and similar colors	1739	403–1208	342	1811
	Total		29155	9661–14491	1296	29385
21/04/ 2017 Campus	Fibers	Dark blue and similar colors	5247	4025–6843	886	4931
		White and similar colors	3450	2415–4428	825	3623
	Fragments	Transparent and similar colors	2085	805–2616	430	2214
		Brown and similar colors	891	403–1811	281	805
	Total		11745	8856–14894	1699	11774
22/0/2017 Intercity bus terminal	Fibers	Dark blue and similar colors	5750	7044–16101	1051	6038
		White and similar colors	3594	6038–11271	783	3824
	Fragments	Transparent and similar colors	1869	2415–5233	714	2013
		Brown and similar colors	1150	805–2415	541	1208
	Total		12363	16705–35020	2223	11673
17/05/ 2017 Campus	Fibers	Dark blue and similar colors	10624	10466–14089	3605	9258
		White and similar colors	8166	8453–11673	2259	7246
	Fragments	Transparent and similar colors	3652	4428–6843	1093	3623
		Brown and similar colors	1567	1610–2415	538	1610
	Total		24008	28580–32605	7252	21737

(continued)

**Table 2** (continued)

MPs		Colors	Average	Range	STD	MED
17/05/ 2017 Intercity bus terminal	Fibers	Dark blue and similar colors	12895	10466–14089	1211	13283
		White and similar colors	10437	8453–11673	1087	10667
	Fragments	Transparent and similar colors	5592	4428–6843	817	5434
		Brown and similar colors	1898	1610–2415	304	2013
	Total		30822	28580–32605	1337	30793

**Fig. 3** Some MPs on steel filter**Table 3** Amounts of MPs in soil samples

MPs in soil		Sampling date		
		22/12/2016	21/04/2017	17/05/2017
Fibers	Dark blue	3	5	6
	Blue	2	2	4
	Black	1	1	1
	Purple	1	0	2
	White, transparent, etc.	1	0	3
Fragments	Brown	1	2	3
	Dark blue	4	4	3
	Blue	2	3	3
	Black and others	1	0	2
	White, transparent, etc.	2	5	2
Total		18	22	29



## 4 Conclusions

Given the small size of MPs reviewed in the study, the results do not reflect all MPs presence in air. The observations carried out outdoors at different time frames revealed that MPs were present in widely varying rates and sizes in the air, and the amount of MPs caught could vary even in very short time frames. The number of MPs in a given setting is determined by a number of factors including but not limited to consumption habits, socioeconomic status, traffic, urbanization. The clothing requirements varying from season to season have also an impact on the amount of MPs in outdoor air, particularly for the case of fibers. Substantial developments in the synthetic fibers industry and extensive use of cheap nonwoven fabrics could also help explain the increase of MPs fibers in the environment. It is also important to note that meteorological factors such as the weather, wind speed, wind direction, humidity, temperature, and cyclones can also play a major role in determining the amount of MPs in the air.

Further studies are required for the development of standard methods regarding the analysis of MPs in the air we breathe and in atmospheric fallout.

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# Commonly Used Disposable Plastic Bags as a Source of Microplastic in Environment

Meral Yurtsever and Ulaş Yurtsever

## 1 Introduction

The use of disposable plastic bags, one of the leading plastic products encountered in daily life, has been mostly impossible to prevent, even though a falling trend is observed in light of taxes and fines introduced in a number of countries. The term microplastics often recalls the break of larger plastics into smaller “plastic fragments.” Yet, “MP fibers” which are severed apart from synthetic textiles and “MP films” which are produced as plastic bags crumble away in time should also come to one’s mind. The smaller the size of a plastic, the larger the number and range of species it could affect and harm. In terms of volume, microplastic fibers and films are particularly smaller, lighter, and less voluminous compared to “plastic fragments.” Hence, plastics in fiber and film forms can be carried away to further distances by water and can be swallowed more easily by primary consumers; making them practically more substantial threats [1–4]. Today, it is necessary to consider microplastics as pollutants and to reduce the use of plastic products (especially disposable plastic bags) in favor of natural equivalents. The consumers’ behavior and governmental policies are pivotal in terms of encouraging people to go for reusable bags [5, 6].

Considering the types of plastic used in the disposable bags, three types of plastic can be mentioned from an environmental point of view. These are ordinary

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(petroleum-based, cheap) plastics [7, 8], oxo-biodegradable plastics (expensive) [9], and biodegradable plastics (more expensive) [10, 11]. Standard plastic polymer bags, such as shopping bags from supermarkets, consist of polyethylene (PE) or polypropylene (PP), the most commonly used plastics. Polyethylene is composed of polymerized ethylene molecules ( $C_2H_4$ ) [12]. Plastics that are obtained from biological natural raw materials (e.g., starch-based, cellulose, chitosan) and degradable without any additive to nature are called biodegradable plastics. The raw materials of oxo-biodegradable plastics (ODP) [13] are oil and petroleum derivatives. However, additives added to these plastics accelerate the degradation of the plastic. The plastic bags in this type have the phrase “100% soluble in nature.” Plastic bags start to crumbling like “glass” due to the additive matter used in such plastics, after a while in nature. “Oxo-biodegradable” plastics (petroleum-based) can break into small fragment, but they do not disappear like “biodegradable” plastics. Small pieces are separated by continuously preserving the plastic features. Biodegradable plastics are defined as materials that are completely degraded to carbon dioxide and water by the action of naturally occurring microorganisms (bacteria, fungi, and algae).

## 2 Materials and Method

### 2.1 Material

The present study compares some characteristics of aged and new polyethylene (PE) plastic bags. In this context, physical properties (such as durability, flexibility, surface morphology, and fragmentation) of three aged bags (grocery bag, shopping bag, and blue thin garbage bag) which were kept under normal conditions in a dark cupboard for 4 years, after approximately 3 months of exposure to sunlight outdoors in spring–summer (annual average relative humidity is 74%, average temperatures are min. 17 °C–max. 28 °C, average insolation time is 8 h), were compared against those of new bags. The thickness of a commonly used thin plastic food bag is about 0.5 mil ( $\cong 12.7 \mu\text{m}$ ). In this study, thicknesses of examined plastic bags are as follows: The grocery bag (oxo-LDPE) is 20  $\mu\text{m}$ , the shopping bag (oxo-HDPE) is 44  $\mu\text{m}$ , and trash bag (LDPE plastic) is 15  $\mu\text{m}$ . Plastic types are oxo-biodegradable low-density polyethylene film (OBD-LDPE) with a density of 0.92 g  $\text{cm}^{-3}$  and oxo-biodegradable high-density polyethylene film (OBD-HDPE) with a density of 0.96 g  $\text{cm}^{-3}$ . Scanning electron microscope (SEM) images were taken to be able to get a better definition of the surfaces of these bags (see Figs. 3 and 4).

Life cycle assessment (LCA) is a systematic perspective aiming to present a detailed picture of the environmental impact of products and services, throughout

the whole life cycle (from the cradle to the grave) [14–16]. Therefore, it is one of the important means to achieve sustainable development. For instance, the environmental risks a product may pose from the production of the raw materials to be used for the manufacturing of the product, to shipping, consumption, and disposal procedures, can be reviewed with reference to a life cycle.

Approximately 0.2% of all oil produced worldwide is consumed for producing plastic bags. According to data for 2012, presented by Wal-Mart Stores Inc., an American chain of retail stores with branches all around the globe, on average each individual uses 216 “disposable plastic bags” per year [17]. Of course, the figure presents some variations from one region to another (e.g., Los Angeles usage, per person 600 bags/year). In addition to all these, considering the fact that plastic bags which began to be used for grocery shopping since 1977, if they are thought to have turned into a waste within about 30 minutes from initial use, it will be better understood how these disposable bags posed a threat to the environment [18, 19].

In Fig. 1, a life cycle assessment of the disposable plastic bag is given. Here, if we make a life cycle assessment for disposable PE plastic bags in general terms, it is likely that a plastic bag will complete its life cycle after these steps: processing of petroleum, acquisition of plastic raw material, melting and processing of raw materials, packaging, transportation, distribution, use, waste formation, reuse, recycling, and finally disposal (landfill, energy recovery, etc.), respectively.

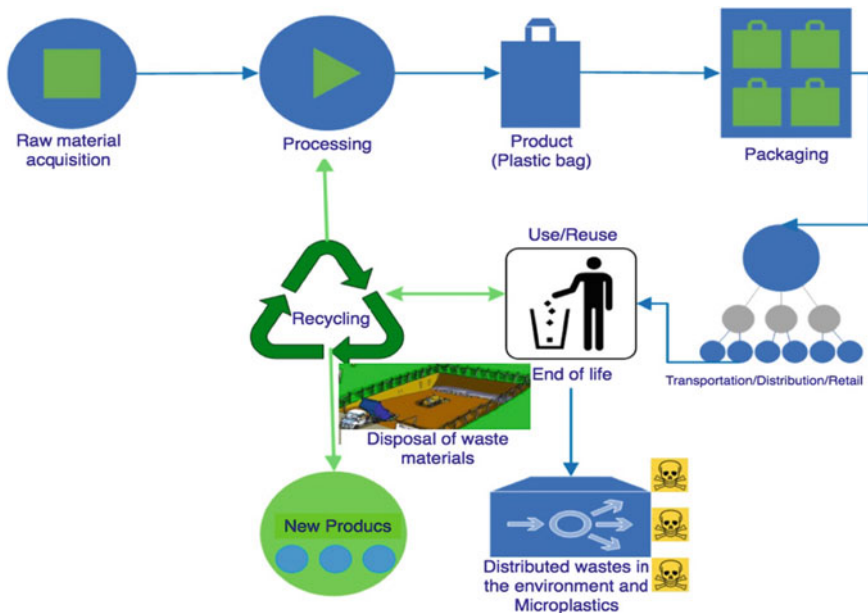


Fig. 1 Life cycle assessment (LCA) of disposable plastic bags

**Fig. 2** Image of aged and fragmented grocery plastic (OBD-LDPE) bag

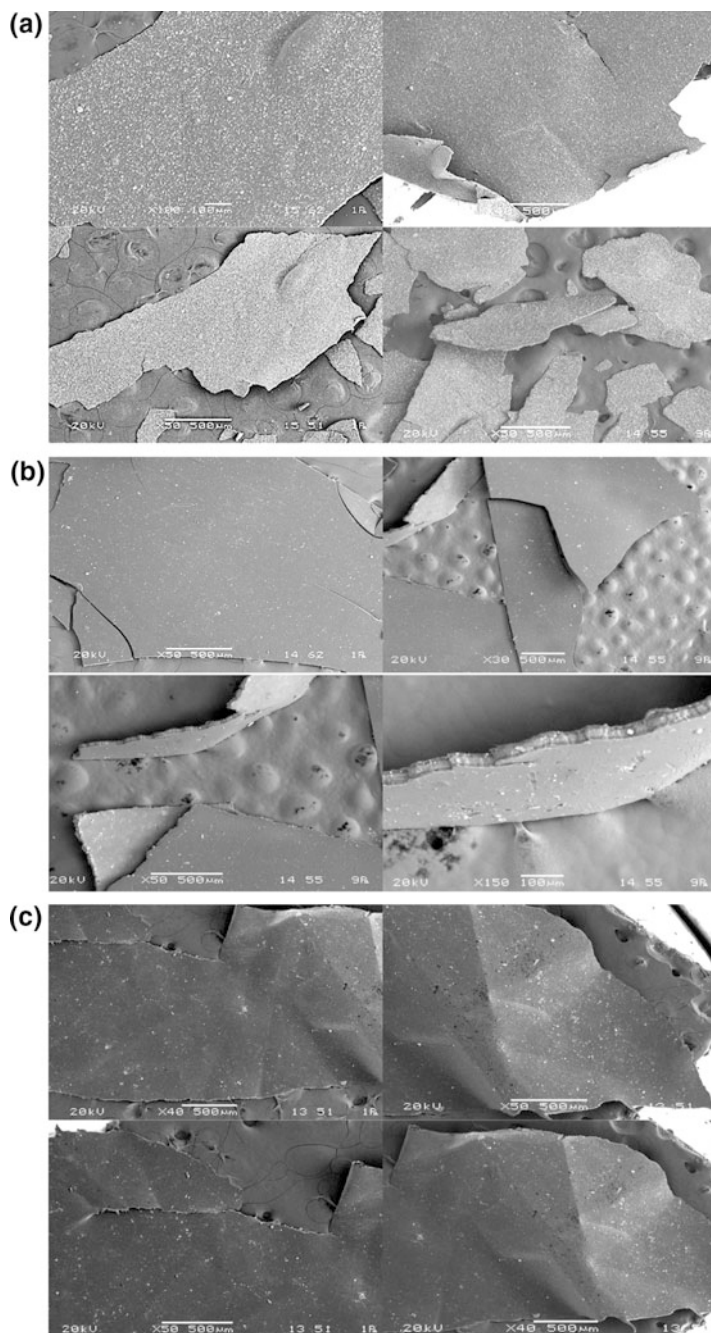


Throughout all stages of their life cycle, plastics find their way into the environment, air, and water. Particularly the time frame to follow the initial use sees the highest generation rates of MPs, as the plastics are shredded into smaller and smaller sizes through a number of environmental factors (sunlight, temperature, humidity, wind, water-wave impact, and anthropogenic activities) in time (see Fig. 2). The life cycle assessment did not entail detailed analyses which may be required for verification purposes, such as mass and energy equilibriums and comparative emission factor analyses.

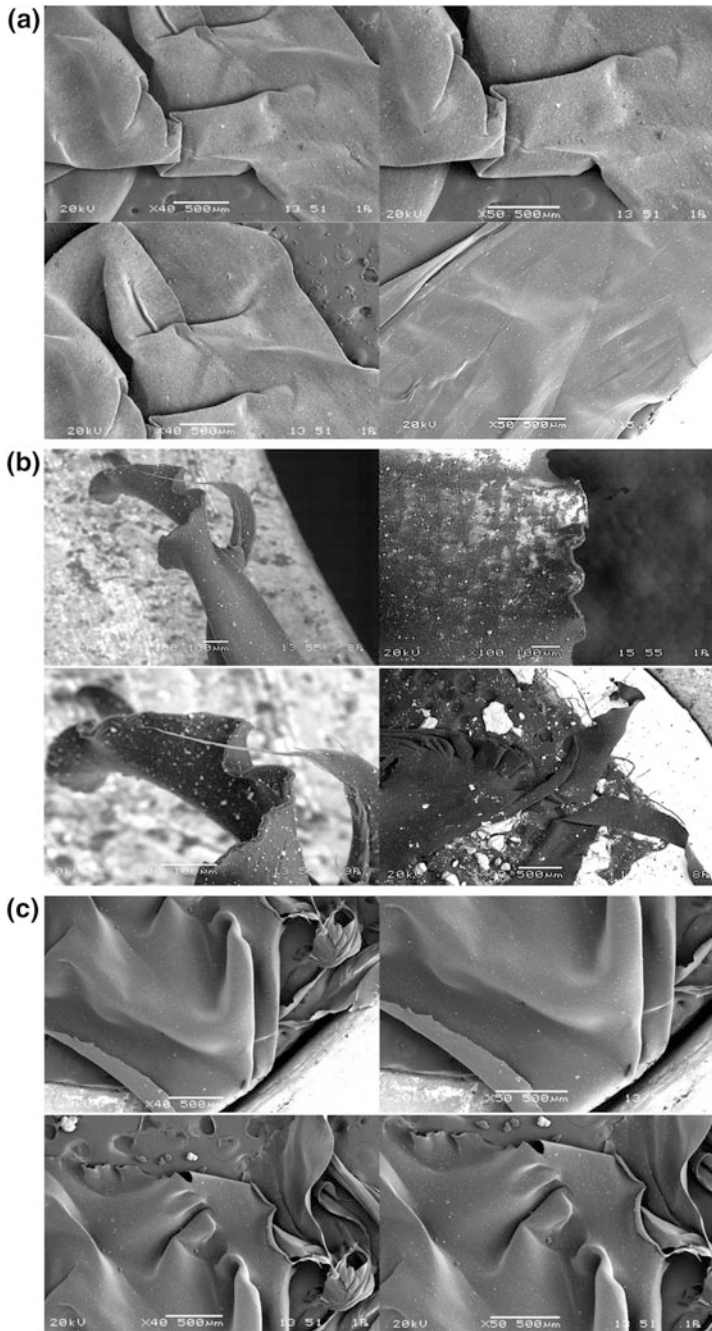
### 3 Results and Discussion

Even degradable plastics may persist for a considerable time depending on local environmental factors, as rates of degradation depend on physical factors, such as levels of ultraviolet light exposure, oxygen, and temperature [20, 21]. The deforming impact of UV in sunlight is well known; the bags exposed to sunlight for a specific term, followed by an extended period of no intervention, were found to be pretty fragile, fragmenting with even the softest of touches (see Fig. 2).

The SEM images reviewed revealed that aged bags (see Fig. 3) were in the form of fragmented MP films with sharp edges and fractures, while new bags (see Fig. 4) resisted tear when pulled with forceps, producing flexible stretches and thinned edges. It can be said that the degradation in the plastic films occurs as superficial abrasion.



**Fig. 3** SEM images of aged bags, **a** grocery bag, **b** shopping bag, **c** trash bag



**Fig. 4** SEM images of new bags, **a** grocery bag, **b** shopping bag, **c** trash bag



## 4 Conclusion

The use of oxo-biodegradable bags (ODP-PE) produced with the use of additives the polymer bonds of which can be degraded through interaction with oxygen, humidity, sunlight, and heat is now commonplace. As oxo-biodegradable bags undergo rapid degradation and dissolution in the nature, they are usually deemed environment-friendly products. However, they continue to be essentially petroleum-based plastics, even after fragmentation down to the smallest sizes they can be observed in, within a time frame of 18–24 months. In other words, the environmental threat they pose will not be affected at all, even after they are reduced to micro-nanoscale. That is why it is necessary to make substantial efforts to raise awareness among people and encourage recycling of oxo-biodegradable plastics, just like the dominant perspective on petroleum-based plastics. Applicable regulations introducing fees and taxes, encouraging durable and natural alternatives instead of single-use plastics with a view to achieving the minimization of waste can help prevent excessive consumption and the development of excessive volumes of waste.

In a nutshell, complete degrading and dissolution of plastic bags exposed to natural elements in the environment is not a very realistic expectation (in the last period of the degradation process), as they unavoidably turn into MPs, which, essentially, is a greater source of environmental pollution. That point will be even more clearer, considering the plastics floating in water sources and oceans, and the biota exposed to this form of pollution.

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# From Coral Triangle to Trash Triangle—How the Hot spot of Global Marine Biodiversity Is Threatened by Plastic Waste

Markus T. Lasut, Miriam Weber, Fransisco Pangalila, Natalie D. C. Rumampuk, Joice R. T. S. L. Rimper, Veibe Warouw, Stella T. Kaunang and Christian Lott

## 1 Introduction

Southeast Asia harbours the highest marine diversity of our planet. At the same time, the countries in the so-called Coral Triangle (CT; Fig. 1) have the highest potential/risk of plastic pollution to the marine environment. Biodiversity research is still struggling with the sheer inventory of biota, as many marine organisms already are under risk of becoming extinct by human influence. Many authors have reported about the occurrence of this type of pollution, including other marine debris in general, in this region, for instances in Bootless Bay, Motupore Island, Papua New Guinea [1], in Indonesia; Kupang [2], Bali [3], Manado [4], Ternate [5]. Most of them described the environmental condition of the areas as being full of waste, especially plastic debris, except Luwuk Peninsula, Indonesia, which Scaps and Runtukahu [6], about ten years ago, described in good condition with no debris.

Indonesia plays an important role in the CT region: it hosts marine mega-biodiversity, called “Amazon of the Ocean” with various resources of coral reefs, mangrove and seagrass ecosystems [8]. At the same time, the country is the second biggest polluter in the world, with an estimated production of about 3.22 million tons of plastic waste in 2010 [9]. This is triggered by the geography with its small-scale areas of regencies, cities and islands, and also, for instance, a medium-sized city like Manado (North Sulawesi, Indonesia) as a case study in the

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**Fig. 1** Coral Triangle (CT) area [7]

CT area. In this city, waste management strategies are rudimentary, baseline data on sources, quantity and quality of plastic waste are lacking, and micro-plastic in special is hardly addressed, thus urgently needed risk assessment and mitigation concepts lack fundamental knowledge.

We provide observational data as beach clean-up reports, remotely operated vehicle (ROV) seafloor surveys and gut content analyses of fish, as the first collection of available information for this region. As a conclusion, we formulate further actions needed for Indonesia and globally to address the problem.

## 2 Waste Management in the City of Manado

### 2.1 Waste Discharges

Manado City (MC), the capital city of North Sulawesi Province, is located at the western part of Minahasa Peninsula in the NE of Sulawesi Island, a K-shaped island, in the middle of the Indonesian archipelago (Fig. 2). It is a medium-sized city, 15,726 ha [10], with a dense population of about half a million people. Right next to the city is Manado Bay and Bunaken Island, one of the iconic world-class destinations of diving tourism, and the Bunaken National Park (BNP). Manado is origin of a stream of mismanaged waste entering the ocean everyday, with different estimations, the highest being an assumed volume of  $250 \text{ m}^3 \text{ d}^{-1}$  in 2004 [11], showing drastic effects to the nearby coastal environment, especially coral reefs and mangroves.



Fig. 2 Manado City with North Sulawesi Province [12]

Leading to the waste discharge, the production of waste is increasing from 828  $\text{m}^3 \text{d}^{-1}$  in 2011 to 980  $\text{m}^3 \text{d}^{-1}$  in 2014 [13]. In 2015, potential waste of the city was estimated about 1037  $\text{m}^3 \text{d}^{-1}$  (assumption of 0.0025  $\text{m}^3 \text{ind}^{-1} \text{d}^{-1}$ ) [14, 15]; up to 984  $\text{m}^3 \text{d}^{-1}$  could be treated by waste dumping as the final destination of the waste, but about 52  $\text{m}^3 \text{d}^{-1}$  were calculated to be released to the environment [14]; this could be end up into Manado Bay. The overall waste production in 2015 of North Sulawesi Province was 391,000 ton year<sup>-1</sup> [16]. In addition, in Bunaken Island, about 21–45 and 50–63% of wastes were in organic and inorganic forms, respectively. Most of the inorganic waste was attributed to tourism activity on the island [17].

After the local government of Manado released the regulation n. 7 in 2006, the management is officially regulated by the government. Since then however, significant changes have not been achieved yet. In fact, most of wastewater discharges occur with insufficient treatments [11] or even without any treatment at all [18].

This causes rivers flowing to Manado Bay through the city being full of garbage, especially long-lasting plastic waste.

There is a wide range of environmental impact due to insufficient management of inland waste and marine debris, especially plastic waste. Based on a survey by Green Eye Project Aquamarine Fukushima, Japan, in 2011, using ROV, they found large amounts of plastic trash on the bottom of Manado Bay and its surrounding area, close to Bunaken National Park (BNP). The assumed source of the trash was mostly from the inland area brought in through six rivers that flow across the city to the bay.

In Indonesia, plastic debris was found in the gut of about 20% of commercially caught fish, this being a drastic proof the Indonesian marine waters are polluted, mainly due to garbage [19]. As an accidental finding, there was the high gut content of plastic in the specimen of “living fossil” *Latimeria menadoensis*, the iconic coelacanth in 2012. This paragraph summed up the first collection of available information for this region.

## **2.2 Best-Practice Environmental Activities**

In MC, in order to overcome this poor environmental condition due to plastic debris, all stakeholders (communities, students, civil servants, army, etc.) are mobilized to do best-practice environmental activities. One of the activities which are being conducted regularly is beach clean-ups. These events are also seen as a manifestation of community awareness to this problem. Such activity is being conducted not only at the city level of Manado but also at the provincial level of North Sulawesi. Moreover, such activity is regularly conducted within the CT countries through the Coral Triangle Day Program [20].

In Indonesia, other best-practice environmental activities are being conducted. In Manado, to manage the general waste, on-site composting of organic waste is conducted by each individual and/or by groups of the community which was initiated by themselves. This form of waste treatment is also the most common in the island of Bunaken [17]. In Bali, participation in waste management is conducted by community on a sociocultural base through a community-based waste management [3].

## **3 Experiences from Waste Management in the Mediterranean Sea**

The Mediterranean Sea also has a high biodiversity, parts of which are already lost or highly threatened. Plastic pollution has a long history, and the problem has been addressed by large only recently. The Coral Triangle could profit from expertise and

concepts developed in the Med and give in change experiences from a region where due to the relative short history of plastic waste, and still spatially restricted heavy urbanization, the impact on near-pristine marine ecosystems can be studied in order to find global solutions.

## **4 Further Actions**

### **4.1 *Indonesia Actions***

Many actions are being conducted as countermeasures to the plastic debris pollution in Indonesia. On the local level of Manado City, it was advised to invest more in prevention, like proper education about the effects of garbage [11]. In 2004, key management strategies were proposed; they were private sector involvement; public awareness, cooperation and participation; and cost recovery and financial resources [21]. On the national level, the Presidential Instruction n. 12, 2016, was published as the direction for the Clean Indonesia Program. Based on this instruction, an action plan “the Indonesia’s Plan of Action on Marine Plastic Debris 2017–2025” [8] was launched. The action recommended three important key aspects in handling marine plastic debris in Indonesia, such as coordination between institutions; application of technology to control plastic debris, including the application of science-based management; and the significant importance of societal efforts to reduce, recycle and reuse plastic debris to be advanced since early stage. The expected goal of this action is to reduce marine plastic debris by 70% in 2025.

In addition, in order to create countermeasures among the CT countries, a joint activity of Coral Triangle Initiatives (CTI) could be conducted. The CTI is a multilateral partnership of six countries (Indonesia, Malaysia, Papua New Guinea, the Philippines, Solomon Islands and Timor-Leste), located in the CT region and formed in 2007 to address the urgent threats faced by the coastal and marine resources of one of the most biologically diverse and ecologically rich regions on Earth [20].

### **4.2 *Global Actions***

Since marine debris does harm many marine organisms and wildlife [1, 22–24], global and local governance responses are needed to effectively manage the plastic marine litter problem [25]. Besides, joint actions and exchange of knowledge should lead to an efficient global strategy.

## 5 Final Remarks

An interdisciplinary action plan for the Manado area should be formulated that can be extended and adapted to the wider region of the Coral Triangle.

A quote from a biodiversity researcher and colleague: “*I really hope that some of the marine debris problems in Bunaken Island are addressed by the authorities in near future, or the island will be considered as another lost paradise*” (Waegele, 2016 pers.comm.).

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# Preliminary Assessment of Microplastic Accumulation in Wild Mediterranean Species

Manuela Piccardo, Serena Fellingine and Antonio Terlizzi

## 1 Introduction

Because of their abundance, persistence, and ubiquity, microplastics (MPs) represent a serious environmental risk, recognized even by the European Union which calls for further investigation. The Mediterranean basin seems to be highly affected by microplastic pollution, and it has been considered the sixth great accumulation area for marine litter [1]. Polyethylene (PE) and polypropylene (PP) are the main polymers found in the Mediterranean Sea, followed by a “soup” of many other synthetic polymers [2].

While it is known the impact caused by the largest pieces of plastic debris and ghost fishing on mega-fauna such as seabirds, turtles, and cetaceans, the knowledge of environmental effects of microplastics—plastic particles  $\leq 5$  mm diameter, as defined by NOAA [3]—is still limited.

Recently, several studies showed that ingestion of microplastics is commonplace throughout marine ecosystems (e.g., [4, 5]), but the effects on health status of marine wildlife, or the impact to higher trophic levels through biomagnification is still to understand.

In this study, we carried out a preliminary assessment of the ingestion of microplastics in a wild fish species, namely the red mullet *Mullus barbatus*, of economic and ecologic interest in the Mediterranean basin. For the first time,

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we searched for microplastics not only in the stomach contents, but also in the gills, in order to understand if these tissues could represent a further route of entry of MPs in biological systems.

## 2 Experimental

This study is part of a larger project aimed at monitoring and evaluating the impact of marine litter on fish populations from the Italian sea coasts.

Twelve individuals of *M. barbatus* ( $16.8 \pm 0.3$  mean length;  $71.9 \pm 2.8$  mean weight) were sampled by trawl nets from the southeastern Ionian Sea in July 2016.

In laboratory, each individual was weighed and measured in order to obtain the principal condition indices. Thus, they were eviscerated and the gastrointestinal tract, gills, liver, and gonads were collected and frozen at  $-28^\circ\text{C}$ , until the analyzes.

### 2.1 Materials and Methods

In this study, only gastrointestinal tracts and gills were processed for the extraction of microplastics. Extractions were carried out according to the protocol developed by [5], which resulted particularly effective for the collection of the two most abundant polymers found in the Mediterranean Sea, namely PE and PP.

Each tissue was thawed and dried in oven at  $50^\circ\text{C}$  overnight. The dried samples were mashed with a pottery mortar and pestle in order to obtain a fine powder of MPs, organic matter, and other contents (e.g., sands). The powder was put in a beaker with 100 ml of hypersaline solution of NaCl ( $1.2\text{ g/cm}^3$ ), stirred and decanted for 1 h. Then, 50 ml of supernatant were collected with a micropipette and directly filtered in a vacuum pump system with  $3\text{-}\mu\text{m}$  nitrate cellulose membrane for intestinal contents and  $0.2\text{-}\mu\text{m}$  nitrate cellulose grided membrane for gills. Filtered materials were digested with a few ml of hydrogen peroxide (15%), and dried in oven at  $50^\circ\text{C}$  overnight, before the microscopic observation.

In order to eliminate airborne contamination of fibers and microplastics, we used only metal, pottery, and glass instruments, previously rinsed with pre-filtered ( $0.45\text{-}\mu\text{m}$  filter) bi-distilled water.

Moreover, all technical procedures (dissection, extraction, and filtration) were carried out under a vertical laminar flow hood and filters were stored in covered petri dish before being transported out of the fume hood for the counting of microplastics under the stereomicroscope. Finally, only cotton clothes were worn during both the dissection and extraction procedures.

The overestimation of microplastics concentrations in samples caused by any possible accidental external contamination was also prevented through the analysis

of control filters, obtained by filtering 50 mL of the pre-filtered bi-distilled water used for the preparations of the hypersaline and hydro-peroxide solutions.

Extracted particles were microscopically observed, counted, photographed, and measured at their largest cross-section and characterized according to both size class (5000–1000  $\mu\text{m}$ ; 1000–500  $\mu\text{m}$ ; 500–100  $\mu\text{m}$ ; 100–50  $\mu\text{m}$ ; 50–10  $\mu\text{m}$ ; <10  $\mu\text{m}$ ) and typology (line, fragment, fiber, pellet) with a stereomicroscope (Nikon SMZ 1270 equipped with a DS-Fi2 camera and NIS Elements image analysis software).

### 3 Results and Discussion

Analysis of control samples showed the absence of microplastic and fibers in all the control filters, so data did not need to be corrected, and it was possible to include the fiber counting in our results.

#### 3.1 Results

Microplastics were detected in all the gills processed and in the 92% of gastrointestinal contents, with a total of 411 MPs counted in gills and 146 in the stomach contents. The average number of microplastics extracted in gills and gastrointestinal tract was  $34.25 \pm 9.89$  and  $14.6 \pm 4.61$ , respectively (Table 1).

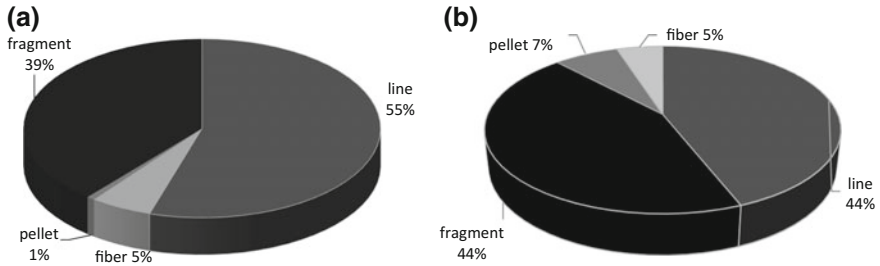
The shape of the plastic particles isolated in both gastrointestinal tracts and gills of all the fish was largely dominated by fragments (47 and 39%, respectively) and line (44 and 55%, respectively) (Fig. 1).

The dimensional distribution analysis showed the dominance of particles between 100 and 500  $\mu\text{m}$  (49%) in gills, whereas pieces lower than 10  $\mu\text{m}$  (53%) represented the most abundant class in gastrointestinal contents (Fig. 2).

For each individual the condition index (CI), the gonadosomatic index (GSI), and the hepatosomatic index (HSI) were calculated (Table 2).

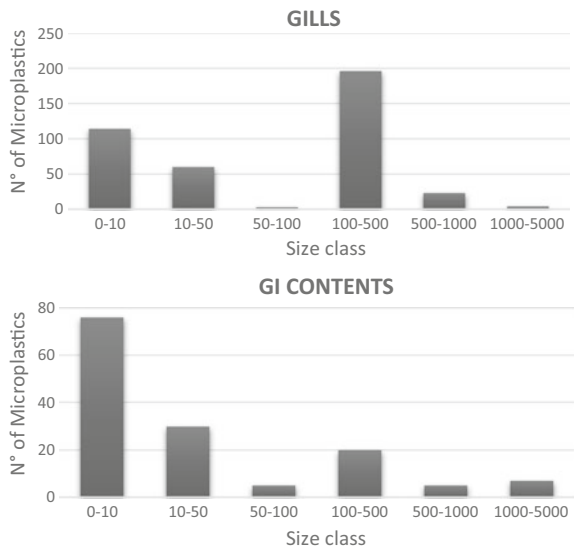
**Table 1** Fish data from individuals ( $n = 12$ ) of *M. barbatus* caught along the Ionian coast of Salento: type of tissue analyzed, average total weight (g), total number of MPs, number of MPs for total weight and for each individual, frequency of occurrence in each tissue

Tissue	Weight tot $\pm$ S.E.	N of MPs	N of MPs/g tot	N of MPs/ind $\pm$ S.E.	% of occurrence
Gastrointestinal contents	71.92 $\pm$ 2.79	146	0.20	14.6 $\pm$ 4.61	92
Gill		411	0.48	34.25 $\pm$ 9.89	100



**Fig. 1** Pie chart showing the types of plastics found and their relative abundance (%) for gills (a) and gastrointestinal contents (b)

**Fig. 2** Number of microplastics in each tissue according to their size class (µm). Particles between 100 and 500 µm are the most abundant in gills, instead particles lower than 10 µm represent the 53% in GI contents



**Table 2** Condition indices of all individuals of *M. barbatus*: CI = condition index; GSI = gonadosomatic index; HSI = hepatosomatic index

SAMPLE	CI	GSI	HSI
TR1	2.30	0.37	1.43
TR2	2.23	0.35	1.42
TR3	2.28	0.37	0.89
TR4	2.21	0.34	1.33
TR5	2.36	0.83	1.47
TR6	2.02	0.48	1.23
TR7	2.23	0.36	1.29
TR8	2.38	0.64	1.49
TR9	2.10	0.41	1.11
TR10	2.13	0.22	1.03
TR11	2.40	0.33	1.25
TR12	2.40	0.45	1.53

### 3.2 Discussion and Conclusions

The occurrence of microplastics (92%) in individuals of *M. barbatus* was higher than that found in Iberian Peninsula (18.8%) [6] and in Adriatic Sea (64%) [5], whereas similar values were observed in the congeneric specimens (*M. surmuletus*) caught along Portuguese coast (100%) [7].

For the first time, we extracted and measured the MPs accumulated in gills of fish, which resulted to be a highly contaminated tissue, previously investigated only in *Uca rapax* specimens [8].

The high amount of MPs found in gills clearly showed that benthic species can adsorb microplastics not only during the feeding process, but also from water column, during breathing.

The analysis of grain size distribution showed a predominance of the smallest size class (<10  $\mu\text{m}$ , 53%) in sampled gastrointestinal contents, while the greatest size class had only a small contribution, accounting for approximately 5%. A completely different distribution was reported for stomach contents of fish from the Adriatic coasts of Italy, showing a predominance of particles of higher dimensions (0.5–1 mm) [5].

Regarding the shape of MPs, similar results to those in the Adriatic Sea [5] were found in this study. The most abundant types of MPs in both investigated tissues are, indeed, fragment and line, whereas fiber and pellet constituted only a little percentage on the total (<7%).

Our results seem to suggest that the Ionian coast of Salento is highly impacted by microlitter debris. Further investigation will be undertaken on a large number of individuals, tissues, species, and locations for better understanding the complex phenomenon of accumulation translocation and transfer of MPs in biota and along the food chain.

Furthermore, this study supports the results obtained along the coasts of the Iberian Peninsula [6] which suggest *M. barbatus* as a good candidate for the monitoring of spatial and temporal trends of ingested microlitter (Descriptor 10) within the Marine Strategy Framework Directive (MSFD).

No significant correlation ( $p > 0.05$ ) was found between the condition indices and the number of microplastics for each individual.

From a technical point of view, the use of vertical laminar flow hood during all phases of extraction solved the problem of airborne contamination allowing the fiber counting. It was demonstrated that a washing machine releases more than 100 synthetic fibers per liter of effluent [9], which could then enter into the food chain; the evaluation of the amount of fibers ingested by marine fauna is an important information in the light of a correct monitoring procedure. For this reason, we suggest to consider this aspect as strictly necessary, during the analyzes of microplastics in environmental impact studies.

There is a great concern about potential impacts caused by microplastics; they could have similar effects to those observed for macroplastics (e.g., intestinal blockage [10], pseudo-satiation resulting in reduced food intake [11]), or exert

ecotoxicological hazard related to the ability of MPs to absorb and/or release organic pollutants (polybrominated diphenyl ethers, phthalates, and bisphenol A) [12].

Further studies are required to assess long-term consequences of microplastic contamination on fish status health, and their possible translocation from stomach contents to edible parts with consequences also on human health.

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# Zooplankton and Plastic Additives— Insights into the Chemical Pollution of the Low-Trophic Level of the Mediterranean Marine Food Web

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## Abbreviations

DMP	Dimethyl phthalate
DEP	Diethyl phthalate
DiBP	Diisobutyl phthalate
DnBP	Di-n-butyl phthalate
BBzP	Benzyl butyl phthalate
DEHP	Bis(2-ethylhexyl) phthalate
TPP	Tripropyl phosphate
TiBP	Triisobutyl phosphate
TnBP	Tri-n-butyl phosphate
TCEP	Tris(2-chloroethyl) phosphate
TCPP	Tris(1-chloro-2-propyl) phosphate (isomers 1 and 2)
TDCP	Tris-(dichlorisopropyl) phosphate
TPhP	Triphenyl phosphate, EHDPP: 2-Ethylhexyl diphenyl phosphate
EHDPP	2-Ethylhexyl diphenyl phosphate
TEHP	Tri(2-ethylhexyl) phosphate

## 1 Introduction

Marine litter such as microplastics pose a variety of problems once they reach the environment via improper waste disposal or spills, among others [1]. While microplastics are often ingested by marine organisms, marine life is not only threatened by the physical damage plastic items can cause but also by the possible chemical pollution resulting from the leaching of plastic additives or other adsorbed

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chemicals on the plastics surface during long-range transport. Plastic additives include plasticizers, flame retardants and colour pigments. The demonstrated toxicity of some of these molecules has led to national and international legislations limiting or banning their use. However, a wide variety of substances are still found in plastic products and little is known about their impact on the marine and terrestrial environment. Marine ecosystems could be particularly at risk since they act as recipients for plastic pollution from terrestrial sources via river runoffs, wastewater treatment effluents or airborne transportation [2]. Representing a pivotal component of the marine food web, zooplankton plays a vital role in the ecosystem functioning, yet the actual impact of microplastic on these organisms and the occurrence and magnitude of potential resulting chemical contamination have been poorly studied so far.

Phthalic acid esters (PAEs) and organophosphate ester (OPE) flame retardants and plasticizers are among the most important plastic additives [3–5]. Previous reports proved their overall occurrence in the aquatic environments [6–8]. Main PAEs detected in seawater are dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DnBP), benzylbutyl phthalate (BzBP), diethylhexyl phthalate (DEHP) and di-n-octyl phthalate (DnOP), with DEHP being generally the most abundant [6]. However, there is still very little information on their concentrations in the first levels of marine trophic webs. Here, we make a first tentative to overcome this knowledge gap by reporting PAE (and OPE for water samples) occurrences and concentrations in zooplankton and seawater samples of the north-western Mediterranean Sea.

## 2 Experimental

### 2.1 Materials

Samples were collected at three different locations in the Bay of Marseille (France). Sampling sites are situated in proximity of a harbour (L'Estaque; 43°20.589' N, 5°18.495' E), at the outflow area of Marseilles wastewater treatment plant (Cortiou; 43°12.708' N, 5°24.166' E) and near a sparsely inhabited island (Frioul; 43°16.317' N, 5°18.828' E). Zooplankton was sampled in March and May 2017 using a 150 µm mesh-sized neuston net (opening area 40 × 70 cm). The net was towed horizontally for about 20 min at a speed of 2–3 knots. Afterwards, the net was carefully rinsed and the content of the cod-end was poured into 1 L pre-combusted glass bottles. Upon arrival at the laboratory, the samples were stored in a cold room (+4 °C) overnight.

Surface water samples were collected in May 2017 using an inox collector and were poured into pre-combusted 1 L glass bottles. Upon arrival in the laboratory, these samples were stored in a freezer (−25 °C) until further analysis.

## 2.2 Methods

### 2.2.1 Preparation of Samples

Zooplankton size classes were established (where possible) using metal sieves and rinsed with ultrapure water (ISO 3696). (Sub-) samples were analysed using a dissecting microscope, and if present, microplastics were removed using tweezers. Each size class was characterized by determining qualitatively the dominant/present/rare taxonomic groups. The samples were then stored in 30 mL glass vials in a freezer ( $-25\text{ }^{\circ}\text{C}$ ) before lyophilization. The dried biomass was weighted, and 70–200 mg, depending on the sites, was extracted. The water samples were thawed overnight and filtered (Whatman GF/F,  $0.7\text{ }\mu\text{m}$ ).

### 2.2.2 Analytical Techniques

Zooplankton and seawater samples were analysed for six phthalate esters (DMP, DEP, DiBP, DnBP, BBzP and DEHP), and seawater samples were additionally analysed for ten organophosphate esters (TPP, TiBP, TnBP, TCEP, TCPP-1, TCPP-2, TDCP, TPhP, EHDPP and TEHP). Accelerated solvent extraction (ASE) was used to extract the target contaminants from the zooplankton samples. The extraction cells were first extracted empty in order to reduce contamination risks. Samples were homogenized with 600 mg of baked ( $450\text{ }^{\circ}\text{C}/6\text{ h}$ ) Florisil, introduced into the extraction cells, spiked with 100 ng of surrogate standards (D27-TBP, D18-TCPP, D15-TDCP, D15-TPhP and D4-DnBP) and extracted (three cycles) using a dichloromethane/acetone (2:1, v/v) mixture. The extracts were evaporated using a RotaVap until a final volume of 2–3 mL was reached. A subsequent clean-up was performed with glass columns containing 3 g of alumina ( $\text{Al}_2\text{O}_3$ ) (3% deactivated), 3 g of silica gel ( $\text{SiO}_2$ ) and about 0.5–1 cm of  $\text{Na}_2\text{SO}_4$ . A first fraction was eluted using 20 mL of hexane and a second fraction with 40 mL of a dichloromethane/acetone (70:30) mixture. Once the volume was reduced to about 0.5–1 mL, the extracts were transferred to 2 mL injection vials and evaporated under a gentle  $\text{N}_2$  stream to a final volume of about 20  $\mu\text{L}$ . Internal standards were added (100 ng sample<sup>-1</sup>; D21-TPrP, D12-TCEP, D7-MAL, D4-DEP and D4-DEHP). Restrictions concerning the required biomass did not allow the establishment of replicates. A blank was made during each extraction series to detect possible contaminations during the extraction and clean-up processes.

Solid-phase extraction (SPE) was applied to seawater samples. After a washing step, the SPE cartridges (Oasis HLB, 250 mg,  $30\text{ }\mu\text{m}$ ) were conditioned using 10 mL of methanol, followed by 10 mL of ultrapure water. One litre of the sample was passed through the cartridge using polytetrafluoroethylene liners, followed by a washing step using 10 mL of ultrapure water. Next, the cartridges were left drying for 1–2 h. The compounds of interest were eluted using 5 mL of hexane, followed by 5 mL of hexane/dichloromethane (50:50, v/v). Extracts were finally evaporated

under a gentle N<sub>2</sub> stream and internal standards were added (100 ng sample<sup>-1</sup>; D21-TPrP, D12-TCEP, D7-MAL, D4-DEP and D4-DEHP). Two blanks were made to detect possible contamination, and a replicate was made for each sampling station to improve results reliability. All samples were quantified by GC/MS (Agilent Technology 7820A-5977E).

### 3 Results and Discussion

#### 3.1 Results

##### 3.1.1 Quality Assurance/Quality Control

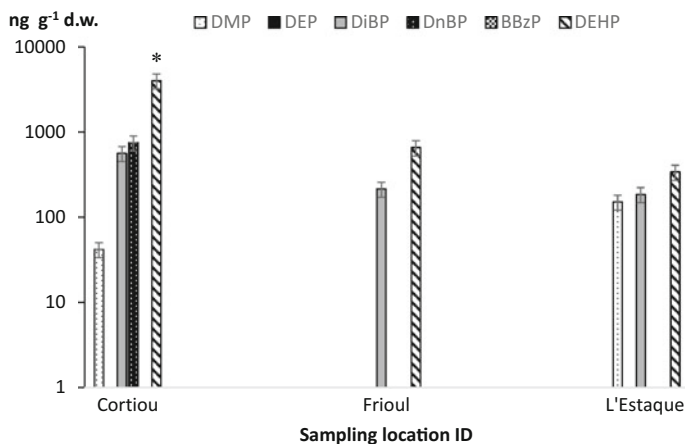
Good method recoveries were obtained for phthalates, ranging from 80 to 131% in zooplankton samples and from 82 to 97% in water samples. OPE recovery rates ranged from 66 to 107%. Only a few PAEs were detected in the blanks and at low levels compared to real samples (<5% of DiBP and <13% of DEHP of real zooplankton sample concentrations).

DMP and DnBP were found with 1.5 ( $\pm 0.05$ ) ng L<sup>-1</sup> in SPE blank replicates and DiBP with 3.1 ( $\pm 0.13$ ) ng L<sup>-1</sup>. For OPEs, 0.4 ng L<sup>-1</sup> of TiBP, TnBP and TPhP, as well as 5.5 ng L<sup>-1</sup> of TCPP-1 were measured in SPE blanks. All contaminant concentrations presented here are blank-corrected. Surrogates tracked extraction accuracy and internal standards ensured analysis accuracy. All SPE procedures were performed in a clean room (class ISO 6) equipped for organic studies at trace concentrations. Plastic materials were completely avoided during sampling, sample preparation and analysis. All glassware and filters were pre-combusted at 450 °C for 6 h.

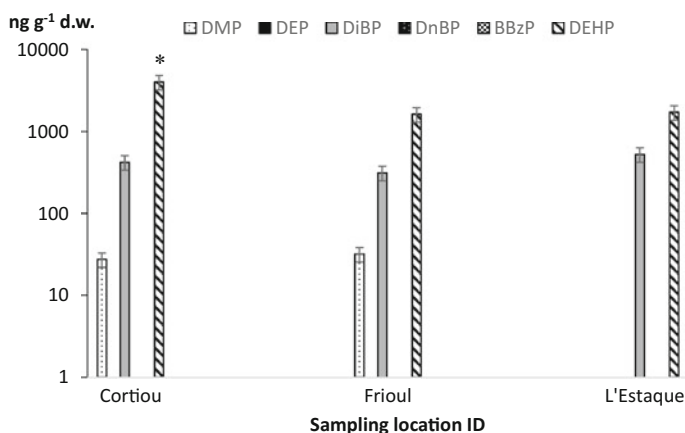
##### 3.1.2 Phthalates in Zooplankton and Seawater Samples

While in zooplankton samples from March, DMP was only found at Cortiou and L'Estaque (Fig. 1), this phthalate was found in Cortiou and Frioul samples collected in May (Fig. 2). The Cortiou sample of March is the only one where DnBP was observed ( $\sim 750$  ng g<sup>-1</sup> dry weight, d.w.). DEHP is the highest concentrated compound in all samples and reaches particularly high values in zooplankton samples from Cortiou ( $\geq 4000$  ng g<sup>-1</sup> d.w.). DEP and BBzP were not detected in samples collected in March 2017, while in May, DnBP was also not detectable.

Total phthalate concentration in seawater samples varied between stations with 179.0 ng L<sup>-1</sup> at Cortiou, 135.7 ng L<sup>-1</sup> at Frioul and 100.7 ng L<sup>-1</sup> at L'Estaque (Fig. 3). At Cortiou, all six analysed phthalates were detected, with DEP being the less abundant one ( $3.8 \pm 0.9$  ng L<sup>-1</sup>). At Frioul and L'Estaque, DEP was not



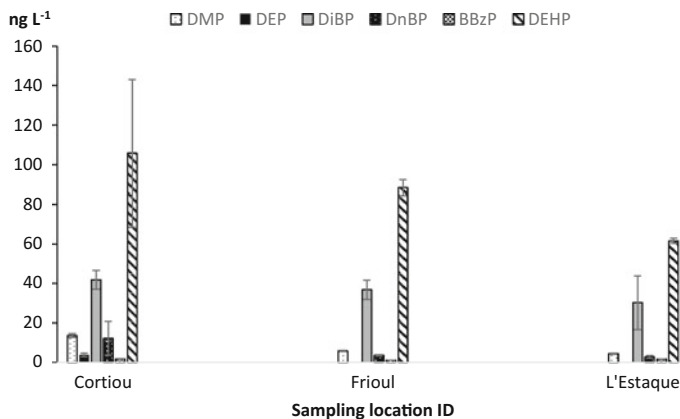
**Fig. 1** Blank-corrected phthalate concentrations (in  $\text{ng g}^{-1}$  d.w.) of zooplankton samples (size class  $\geq 1$  mm) collected on 14 March 2017. Error bars represent  $\pm 20\%$  of measured concentrations, based on surrogate recoveries. \* $[\text{DEHP}] \geq 4000 \text{ ng g}^{-1}$  d.w. Further analysis will clarify the exact concentration and will be presented during the  $\mu\text{MED}$  conference



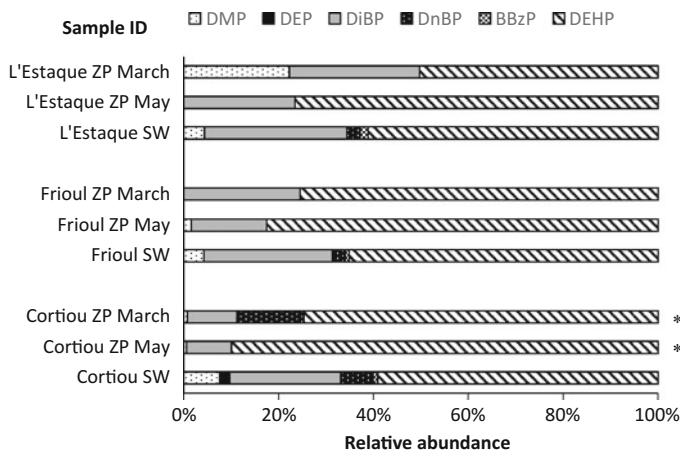
**Fig. 2** Blank-corrected phthalate concentrations (in  $\text{ng g}^{-1}$  d.w.) of zooplankton samples (size class  $\geq 1$  mm) collected on 9 May 2017. Error bars represent  $\pm 20\%$  of measured concentrations, based on surrogate recoveries. \* $[\text{DEHP}] \geq 4000 \text{ ng g}^{-1}$  d.w. Further analysis will clarify the exact concentration and will be presented during the  $\mu\text{MED}$  conference

detected; DMP, DiBP, DnBP, BBzP and DEHP were however measured, in variable amounts.

The most abundant phthalate in all samples was DEHP (Fig. 4), with its relative abundance ranging from 50 to 90%, while DEP was only found in the Cortiou seawater sample where it represents 2% of the total phthalate concentration. DiBP was present in all samples with its relative abundance ranging from 9 to 30%.



**Fig. 3** Blank-corrected phthalate concentrations (ng L<sup>-1</sup>) of seawater samples collected on 9 May 2017. Error bars represent standard deviations based on analysis of the two replicas per station



**Fig. 4** Relative abundance of the six phthalates analysed in zooplankton (ZP) and seawater (SW) samples. \*Exact determination of the very high ( $\geq 4000$  ng g<sup>-1</sup> d.w.) DEHP concentration in these samples might increase its relative abundance

### 3.1.3 Organophosphate Esters in Seawater Samples

The seawater was also analysed for ten organophosphate esters (Table 1).  $\sum_{10}$  OPEs were highest in seawater collected at Cortiou ( $726.5 \pm 37.6$  ng L<sup>-1</sup>), while at Frioul only  $51.6 (\pm 0.8)$  ng L<sup>-1</sup> of total OPEs were detected. TCPP-1 was the most abundant OPE at all sampling sites, followed by TCPP-2. TPP is the only OPE which was never detected.

**Table 1** Blank-corrected OPE concentrations (ng L<sup>-1</sup> ± SD) of seawater samples collected on 9 May 2017

	TPP	TtBP	TnBP	TCEP	TCPP-1	TCPP-2	TDCP	TPhP	EHDPP	TEHP	Σ <sub>10</sub> OPEs
Cortiou	n.d.	16.3 ± 1.1	5.7 ± 0.8	26.8 ± 1.0	535.1 ± 29.0	63.0 ± 3.3	57.3 ± 1.0	16.4 ± 0.6	6.0 ± 0.8	n.d.	726.5 ± 37.6
Frioul	n.d.	4.4 ± 0.2	0.7 ± 0.1	n.d.	23.0 ± 0.2	21.6 ± 0.1	n.d.	0.2 ± 0.0	1.5 ± 0.2	0.3 ± 0.0	51.6 ± 0.8
L'Estaque	n.d.	3.6 ± 1.2	1.5 ± 1.0	n.d.	36.9 ± 9.8	29.6 ± 4.6	0.7 ± 1.0	0.6 ± 0.2	1.4 ± 0.4	0.3 ± 0.4	74.6 ± 8.6

n.d. = not detected

### 3.2 Discussion

Highest total phthalate and organophosphate ester concentrations were found at Cortiou for both sample types which could reflect a strong influence of the wastewater plant discharges as well as high plastic abundances in the seawater and/or sediment. The most abundant OPE hereby was TCPP (535.1 ng L<sup>-1</sup> at Cortiou). At the effluent of Swedish sewage treatment plants, up to 24,000 ng L<sup>-1</sup> of TCPP were measured [9], highlighting the influence sewage disposals can have on environmental contaminant concentrations.

DEHP was the most abundant phthalate observed, reaching concentrations of 4000 ng g<sup>-1</sup> d.w. or more in both zooplankton samples from Cortiou. This indicates an important contaminant burden for the local zooplankton community and possibly for the whole food web. In seawater samples, DEHP was also the most abundant phthalate and concentrations were highest at Cortiou. Analysing the same phthalates at the same sampling site, Paluselli et al. measured only 56.2 ng L<sup>-1</sup> at Cortiou in 2014, but their total phthalate concentration reached 212.6 ng L<sup>-1</sup> [6]. This might indicate daily, seasonal or annual variabilities in local phthalate concentrations and relative abundances. With a maximum DEHP concentration of 0.11 µg L<sup>-1</sup>, our seawater samples did not exceed the EU guideline for environmental quality of marine waters of 1.3 µg L<sup>-1</sup> [10].

## 4 Conclusion

Future sampling campaigns will show if the observed trends are being confirmed and whether—additionally to the spatial variability—seasonal differences in PAE and OPE concentrations in zooplankton and seawater samples can be observed.

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# Microplastics in Juvenile Commercial Fish from an Estuarine Environment

Filipa Bessa, Pablo Barría, João M. Neto, João P. G. L. Frias, Vanessa Otero, Paula Sobral and João Carlos Marques

## 1 Introduction

Marine plastic debris is considered nowadays a global threat to aquatic ecosystems [1, 2] with recent studies estimating that currently there are more than five trillion plastic pieces weighing over 250,000 tons floating in pelagic habitats [2]. Additional concern has been devoted to microplastics, i.e. any plastic particle with less than 5 mm in diameter [3]. Microplastics are considered a widespread environmental contaminant in aquatic ecosystems and have been reported in all compartments of the marine environment including the surface ocean, water column, coastline, the deep sea sediments and due to their small size may be ingested by biota [4–7].

Microplastic ingestion has been reported by a range of marine species but less known is the level of microplastic contamination by these species when they live in transitional ecosystems such as estuaries [8]. These ecosystems are rich in productivity, and fish have an important ecological and economic role, but can also experience high levels of plastic contamination. The aim of this study was to assess

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the ingestion of microplastics by three important commercial fish species: the sea bass (*Dicentrarchus labrax*), the common two-banded seabream (*Diplodus vulgaris*) and the European flounder (*Platichthys flesus*) from a transitional estuarine habitat (Mondego estuary, Portugal).

## 2 Experimental

### 2.1 Methods

Fish were collected from June to October 2014 in two areas from the Mondego estuary in order to assess the potential marine and riverine influence on MPs ingestion by fish.

Fishing took place during the night, at high water of spring tides, and was performed using a  $2 \times 0.5$  m beam trawl with one tickler chain and 5 mm mesh size in the cod end. At each sampling station, three hauls were towed at an average of 2–3 knots during 3 min, covering an area of about 500 m<sup>2</sup>. Samples were transported in iceboxes to the laboratory for further measurements. Approximately 40 individual fish for each species were selected and stored at  $-20$  °C until further laboratory procedures.

#### 2.1.1 Preparation of Samples

In the laboratory, fish were thawed at room temperature before examination. For each fish identified, basic measurements including total body length (TL), from mouth to central point of caudal fin (mm), and body weight (W) (g) were determined. Subsequently, each fish was dissected to remove the whole gastrointestinal tract (GIT).

#### 2.1.2 Analytical Techniques

In order to extract potential plastic debris from the samples, the gastrointestinal tracts were subjected to analyses in two steps: (1) visual inspection of the whole gastrointestinal tract under a stereomicroscope during 10 min and extraction of anthropogenic debris and (2) digestion of the whole GIT with a KOH 10% solution during 5 days. The solution added was at least three times the volume of the biological material, and the samples were incubated at 60 °C. After the incubation period, the remaining liquid was vacuum filtered through 1.2- $\mu$ m Whatman GF/C microfiber filter papers and the filters were oven-dried at 60 °C before analysis at the stereomicroscope again using the same approach (visual inspection) as described before. Laboratory blanks used during extraction and identification stages in

order to test for cross-plastic contamination have resulted negatively with no MPs in the blank tests. All particles extracted were observed and photographed by using a stereomicroscope LEICA M80 (Leica Microsystems GmbH, Wetzlar, Germany) with image analysis system IC80 HD camera with Leica Application Suite (LAS) software. In order to identify the composition of plastic polymers, selected microplastics were analysed by micro-Fourier transform infrared spectroscopy ( $\mu$ -FTIR) in transmittance mode. Spectra were analysed using OMNIC software to compare spectra to a database of reference, and the most appropriate match was selected based on matching peak wavenumber positions. Only polymers matching reference spectra for more than 85% were considered.

To minimize contamination, all labware used during the sample processing was cleaned with ethanol prior to use and the usage of plastic material was avoided whenever possible. In addition, during the digestion procedures, in order to account for any possible (handling and airborne) contamination, three procedural blanks were also run without tissues in parallel with samples containing the digestion solutions.

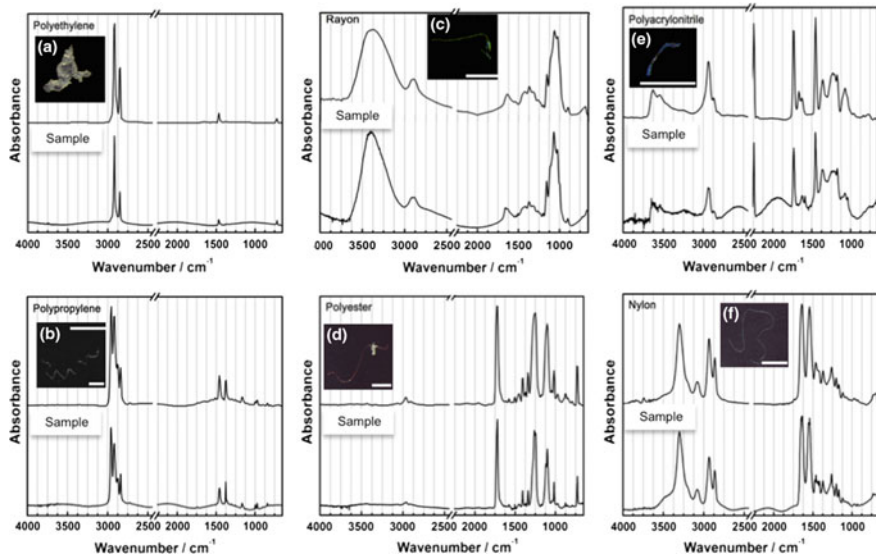
### 3 Results and Discussion

A total of 120 individuals from three fish species with similar length and weight were analysed, and the frequency of microplastic ingestion is reported in Table 1.

A total of 157 microplastics were extracted from the gastrointestinal tract of all fish, with an average of  $1.67 \pm 0.27$  (SD) particles per fish. Plastic particles were identified as fibres (96%) and fragments (4%), of variable size and colour. A sub-sample of the retrieved particles was analysed under a FTIR spectrometer revealing that the most dominant plastic polymer types found were polyethylene (6%), polypropylene (14%), polyester (31%), nylon (5%) and rayon (30%) (Fig. 1). No significant spatial difference was found between the occurrences of microplastics

**Table 1** Fish data from the Mondego estuary: species composition, average weight and length, corresponding percentage of individuals with microplastics and the average microplastics per fish. Standard deviation (SD) values in parentheses

Species	Average weight (g)	Average length (cm)	% with ingested microplastics (downstream; upstream)	Mean microplastics per fish
<i>Platichthys flesus</i>	31.84 (3.43)	12.04 (0.42)	(33; 26)	0.18 (0.55)
<i>Diplodus vulgaris</i>	38.17 (23.60)	12.49 (2.89)	(67; 74)	3.14 (3.25)
<i>Dicentrarchus labrax</i>	59.51 (15.41)	14.25 (1.32)	(25; 41)	0.30 (0.61)



**Fig. 1** FTIR spectra of representative microplastic polymers (and their reference spectra) found in the gastrointestinal tract of fish from the Mondego estuary: **a** polyethylene, **b** polypropylene, **c** rayon, **d** polyester, **e** polyacrylonitrile and **f** nylon. The scale bar represents 1 mm

in fish along the estuary, but the higher number of fibres (80%) was found in the GI tract of the common two-banded seabream (*D. vulgaris*), a very voracious and fast species (Table 1).

### 3.1 Discussion

The present study constitutes the first report of microplastic ingestion by fish from an estuarine environment in Portugal (Mondego estuary). On the basis of the proportion of commercial fish analysed here, the common two-banded seabream (*D. vulgaris*) presented the highest ingestion of microplastic (73%), followed by the sea bass (*D. labrax*) with 23%, whereas the European flounder (*P. flesus*) presented the lowest levels (13%).

The location (riverine/marine influenced area) did not influence the levels of microplastic ingestion reported, which could be also related to the small area of the estuary and the natural mobility of species. The highest level of microplastics in *D. vulgaris* may be related to their feeding behaviour being a benthopelagic voracious and fast species, which can ingest microplastics in the water column along the estuary.

In agreement with previous studies in marine and freshwater habitats, extracted microplastics were mostly fibres (96%), but also fragments (4%) were recorded with different colours (mainly blue and black). In this study, most of the fibres and polymers detected (nylon, polyethylene, polypropylene, rayon, polyester and polyacrylonitrile) may be related to textiles and the fishing industry, which is one of the main activities in the estuarine area. The verification of potential plastic particles and their chemical composition is crucial for the generation of reliable data regarding potential sources of plastic contamination.

## 4 Conclusions

We report some of the first findings on the presence of microplastic pollution in juvenile commercial important fish from the Mondego estuary. This study represents a baseline to determine the potential implications on microplastic ingestion on fish health and the potential transfer of microplastics along the estuarine and marine food webs that still needs to be assessed.

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# Plastic Soles: Microplastic Litter in the Gastrointestinal Tract of *Solea solea* from the Adriatic Sea

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## 1 Introduction

Plastic is widely used in everyday life being one of the more versatile materials ever produced. The rising demand of plastic items to support the societal development has dramatically boosted the annual plastic production from 1.5 in the 1950s to 311 million tonnes in 2014 [1]. The dark side of the plastic revolution is the marine pollution, with an estimated amount of 9.5 million tonnes of new plastic waste flowing into the oceans each year [2].

In the literature, a distinction is made between macro-, micro-, and nano-plastics. Even if there is not a clear definition of microplastics (MPs), many contexts—and also in this work—MPs are plastic pieces having a size ranging from 1  $\mu\text{m}$  to 5 mm [3]. MPs can be categorized as primary and secondary according to their formation

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mechanism [4]. Primary MPs are directly released into the environment in the form of small particulates. Secondary MPs derive from the degradation of larger plastic items into smaller fragments once exposed to the marine environment.

MPs distribution is affected by sea currents and other chemical/physical oceanographic conditions [5, 6], as well as by their intrinsic characteristics. Factors like the polymeric composition, additives, particle morphology, and the degree of biofouling on particles' surface may have a great impact on where MPs concentrate in the marine environment [7]. Plastics with a density exceeding the seawater one sink and therefore tend to accumulate in the sediments [8]. On the other hand, low-density particles tend either to float on the sea surface or in suspension in the water column [9, 10]. The occurrence of biofilms in floating particles may improve the sinking property of low dense polymers such as polyethylene and polypropylene [11]. Plastics with smaller range can be taken up in aquatic organisms by oral ingestion or through the gills [12, 13]. For example, indiscriminate feeders, such as filter-feeding zooplankton and other planktonic organisms, can take up plastic particles as they are mistaken for prey [14, 15]. The consequences of MPs ingestion may affect the organism's ecophysiology such as feeding activity, respiratory functions, reproductive output, and also modulate several molecular and cellular pathways [16, 17].

According to their different sizes, MPs can transport contaminants faster and more effectively through biological membranes and ultimately inside cells of marine organisms by a Trojan horse-like effect. This promotes contaminants' bioaccumulation and biomagnification in the food web as well as adverse biological effects ranging from the loss in the reproductive output up to the disruption of key molecular and cellular processes in vertebrate/invertebrate marine organisms [18, 19].

The Mediterranean Sea was defined as one of the most impacted areas in the world by plastic pollution [20, 21] and several studies have been carried out on the ingestion of MPs in marine organisms, ranging from zooplankton to top predators [22–25].

In the context, the present study aimed to analyze the occurrence of MPs in the gastrointestinal content of wild-caught samples of *Solea solea* (Linnaeus, 1758) collected in the northern/central Adriatic Sea and give a characterization (in terms of size, shape, and polymer). It represents the first work carried out on this species in the Mediterranean Sea as well as the first one dealing with the MPs content in a finfish species in the Adriatic Sea. This benthic flatfish was selected for its wide distribution, the high ecological and economic value, and its relevance in the human seafood consumption. *S. solea* is one of the most important commercial species of the FAO General Fisheries Commission for the Mediterranean (GFCM) area 37 (Mediterranean and Black Sea) [26].

## 2 Materials and Methods

### 2.1 Study Area

The Adriatic Sea is an elongated semi-enclosed basin within the Mediterranean Sea, extending for 138,600 km<sup>2</sup> [27]. It consists of three basins decreasing in depth from the southern to northern one. An 800 m deep sill partly separates the southern basin (deeper than 1200 m) from the Ionian Sea. A second sill, of around 130 m depth, separates the southern basin from the central one where the maximum depth (260 m) is reached in the Pomo Pit. Northward, the seabed gradually rises until to reach an average depth of around 35 m. Two main currents dominate the Adriatic circulation: the West Adriatic Current (WAC) flowing toward southeast along the western coast, and the East Adriatic Current (EAC) flowing northeast along the eastern coast. Two main cyclonic gyres occur, one in the northern part and the other in the South.

### 2.2 Field Sampling

Individuals of *S. solea* were sampled in the GFCM Geographical Sub-Area 17 (central and northern Adriatic Sea). Sampling was carried out in fall (November–December) 2014 and 2015 within the framework of the “*rapido*” Trawl Survey SoleMon [26], onboard of RV G. Dallaporta. Samples were caught with *rapido* trawls (a modified beam trawl) at a total of 60 stations in 2014 and 22 in 2015 distributed over the area following a depth-stratified random design (Fig. 1).

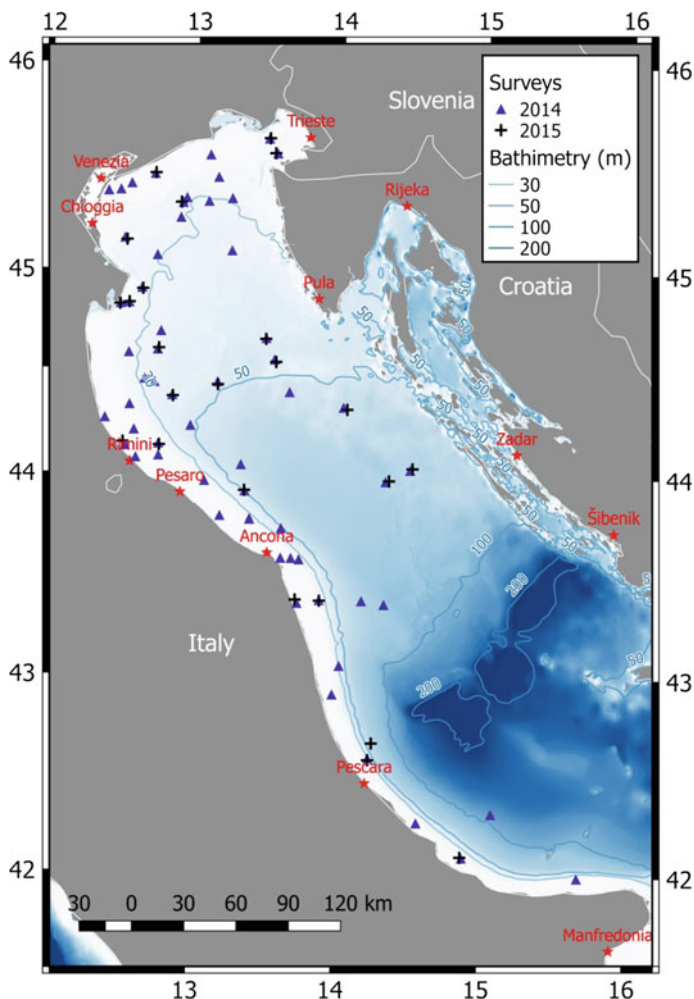
From two to six individuals were sampled at each station, for a total of 423 soles in 2014 and 110 in 2015.

Fish were measured and weighed, dissected fresh on board and whole gastrointestinal tracts were collected and frozen at  $-20\text{ }^{\circ}\text{C}$  until the analyses.

### 2.3 Laboratory Analysis

All tools and glassware used for the digestion tests were carefully rinsed with bi-distilled water filtered through 1.6  $\mu\text{m}$  GF/A grade glass fiber filters (Whatman, Oslo). Reagents were also filtered with the same GF/A filters. After thawing, in the laboratory, the content of esophagus, stomach, and intestine of each specimen was weighed, put into 250 mL Pyrex bottles and filled with a 10% KOH solution. The amount of KOH added was at least five times the volume of the biological material. Samples were incubated at 55  $^{\circ}\text{C}$  for 36 h. Once the organic material was degraded,





**Fig. 1** Maps of the stations sampled during the Solemon surveys. It was represented divided by years (2014 and 2015), and it has been added the bathymetry. The main city was also reported

the digestates were filtered with GF/A filters. In the case of the presence of debris in the digestate, a density-based separation step using sodium tungstate was subsequently added. Briefly, a sodium iodide (NaI) solution ( $d\ 1/4\ 1.8\ \text{g/cm}^3$ ) was added to the digestate (3:1, v/v) right after the 36 h digestion. The mixture was then thoroughly stirred for 20 min before being left to settle for 2 h. The supernatant containing the floating plastic particles [28] was subsequently collected and filtered, as previously described. Extracted particles were microscopically observed, photographed, measured at their largest cross section through an ocular micrometer, and categorized according to three size classes ( $<100\ \mu\text{m}$ ;  $100\ \mu\text{m} < X < 500\ \mu\text{m}$ ;

>500  $\mu\text{m}$ ) and fibers. Plastic particles were counted per individual fish, and color and shape described. Particles were then characterized by  $\mu\text{FT-IR}$  spectrometry for polymer composition. Analyses were performed using a Bruker Hyperion 3000  $\mu\text{-FTIR}$  system which allowed the characterization of MPs greater than 0.2  $\mu\text{m}$ . Following background scans, scans were performed and  $\text{CO}_2$  interference (adsorption at approx. 2300–2400  $\text{cm}^{-1}$ ) was removed for clarity; for each particle, scans were performed with a 4  $\text{cm}^{-1}$  resolution.

## 2.4 Data Analysis

The mean number of MPs particles (split according to size, shape, and polymer composition) per specimen was computed for each sampling station in 2014 and 2015. A fourth-root data transformation was performed and the similarity matrix calculated using the Gower S15 index [29]. Statistical difference between 2014 and 2015 in terms of MPs abundances at the stations sampled in both years was investigated by a multivariate analysis using a PERmutation Multivariate Analysis Of Variance (PERMANOVA) [30]. A hierarchical cluster analysis, using the same similarity matrix, was carried out and validated through a Principal Coordinates Analysis (PCO) to evidence similarity among stations basing on the occurrence of the different MPs polymer per size.

A Principal Component Analysis (PCA), for both years, was also performed to evidence pattern distribution of MPs sizes for each polymer. Since data of 2014 referred to a greater number of samples and a wider geographical coverage in respect to those of 2015, maps showing the spatial distribution of the different polymers as well as of MPs size classes were produced only using 2014 data. Spearman's rank correlation coefficient was used to check for the presence of a relationship between the length of sole specimens and MPs size.

The same correlation test was performed to highlight eventual correspondences between the occurrence of MPs and the plastic marine litter reported in the same area in 2014 [31].

All statistical analyses were performed using Primer V6 with the add-on package PERMANOVA+ [30] and R (<https://cran.r-project.org>).

## 3 Results

MPs were recorded in 95% of the 533 fishes sampled in 2014 and 2015, with at least two MPs items found in around 80% of specimens.

The most common polymers were polyvinyl chloride (PVC), polypropylene (PP), polyethylene (PE), polyester (PET), and polyamide (PA) for a total amount of

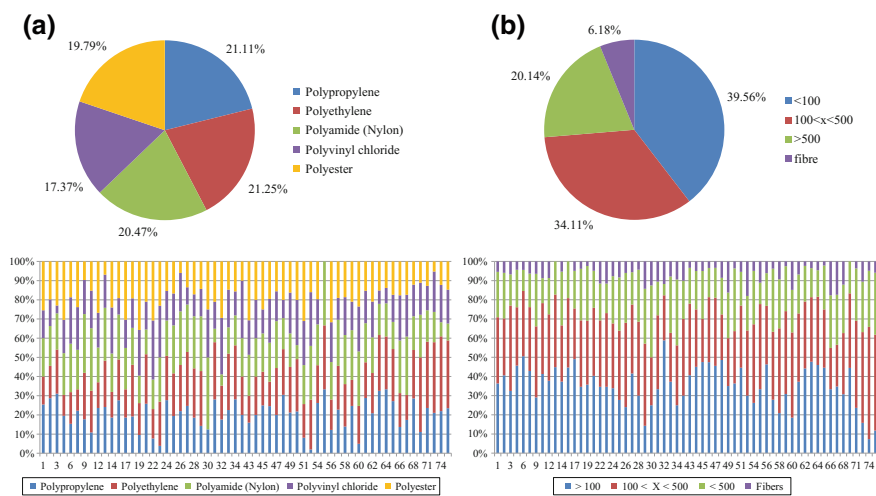
4566 microplastic items (3665 in 2014 and 901 in 2015) and an average of  $1.73 \pm 0.05$  MPs items per fish in 2014 and  $1.64 \pm 0.1$  in 2015. No statistical difference between years was evidenced in the MPs abundances recorded at the 22 stations sampled in 2014 and 2015 ( $p$ -value = 0.1236).

Most of MPs were found as fragments of various size (72%) and the remaining ones as fibers (28%).

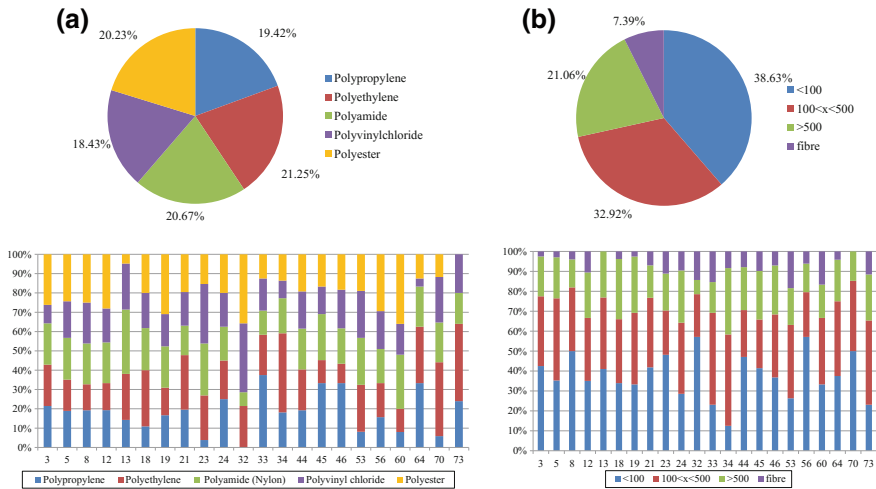
No differences were observed in the occurrence of the different polymer typologies in 2014, as each of them contributed from 17 to 21% to the overall MPs amount recorded in that year (Fig. 2a). The various polymers showed an average concentration per fish ranging from  $1.45 \pm 0.04$  (PVC) to  $1.85 \pm 0.04$  (PP and PE). Similar values, ranging from  $1.50 \pm 0.06$  (PVC) to  $1.72 \pm 0.08$  (PP), were recorded in 2015.

All polymers were found at almost all stations with the exceptions of stations 30 and 54 where PE and PET were not found, respectively (Fig. 2a). In terms of size, the two smallest dimension classes (<100 and  $100 < X < 500 \mu\text{m}$ ) were the most abundant accounting for 50–85% of the total in most stations (Fig. 2b). Similar percentages were observed in 2015 (Fig. 3).

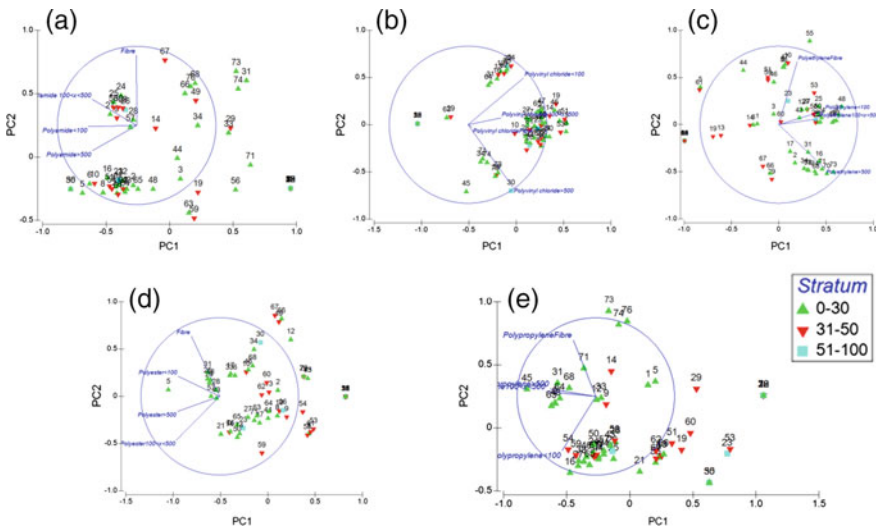
Multivariate analysis and maps applied to 2014 data showed a spatial subdivision of the different kinds of MPs (Figs. 4, 5 and 6). PVC and PA followed similar distribution patterns with the highest densities in the northern Adriatic Sea, both inshore and offshore. PE, PP, and PET appeared more concentrated in coastal areas with the highest values offshore Rimini (Fig. 5).



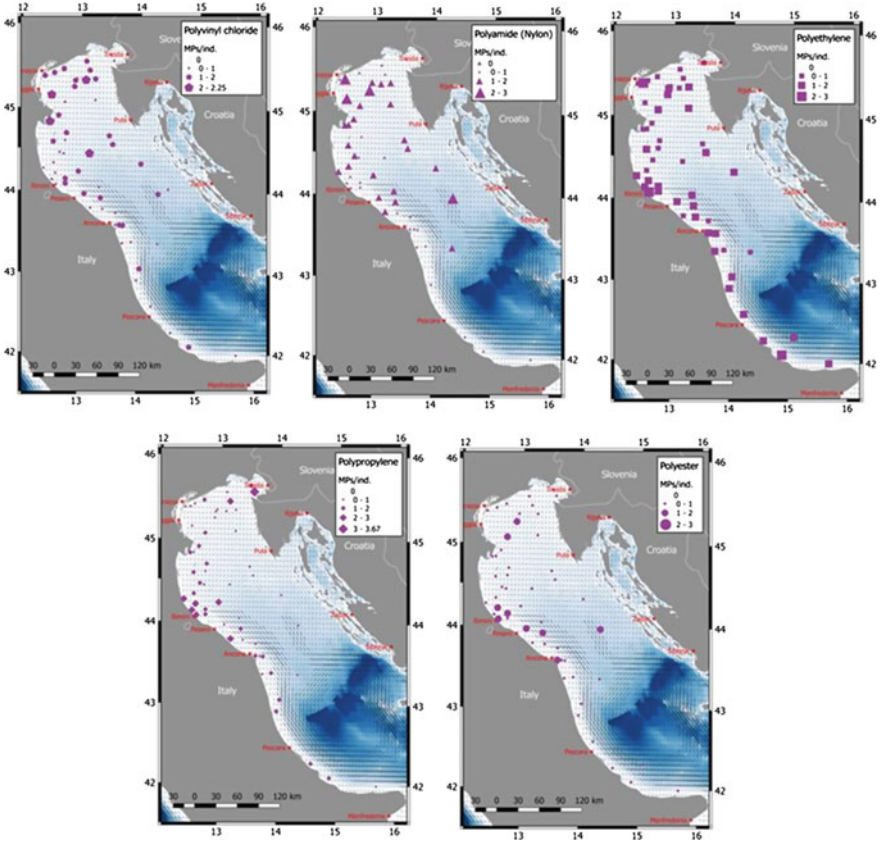
**Fig. 2** Percentage microplastic concentration in 2014. **a** On the top, the total percentage of MPs typologies concentration, and on the bottom, it was divided by station; **b** on the top, the total percentage of MPs size concentration, and on the bottom, it was divided by station



**Fig. 3** Percentage microplastic concentration in 2015. **a** On the top, the total percentage of MPs typologies concentration, and on the bottom, it was divided by station; **b** on the top, the total percentage of MPs size concentration, and on the bottom, it was divided by station



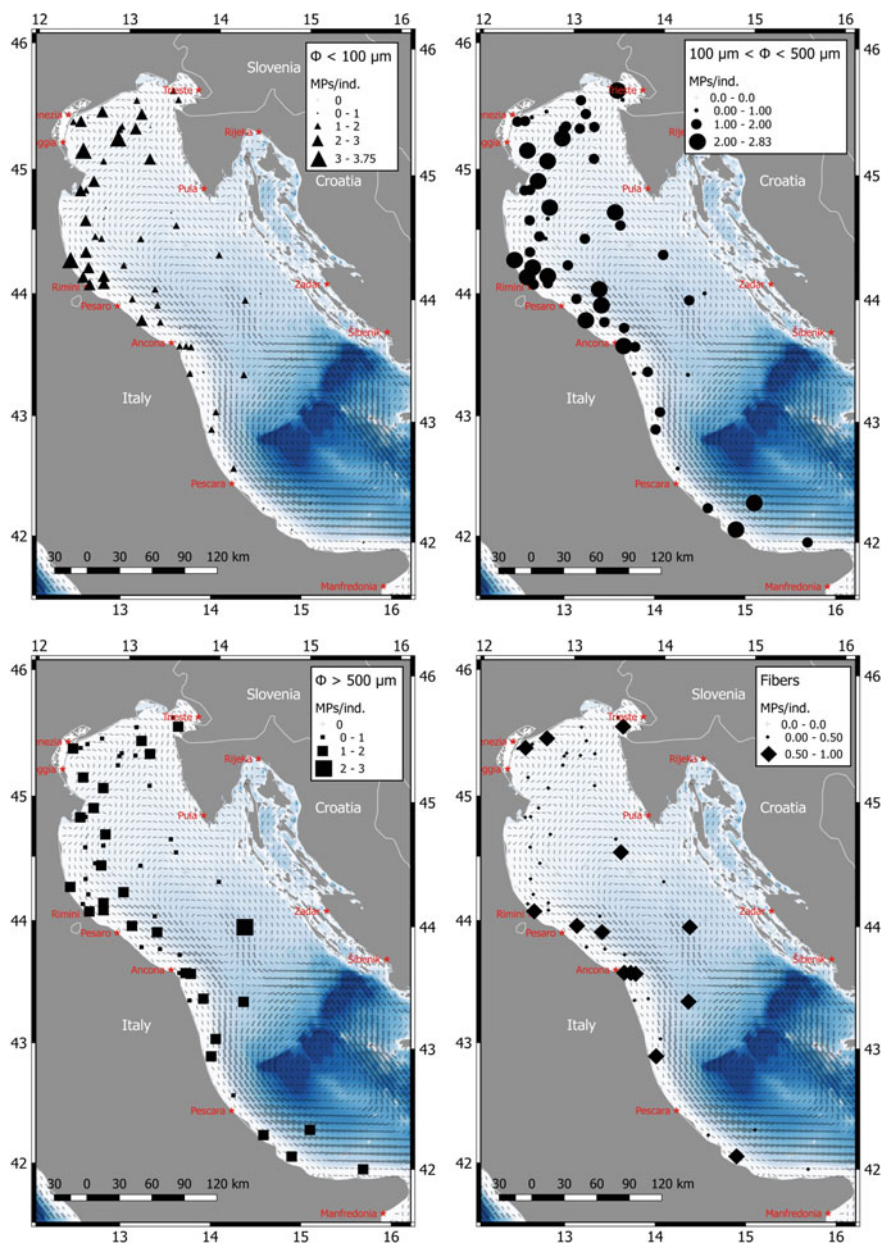
**Fig. 4** Principal component analysis (PCA) of MPs in 2014. The straight line represents the different MPs size classes; the station was divided by three bathymetric layers (0–30 m, green; 31–50 m, red; 51–100 m, light blue). Polyamide (a), polyvinyl chloride (b), polyethylene (c), polyester (d), polypropylene (e)



**Fig. 5** Maps of spatial distribution of the different polymers. Every symbol represents the average MPs concentration per fish in each station. The arrows identify the current pattern and the main city was also reported

MPs  $<100 \mu\text{m}$  appeared more concentrated in coastal waters with a tendency to decrease either in spatial range and quantitative terms from north toward south. Those  $>500 \mu\text{m}$  resulted homogeneously distributed from the coast up to around 40 km offshore with a peak in the middle of the Adriatic Sea, while MPs included in the size class  $100\text{--}500 \mu\text{m}$  and fibers showed a uniform pattern on the overall investigated area (Fig. 6).

The Spearman test applied to 2014 data showed a significant result only for MPs  $<100 \mu\text{m}$  ( $p\text{-value} = 0.027$ ;  $\rho = -0.04789$ ), highlighting an abundance decrease with the increase of sole dimension, expressed as Total Length (TL). Finally, no significant relationship was evidenced between the spatial distribution of MPs and the plastic marine litter ( $p\text{-value} = 0.064$ ).



**Fig. 6** Maps of spatial distribution of the different size. Every symbol represents the average MPs concentration per fish in each station. The arrows identify the current pattern and the main city was also reported

## 4 Discussion

Biota represents a reservoir for MPs mass estimates [32]. Moreover, the MPs identification in digestive contents of marine organisms indirectly reflects the presence of these pollutants in the marine environment and could serve as a starting point for assessing marine biota and determining their potential effects in fish.

The occurrence of small plastic particles on beaches and in coastal waters was first reported in southern New England in the 1970s [33]. However, in the European seas, the interest toward these pollutants and their possible transfer into the marine food chains strongly increased after the EU Marine Strategy Framework Directive (MSFD) entered in force in 2008.

In this work, MPs were found in 95% of the 533 specimens of *S. solea*, representing a higher percentage in respect to those reported for other finfish species, e.g., 35% for five mesopelagic and one epipelagic fish in the North Pacific Gyre [34], 36.5% for pelagic and demersal species in the English Channel [35], and 67.7% for *Boops boops* around the Balearic Islands [36].

Moreover, differently from other works focusing on the occurrence of MPs in the digestive tract of a few finfish species [34, 35], the majority of MPs were particles.

Unlike other Authors [37, 38], in the present study, the MPs occurrence in the sole digestive tract did not appear strictly related to the feeding strategy of the species. The negative correlation between MPs <100 µm concentration and the sole size can be explained with the match of spatial distribution of this species in the Adriatic Sea. In fact, juveniles mostly concentrate along the Italian coastal waters up to 30 m depth and from the northerner part of the basin to south of the Po River mouth where this MPs size was recorded. The absence of a correlation for the other MPs categories, although the diet shifts toward greater preys as the soles grow [39, 40], appears to confirm the hypothesis that the main reason of the MPs presence or absence in the gastrointestinal tract of this species is the MPs spatial distribution and abundance.

PE, PP, and PET spatial distribution could be mostly explained by the Adriatic Sea hydrodynamic circulation, especially by the near-shore currents and gyres. Anyway, it may be difficult to identify eventual MPs sources. Also, PVC and PA distribution are surely affected by the hydrodynamic features of the basin but, in this case, their high concentration near the Po mouth and at South of the Venice Lagoon could be linked to the high occurrence of mussel farms in those areas [41].

However, the absence of correlation between the plastic marine litter and MPs concentrations in the investigated area makes reasonable to hypothesize different dispersion flows by currents due to the smaller dimensions of MPs with respect to marine litter items.

The findings of this study and the maps produced can assume a relevant role to provide appropriate responses to the MSFD 2008/56/EC criteria 10.1.3 (“...trends in the amount, distribution and, where possible, composition of microparticles, in particular microplastic”) and 10.2.1 (“...trends in the amount and composition of litter ingested by marine animals, e.g., gastrointestinal analysis”). Moreover, these

results represent a further step in the investigations on MPs contaminant transfer across the food web to understand the possible ecological and biological consequences also for humans. Indeed, taking into account the results of a few studies about MPs translocation from gastrointestinal tract to other body parts in bivalves and terrestrial mammals [42, 43], it is conceivable that the same might happen in fish entering consequently in the human diet.

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# Size-Selective Feeding by Mesopelagic Fish Can Impact Ocean Surface Abundance of Small Plastic Particles

John van den Hoff, Cecilia Eriksson, Harry Burton and Martin Schultz

## 1 Introduction

One modelled estimate of global plastic pollution suggests a minimum of 5.25 trillion plastic particles weighing 268,940 metric tons which have spread throughout the worlds' oceans [1]. Of the total tonnage, about 35,540 tons (13%) are small microplastic (1–5 mm) particles. The frequency distribution of plastic particles ranging in size from <1.0 mm to about 1000 mm is thought to be uniform across the major oceans [2].

There have been very few surveys of plastic debris distribution at sea in the vast and remote Southern Ocean (SO) to date. Consequently, little is known of the origins, concentrations, distribution, impacts and fate of plastic particles in this area [3]. However, it has been recognised that fur seals (*Arctocephalus* spp.) at Macquarie Island (54°S, 159°E), a subantarctic island in the middle of the SO, consume fish that themselves have consumed plastic particles [4]. Another study of marine debris in the SO [5] made use of the unique geographic positioning of a west-facing beach on the coast of Macquarie Island to make land-based collections of marine-derived debris. We consider Macquarie Island is a natural sampling site for plastic debris circulating within the Antarctic Circumpolar Current (AAC) of the SO.

In this study, we examined the 2–10 mm fraction of plastic particles washed ashore at Macquarie Island and also recovered from fur seal scats at the same island. Our objectives were to determine the size frequency distributions and abundances of plastic particles recovered from the two plastic sampling platforms.

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## **2 Methods**

### ***2.1 Study Areas and Methods***

This study of plastics in the Southern Ocean ecosystem was undertaken at Macquarie Island (MI; 54°30'S, 158°56'E). The island is approximately 34 km long and lies in a north–south orientation north of the Antarctic Polar Front midway between Tasmania and Antarctica. The west coast of the island is exposed to the predominantly westerly surface winds of the West Wind Drift (WWD) and the persistent easterly flow of waters in the ACC [6].

### ***2.2 Beach-Washed Plastic***

Sandell Bay is situated on the west coast of the island. The bay is 4 km long and oriented in an approximate NNE-SSW alignment. Five 50 m transects of shoreline were marked out for daily clearance collections of plastic items smaller than 10 mm washed ashore on the diurnal high-tide strand line during November 2000 to March 2001. The five transects were sited on sand or small diameter shingle beach substrates.

### ***2.3 Plastics in Fur Seal Scats***

Scats from fur seals were collected from their breeding colonies above the high-tide zone on North Head, Macquarie Island. Collections were made during the austral summers (November to April) of 1990–1991, 1996–1997, 1999–00, 2000–01 (the same year as Sandell Bay was sampled) and 2001–02. Breeding season scats were selected because adult seals make short (2–4 day) foraging trips near (50–100 km) to the island when provisioning their pups [7].

The scats were kept frozen at  $-18\text{ }^{\circ}\text{C}$  until sorting. Prey remains were separated by washing each thawed scat in a series of nested sieves (1.0 and 0.5 mm mesh size). Recovered otoliths, fisheye lenses and plastics were stored dry in small plastic bags.

### ***2.4 Fur Seal Foraging Volume***

The volume of ocean within which fur seals foraged during their breeding seasons, when the scats and beach-washed plastics were collected for this study, was determined from satellite telemetry location data collected at sea by the seals

(Simon Goldsworthy, unpubl. data). The foraging area ( $m^2$ ) was measured and the result multiplied by 10 (= the median diving depth for satellite tracked female fur seals from the island [7]) to determine the seals' foraging volume in  $m^3$ .

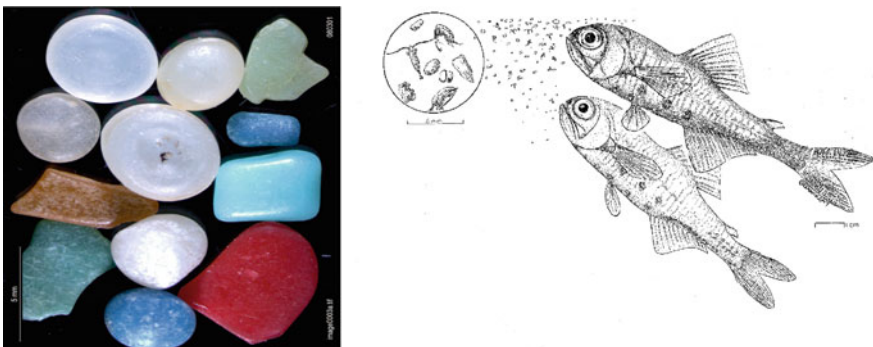
## 2.5 Statistics

A Bayesian multinomial distribution was fitted to the beach plastic size frequency data to infer the probability of membership to any bin size and thus infer if any deviations from the frequency distribution were/weren't an artefact of sampling. The model provided approximate confidence limits for bin counts.

## 3 Results

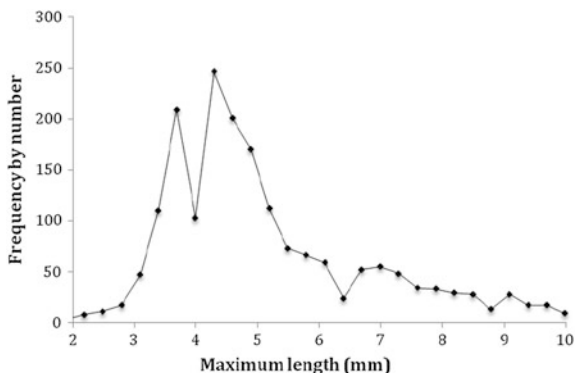
### 3.1 Beach-Washed Plastic

A total of 1829 plastic particles ranging from 2 to 10 mm maximum length were collected from Sandell Bay beach. The total included near equal proportions of irregularly shaped plastic fragments ( $n = 914$ ) and more regularly shaped "feed-stock" pellets ( $n = 915$ ) (Fig. 1). The size frequency distribution for both particles and pellets combined showed significantly negative departures at about 4 mm and 6.5 mm (Fig. 2). Results of the Bayesian multinomial model showed the 95% confidence limits (for  $p = 0.01$ ) for bins centred on 4.1 mm and 6.5 mm could not be reasonably attributed to sampling variation (model output not shown). When plastics were separated into fragments and particles, negative departures from the

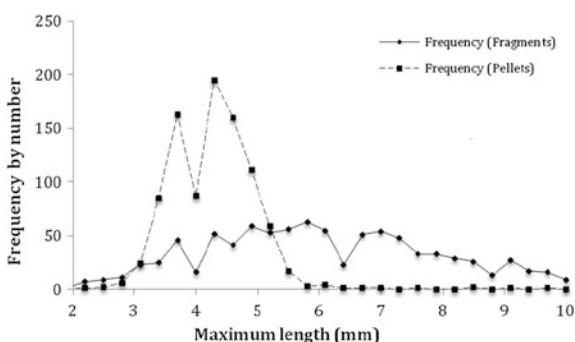


**Fig. 1** Left panel. Examples of plastic particles recovered from Sandell Bay beach and fur seal (*Arctocephalus* spp.) scat at Macquarie Island. Right panel, illustration of the big-eye lanternfish *Electrona subaspera* (Myctophidae) relative to the size of its prey and the plastic particles in this figure. Note the different scales

**Fig. 2** Frequencies of plastic particles (2–10 mm) collected at Sandell Bay, Macquarie Island, 2000–2001 (n = 1829; bin size = 0.3 mm increments)



**Fig. 3** Frequencies of plastic pellets (n = 915) and fragments (n = 914) (2–10 mm) collected from Sandell Bay, Macquarie Island, 2000–2001 (n = 1829; bin size = 0.3 mm)



frequency distribution were observable at both the 4.0 and 6.5 mm bins but only at 4.0 mm for pellets (Fig. 3).

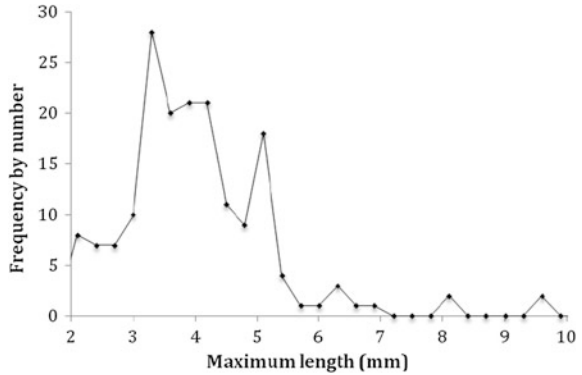
By interpolating between points spanning low-abundance bins in the frequency histogram (Fig. 2), we determined that approximately 7.3% of the total potential particles were missing, 5.7% in the 4.0 mm bin and 1.6% in the 6.5 mm bin. The figure of 5.7% for the 4.0 mm particles could be an underestimate because we could not determine the precise location of the peak in the frequency distribution.

### 3.2 Fur Seal Scats

The 332 fur seal scats yielded 6192 fish otoliths or a minimum of 3096 individual fish. Lanternfish (Myctophidae) dominated (99.3%) the fish prey recovered. Big-eye lantern-fish *E. subaspera* (Fig. 1) accounted for 73.3% (n = 2269 individuals) of all fish and 75.6% of all myctophids in the scats.

The 332 fur seal scats also yielded 47 plastic particles and all scats-containing plastic particles also contained otoliths of *E. subaspera*. Almost all (>99%) plastic particles can be assumed to have been ingested by myctophids. Based on relative

**Fig. 4** Frequencies of plastic particles (2–10 mm) recovered from fur seal (*Arctocephalus* spp.) scats on Macquarie Island, 1999–2002. (n = 177; bin size = 0.3 mm). Mean particle size = 4.1 mm



fish abundances, *E. subaspera* consumed most (74.4%, n = 35) of the particles. We estimate approximately 1.5% (35/2269) of the wild *E. subaspera* population sampled via the fur seals likely contained plastic particles in the range 2–10 mm. From the total number of otoliths recovered, we calculated an average of 0.04 (range 0.03–2.0;  $\pm 0.2$  st.dev.) plastic particles were present per individual *E. subaspera*.

If the population of *E. subaspera* within the fur seals' foraging space near Macquarie Island approximated  $37 \times 10^6$  individuals ( $= 374 \times 109 \text{ m}^3$  of foraging area  $\times 0.0001$  individual *E. subaspera*  $\text{m}^3$  [8]) and 1.5% of those fish contained plastic, then about 560,000 fish contained particles of plastic. The total number of plastic particles in the *E. subaspera* population in the 2–10 mm size range is estimated to be 22,500 particles (Fig. 4).

## 4 Discussion

The plastic particles washed ashore at Sandell Bay had a size frequency distribution shape (Fig. 2) very similar to those summarised by Cozar et al. [2] from all oceans. This overall similarity in distributions supports the view that daily beach collections can be used to reflect the size range frequency distribution of plastic particles in the nearby ocean. Moreover, we found the beach collections had reduced numbers of particles at 4 and 6.5 mm size classes compared with Cozar et al. [2]. These significant losses of particles within the specified size classes suggested their removal was size selective and due to selective predation.

Fur seal scats collected at Macquarie Island contained plastic particles with a mean size of 4.1 mm (the size frequency bin missing from the beach samples) suggesting a probable link. The principal prey for the fur seals was big-eye lantern-fish *E. subaspera* (Fig. 1). It has been suggested that lanternfish that feed in surface waters tend to have larger eyes due to the need to compensate for brighter surface light conditions [9]. Indeed, this fish species has a circumpolar distribution in the upper waters (<20 m) at night within the Subantarctic Zone between the

**Table 1** Selected zooplankton species reported in the diet of the big-eye lantern-fish *Electrona subaspera* with a size range similar to plastic particles recovered from fur seal (*Arctocephalus* spp.) scats at Macquarie Island (Data source <http://www.fishbase.org> accessed 15/06/2017)

Zooplankton species	Plankton group	Adult size range (mm)
<i>Calanus simillimus</i>	Copepod	2.0–3.8
<i>Limacina</i> sp.	Pteropod	2.5
<i>Metridia lucens</i>	Copepod	1.6–3.0
<i>Pleuromamma robusta</i>	Copepod	3.0–4.3

Subtropical Convergence and Antarctic Polar Front [8, 10, 11] where fur seals from Macquarie Island also feed. At night about 95% of all fur seal dives were shallow to median depths of 10 m [7] and *E. subaspera* migrates vertically at night into the upper waters (<20 m) to feed on diurnally migrating zooplankton of a size range similar to the plastic particles “missing” from the Sandell Bay collections (Table 1, Fig. 2). The suite of zooplankton species reported in the diet of the fish (Table 1) is in accordance with the regional species composition determined from numerous transits of Continuous Plankton Recorders through these latitudes [12]. The vertical migration by the fish likely explains why *E. subaspera* is so numerous in the fur seal scats and also why *E. subaspera* consumed the majority of the plastic particles drifting close to the ocean’s surface within the fur seal foraging volume. The relative absence of particles in the 6.5 mm fraction, compared to the beach collections, suggests that a larger predator, not consumed by the seals, may be responsible [13].

From the samples collected, we determined that approximately 2% of the population of *E. subaspera* present in the seals foraging area contained plastic particles in the range 2–10 mm. We suggest the removal of these particles from the ocean’s surface by the fish resulted in the non-uniform size frequency distribution of plastic particle washed ashore on Macquarie Island. We estimated about 30,000 plastic particles ranging from 2–10 mm were removed from the ocean’s surface (upper 20 m) within the seals’ foraging area by the *E. subaspera* population during the scat sampling period. The value we take of the standing concentration of plastics (2–10 mm) in the seas west of Macquarie Island is a recent one [14]. We took their measured value of approximately  $1 \times 10^{-3}$  pieces  $m^{-3}$  at particle length of 3.5 mm size length and modelled the total particles (2–10 mm) following the size distribution they gave for the better known seas off Japan. Future work will undoubtedly produce many more estimates and will approach values with better error estimates, but this study serves to illustrate the potential of the approach.

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# Dynamics in Microplastic Ingestion During the Past Six Decades in Herbivorous Fish on the Mediterranean Israeli Coast

Noam van der Hal, Erez Yeruham and Dror L. Angel

## 1 Introduction

Since plastic was first commercially introduced in the late 1940s, the amounts of plastics used and the types of different polymers have vastly increased. Initial evidence of microplastics in the sea was first documented during the early 1970s [1]. Further work summarizing the presence of marine plastics in the periods 1972–1987 and 1999–2010 from the North Pacific Subtropical Gyre and from Continuous Plankton Recorder (CPR) surface samples from the North Atlantic and the North Sea suggest that the abundances of marine microplastic debris are increasing temporally [2, 3]. Microplastic distribution in the eastern Mediterranean has only recently been recorded [4], indicating a high abundance as compared to the western part of the Mediterranean and other locations worldwide [5]. Studies from the past two decades show that the exposure of various marine species from different trophic levels to plastics is widespread [6–9] and may exert various effects on them [10, 11], many of which are deleterious.

*Siganus rivulatus* and *Siganus luridus* are small-to-medium-size, schooling, herbivorous fish, that migrated to the Mediterranean Sea from the Indo-Pacific

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Ocean. They were first recorded in the Mediterranean in 1924 and 1955, respectively, and since then they have become very abundant. Along the Israeli coast they comprise 95% of the herbivorous fish and one-third of the total fish biomass in rocky habitats [12]. In the northern rocky coast of Israel, where benthic algae proliferate, the diets of the two siganid species tend to diverge, while in the southern sandy coast their diets are more similar [13]. Furthermore, *S. rivulatus* dominates the more shallow waters (0–10 m) and is more abundant than *S. luridus* which is found in deeper coastal water (0–40 m). In addition to their consumption of algae, recent findings [9] have shown that 60% of the *S. rivulatus* examined in Turkish coastal waters had microplastics in their digestive tract. Other research on the rabbitfish family shows evidence of plastic consumption in the Philippines [14] and in Indonesia [15].

Our research compared the amounts of microplastic particles found in the digestive tracts of the two Israeli Mediterranean siganid species over the course of several decades to determine whether there has been a temporal change in plastics consumption, types of particles consumed, etc. The working hypothesis was that there was a gradual increase in the abundance of microplastics in the sea and that the proportion of rabbitfish with ingested plastics will reflect this. These findings could be used to argue that it is crucial to reduce the abundance of microplastics in the marine environment because these eventually return to us in the fish and other biota that we remove from the sea and consume. Moreover, by making the Israeli public and government more aware of the subject, this might help pave the way to take (policy) measures to prevent or reduce plastics and microplastics from reaching the Mediterranean Sea.

## 2 Materials and Methods

The research compared 88 digestive tracts from siganids fished from the Ashdod, Tel Aviv region in October 2016, with 88 siganid digestive tracts, fished from the same region or adjacent, received from the Zoological Museums of the Tel Aviv University and the Hebrew University (49 and 39 digestive tracts, respectively), ranging from the 1960s up to 2014. All digestive tracts were weighed, while the archived fish were also measured for their length and total weight.

Each digestive tract was inserted into a separate beaker with 10% KOH for a period of 2 weeks in order to dissolve all organic matter and facilitate separation of microplastic particles from the organic matter<sup>11</sup>. After the 2 week digestion, contents of the beakers were poured onto a 125  $\mu\text{m}$  sieve and the remains were resuspended in 30% alcohol prior to analysis. Microplastic particles present in the fish digestive tracts were counted, measured, and recorded for color and “class” of particle (e.g., fiber, film) [16].

### 3 Results

#### 3.1 Microplastic Abundances in Siganids: A Temporal Review

Microplastic particles were found in the digestive tracts of 81 out of 88 rabbitfish (92%) obtained from the fish store. The abundances of microplastic particles (see Table 1) varied among the fish and almost two-thirds (62.5%) of the fish had 10–99 particles per fish gut.

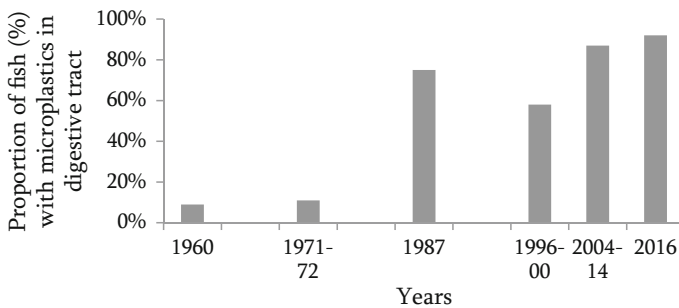
The proportion of fish with microplastics in their digestive tracts has increased over the past 60 years, from roughly 10% in the 1960s–1970s, to about 80% from the late 1990s to the present (see Fig. 1).

Considering the fact that the fish were taken from the national collections, the number of fish sampled from 1960 to 1972 (N = 38) is fairly comparable to the number of samples examined from 1987 to 2014 (N = 50) (see Table 2).

Throughout the years, there was no real difference in the proportion of fish with microplastics in their guts among the two siganids, *S. rivulatus* and *S. luridus* (49 and 50%, resp.). Nevertheless, the abundance of microplastic particles per fish was higher in *S. rivulatus*. As a matter of fact, *S. luridus* was never found with abundances higher than ten particles per fish.

**Table 1** Abundances (ranges) of microplastic particles in the digestive tracts of siganids examined

Number of particles	Number of fish
0	7
1–9	12
10–99	55
100–548	14



**Fig. 1** Proportion (%) of fish with microplastic particles in their digestive tracts, from 1960 to 2016

**Table 2** Number of siganids examined for each period

Years	Number of fish
1960	11
1971–1972	27
1987	4
1996–2000	38
2004–2014	8

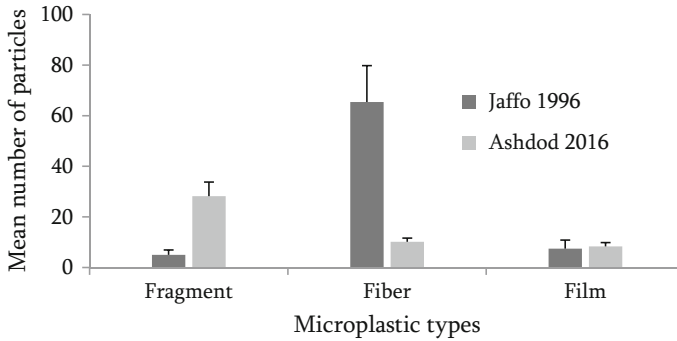
### 3.2 Abundances and Sizes of Ingested Microplastic Particles

Fish weight and digestive tract weight were positively correlated ( $p < 0.01$ ), and the range in wet weight of fish digestive tracts was from 0.3 to 18.2 g (see Table 3). Fish with digestive tracts ranging from 2 to 14 g had the greatest abundances of microplastic particles; fish with guts weighing  $<2$  g or  $>14$  g had much fewer particles (Table 3).

Microplastic particles found inside the siganid digestive tracts ranged from 0.3 to 5 mm, and there was no relationship between the fish length and weight and the size of microplastic particles in its gut. In some cases, particles were smaller than 0.3 mm or larger than 5 mm, but those were ignored because we included only particles in the size range 0.3–5 mm. Moreover, the abundance of the particles outside this range was negligible. It is noteworthy that in some cases, particles of the same type (i.e., fragments, fibers, and filaments) were found in large quantities (i.e., 5–20 particles) in a single digestive tract.

**Table 3** Mean abundances ( $\pm$  standard error, S.E.) of microplastics in the digestive tracts of rabbitfish, by weight range

Weight range of digestive tract	Number of fish examined	Mean abundance of microplastics per digestive tract	S.E. ( $\pm$ ) of microplastic abundance
0.3–2 gr	21	5.8	2.69
2–4 gr	43	38.9	10.98
4–6 gr	42	42.83	9.3
6–8 gr	21	68.09	26.91
8–10 gr	16	41.43	18.13
10–12 gr	13	22.69	11.51
12–14 gr	8	31.5	9.73
14–16 gr	7	4.57	0.87
16–18.2 gr	5	1.47	5.21



**Fig. 2** Mean abundance (+S.E.) of fragments, fibers, and films (types of microplastic) particles found in fish from 1996 in Jaffo and from 2016 in Ashdod

### 3.3 Temporal Changes in Types of Microplastics

Fragments, fibers, and films are the most abundant forms of microplastic particles in the world's oceans. In this study too, these particle types were dominant in the fish digestive tracts that contained microplastics. Nevertheless, the ratio between fragments and fibers varied throughout the temporal survey. A good example may be found when comparing data from Jaffo in 1996 with the fish digestive tracts from Ashdod in 2016 (see Fig. 2). Both Jaffo and Ashdod are port cities with fisheries, which means we would expect to see large abundances of plastic fibers that shed from nets and ropes. Whereas this pattern applies to Jaffo, in Ashdod the microplastics observed were mainly fragments.

## 4 Discussion and Conclusions

Microplastics may be found in fish occupying a variety of trophic levels [17–19], however, there are insufficient data thus far to draw firm conclusions on the uptake of microplastics by fish [19]. In this study, we found that 2 species of a herbivorous fish consistently consume microplastic particles in substantially large amounts. Recent findings regarding the abundances of microplastics along the Mediterranean coasts of Israel [4] indicate that marine species in this region are exposed to large amounts of microplastic particles which may affect them in a variety of ways.

There is currently some debate over the toxicity of microplastics related to exposure of the fish to hydrophobic organic carbons (HOC) that are adsorbed to and concentrated on the microplastics [20]. Whereas many finfish may consume small numbers of microplastics and may therefore experience only a small dose of HOC,

it is possible that the large abundances of microplastics in *S. rivulatus* expose the rabbitfish to a greater HOC toxic load during passage of the particles via the digestive tract.

An analysis of the abundance of microplastic particles in different size digestive tracts showed that the larger fish in this study, with digestive tracts >14 g, generally did not consume microplastics at all. This phenomenon might be explained by the change in diets between young and mature fish. In some cases, large numbers of particles of a specific kind (i.e., fragments, fibers, and filaments) were found inside a single digestive tract. It is impossible to distinguish whether such particles entered as a larger piece and were broken during passage through the digestive tract, or whether they were consumed as small particles before entering the digestive tract. More research is clearly needed in order to understand siganid consumption of plastics. Furthermore, research on microplastic types that might adhere to marine algae (the main food of siganids) could provide a better understanding on this subject.

Although we do not have historical records of the abundances of microplastics in our coastal waters, the large proportion of siganids that contained large abundances of plastics in their digestive tracts suggests that rabbitfish may serve as “bio-indicators” of the abundances of plastics in the environment.

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# Nanoplastic Impact on Human Health—A 3D Intestinal Model to Study the Interaction with Nanoplastic Particles

Roman Lehner, Alke Petri-Fink and Barbara Rothen-Rutishauser

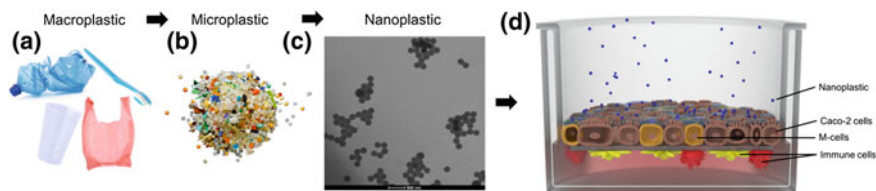
## 1 Introduction

Much attention has been paid in recent years to the fate of microplastics in the environment. Several studies have shown that microplastics can be taken up by a variety of organisms (e.g., fish, mussels, zooplankton, sea urchin, birds) and thus can cause adverse effects such as death due to ingestion and entanglement, as well as pro-inflammatory responses. However, recent studies point out that the process of plastic degradation progresses beyond the formation of microplastics. Lambert et al. [1] have shown the formation of nanoplastic particles from disposable coffee cup lids made out of polystyrene during the decomposition by UV light, which might then end up in the ecosystems. Therefore, microplastic particles are not the end of the chain but a continued disintegration into nanoplastic particles has to be expected. However, up to now, the knowledge of nanoplastic is very sparse since there are only a handful of scientific publications on the subject published focusing on aquatic organisms [2–5]. Very limited data is available regarding the impact of these plastics on human health [6], although their transfer to humans via the food chain is increasing [7]. Cellular uptake, accumulation in gastric intestinal tissue, and inflammatory reactions by nanoplastics could be shown by in vitro studies [8, 9]. Particles in the nano-range show changes in their chemical–physical properties, which is of specific concern regarding in vivo distribution as well as cellular uptake of the particles. As seen with microplastics, nanoplastics can also adsorb hydrophobic chemicals such as polychlorinated biphenyls, thus influencing their potential biological and toxicological impacts to the environment [10].

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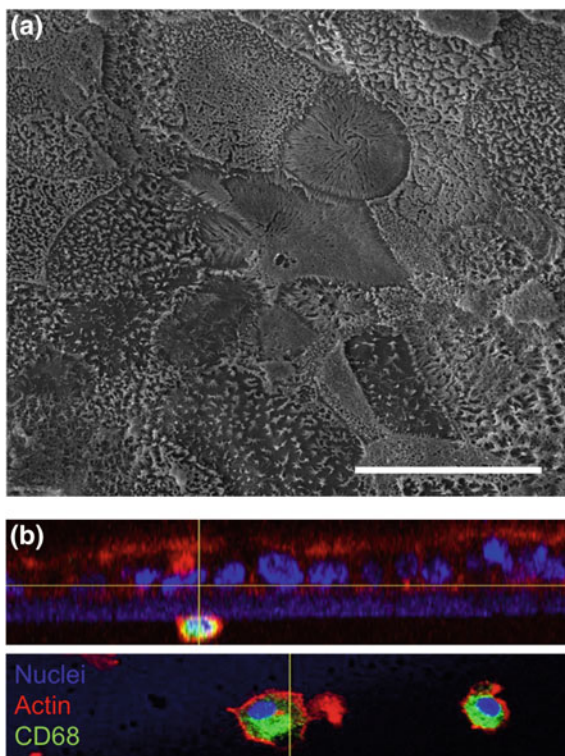
**Fig. 1** **a–c** Plastic degradation in the environment progresses from macro- to micro- down to nanoplastic. **c** Transmission electron microscopy image of nanosized polystyrene particles. **d** Scheme of the intestinal 3D cell model to study the interactions with nanoplastics particles

In our project, we, therefore, aim to demonstrate the effects of nanosized plastic particles on the human intestine tissue barrier, since the gastrointestinal tract represents the main possible uptake route of nanoplastic by the humans (Fig. 1).

## 2 Material and Methods

For the planned experiments, we developed a co-culture model of the intestinal tissue by using the human adenocarcinoma intestine cell line Caco-2, the human colon adenocarcinoma mucus-secreting cell line HT29-MTX, human Raji B

**Fig. 2** **a** Microvilli structure imaged by scanning electron microscopy (scale bar = 20  $\mu\text{m}$ ). **b** Detection of immune cells and epithelial cell monolayer formation

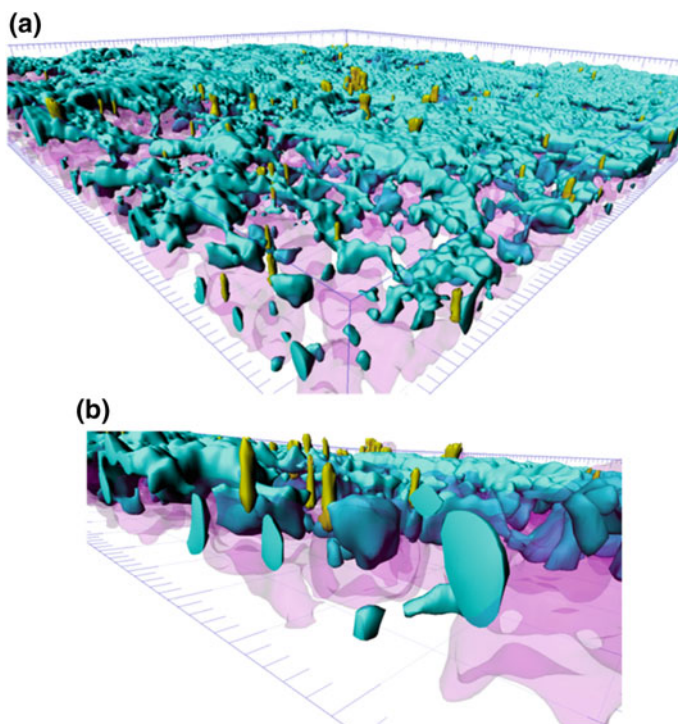


lymphocytes for the differentiation of M cells, and various relevant immune cells. The spatial cell arrangement and cell morphology will be evaluated by confocal laser scanning microscopy (CLSM), scattering electron microscopy (SEM), and atomic force microscopy (AFM). The barrier properties of the epithelial cell layer will be determined before and after nanoplastic exposure. Possible adverse effects of nanoplastic particles will be assessed after exposure for various time points, i.e., 24 and 48 h, and various concentrations. Cellular effects such as cytotoxicity, oxidative stress, and pro-inflammation will be assessed by various assays.

First, co-cultures have been assessed and laser scanning microscopy images showed that the Caco-2 cells have formed a confluent layer complemented with single immune cells on the lower side of the insert as well as the formation of microvilli structures as seen by scattering electron microscopy (Fig. 2).

### 3 Outlook

The planned research project will address a knowledge gap by performing a state-of-the-art assessment of the hazards posed by nanosized plastic particles in an advanced human intestine model. The project will start to investigate the



**Fig. 3** a and b 3D views showing polystyrene nanoplastic particles (yellow,  $d = 200$  nm) exposed to Caco-2 cells (cyan = actin, violet = cell nuclei) after 24 h

decomposition of common plastics, e.g, polyethylene (PE) and polystyrene (PS) (Fig. 3). In a second phase, we will characterize and study the interactions and reactions of the nanosized compounds as related to biocompatibility, cellular uptake of nanoplastic, and possible induction of cell responses such as oxidative stress or pro-inflammation.

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# Imitating the Weathering of Microplastics in the Marine Environment

Kathrin Oelschlägel, Jenny Pfeiffer and Annegret Potthoff

## 1 Introduction

The existence of microplastic particles (polymer particles <5 mm) in our environment has already been proven by several studies [1, 2]. It is known that the interactions between particles, meteorological (sun radiation [ultraviolet light, UV] and air temperature), and marine effects (water temperature, salinity and turbulences) lead to alterations in physical–chemical properties of plastic products or briefly to their degradation and fragmentation [3, 4]. By understanding the individual steps of the weathering process and the respective consequences for material properties (size, shape, density, crystallinity, and surface properties), it is possible to predict the distribution, behavior, and interactions of the particles in the environment and finally to assess their risk toward nature [5]. The aim of this work is to mimic the weathering of microplastics in the marine environment under laboratory conditions and to evaluate its influence on material properties. This study is focusing on abiotic weathering processes including UV radiation and mechanical stress. Important data for weathering processes include the initial state of microplastics, the energy source and input during weathering, and the state of microplastics after weathering. As model polymers, two materials (low-density polyethylene, polyethylene terephthalate) are used, which differ in their chemical structure and density. It is expected that the weathering process of the materials depends on these properties.

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## 2 Experimental

### 2.1 Materials

Investigations were performed on two commercially available polymers with low additive concentrations: low-density polyethylene (LDPE, granules, ET316300, Goodfellow Cambridge Ltd., UK) and polyethylene terephthalate (PET, granules, ES306312, Goodfellow Cambridge Ltd., UK). The granules were processed into powder by cryomilling (pin mill, Alpine C160, Messer Group GmbH, Germany).

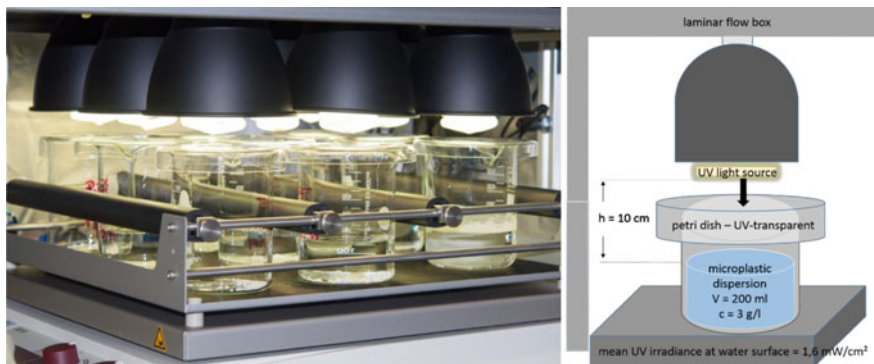
For the preparation of the polymer suspension, a salt media (artificial seawater) based on ultra-pure water with a final salt concentration of 6 g/l was used.

### 2.2 Methods

#### 2.2.1 Weathering Procedure

For the weathering process, suspensions containing artificial seawater and polymer particles were prepared and positioned under UV light sources. The experimental setup is shown in Fig. 1.

Fluorescent bulbs (Reptile UVB 200, 25 W, Exo Terra, HAGEN Deutschland GmbH & Co. KG, Germany) were used as UV light source. The light sources are characterized by a high UV-B-light portion and natural UV light intensities. The beakers with the suspensions were placed under the UV light source in a darkened box. To avoid evaporation of the media during the weathering, all samples were covered with UV light-transparent petri dishes. The samples were weathered at room temperature (25 °C). Illumination was performed for 24 h, 7 days a week over a period of maximum 28 days.



**Fig. 1** Experimental setup for the weathering study

## 2.2.2 Sample Preparation and Analytical Techniques

The initial state of the polymer particles was determined by measuring the particle size distribution (PSD), density, crystallinity, and phase composition of LDPE and PET powders. For density measurements, helium pycnometry (Penta-Pycnometer, Quantachrome GmbH, Germany) was applied. Sample crystallinity was analyzed by differential scanning calorimetry (DSC 7, PerkinElmer, Inc., USA), whereas the chemical composition was determined by X-ray diffraction (Bruker D8, Bruker Corp., USA). In next step, polymer suspensions were prepared by mixing the particles with the artificial seawater. Of each sample, the pH value and conductivity (25 °C, SevenMulti, Mettler Toledo, USA) were measured. After the weathering, the samples were analyzed. For the particle analysis, a homogenous suspension is necessary; therefore, a commercially available surfactant was added. Prior to the measurement, all samples are diluted to a concentration of 1 g/l, based on the batch concentration. Dynamic imaging (QICPIC, Sympatec GmbH, Germany) was performed to determine PSD and particle shape distribution of particles in the size range from 5 to 1700  $\mu\text{m}$  [6]. For the evaluation of the data, the Feret mean diameter [7] was used. Afterward the analysis of small particles followed via ultrafiltration with cellulose acetate membranes, particles  $>1.2 \mu\text{m}$  were cut off. The resulting filtrate was analyzed with Nanoparticle Tracking Analysis (NTA, ZetaView, Particle Metrix GmbH, Germany) to determine PSD and particle concentration for particles  $<1.2 \mu\text{m}$  [8]. While the whole sample is analyzed by dynamic image analysis, only a subsample of filtrate can be analyzed by NTA. The filter cake of the filtration was dried und used for density and crystallinity measurements.

## 3 Results and Discussion

### 3.1 Results

#### 3.1.1 Phase Composition, Crystallinity, and Density

The characteristics of the PSD of LDPE and PET powder are listed in Table 1.

The X-ray diffraction analysis of both polymer types confirms that the polymers are low-density polyethylene and polyethylene terephthalate. The crystallinity of the unaged LDPE and PET in artificial seawater is  $32\% \pm 1\%$  and  $41\% \pm 1\%$ , respectively. Weathering the polymer particles in artificial seawater for 28 days

**Table 1** Number-weighted quantiles of the PSD of LDPE and PET powder determined by dynamic imaging

Polymer powder	$x_{0,10}$ in $\mu\text{m}$	$x_{0,50}$ in $\mu\text{m}$	$x_{0,90}$ in $\mu\text{m}$
LDPE	7	66	263
PET	7	37	101

under UV light leads to an increase in powder crystallinity for LDPE ( $36\% \pm 1\%$ ) and PET ( $44\% \pm 1\%$ ). Measuring the density of the unaged polymer powders resulted in the following values: LDPE =  $0.92 \text{ g/cm}^3 \pm 0.02 \text{ g/cm}^3$  and PET =  $1.43 \text{ g/cm}^3 \pm 0.02 \text{ g/cm}^3$ . After the exposition with UV light, no significant changes in the density of the polymers were detected (LDPE =  $0.94 \text{ g/cm}^3 \pm 0.02 \text{ g/cm}^3$  and PET =  $1.44 \text{ g/cm}^3 \pm 0.02 \text{ g/cm}^3$ ).

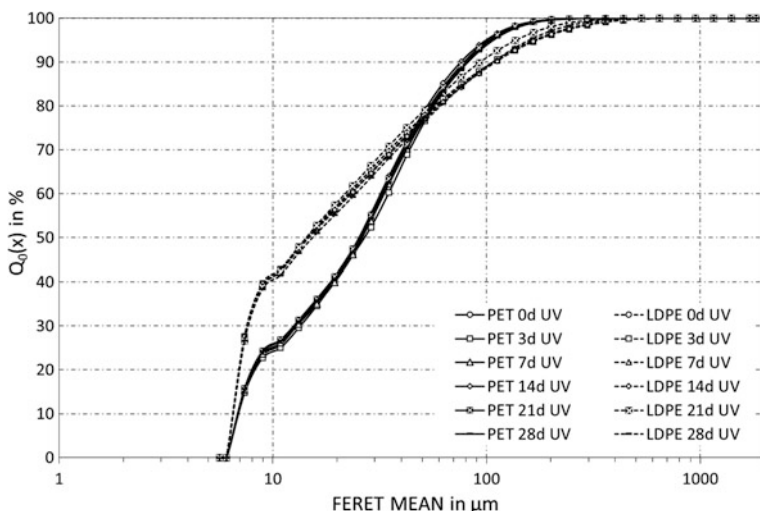
### 3.1.2 Particle Size Distribution

In Fig. 2, the PSDs of LDPE and PET measured by dynamic imaging are shown. For both materials, no significant changes in the particle size range of 5–1700  $\mu\text{m}$  are detected over 28 days of weathering.

In Fig. 3, the PSDs of the weathered LDPE samples analyzed by NTA are shown. The particle size increases from 0 to 3 days of UV exposure. Afterward a successive decrease from 3 to 21 days is observed.

In Fig. 4, the particle concentration of weathered LDPE samples is shown. With exception of the seven day sample, a nearly linear increase of the particle concentration is observed.

In Fig. 5, the PSD of weathered PET samples is shown. Only small variations of the particle size can be detected when comparing samples 0, 3, and 7 days. After 14 days of weathering with UV light, a decrease in particle size is observed.



**Fig. 2** Number-weighted PSD of LDPE and PET weathered under UV light for 0, 3, 7, 14, 21, and 28 days determined by dynamic imaging. The standard deviation of the measuring points is lower than  $\pm 0.8 \mu\text{m}$



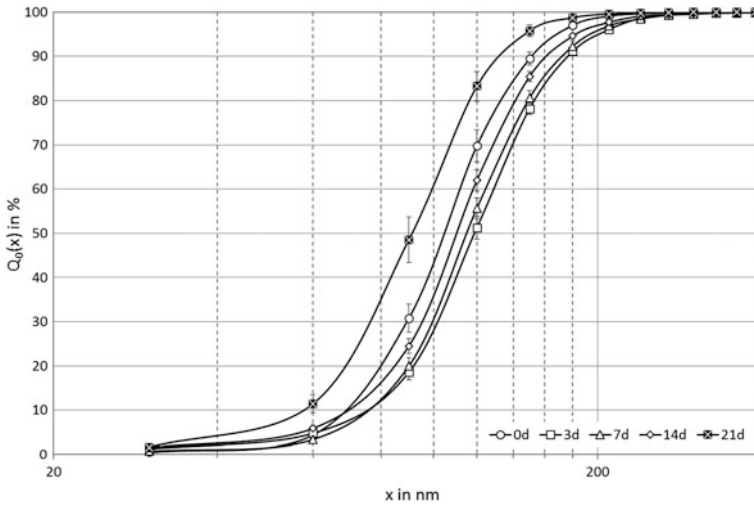


Fig. 3 Number-weighted PSD of weathered LDPE samples determined by NTA

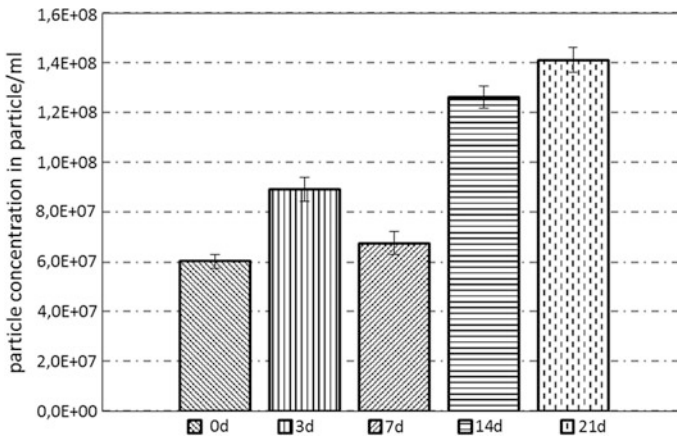


Fig. 4 Particle concentration of weathered LDPE samples detected by NTA

Comparing statements concerning the twenty-one day sample are not reasonable, because of the high standard deviation of the measuring points. The particle concentration of the weathered PET samples is shown in Fig. 6. No significant differences were measured for the sample weathered for 3, 14, and 21 days. The maximum particle concentration was measured after 7 days of weathering, the minimum particle concentration after 0 days.

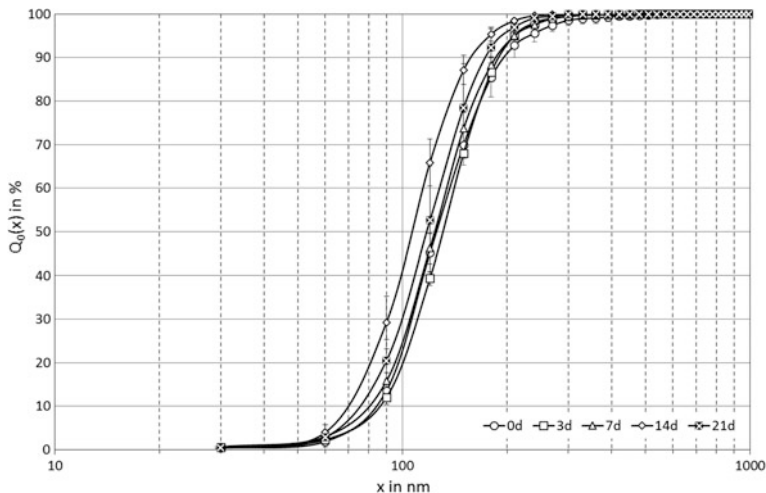


Fig. 5 Number-weighted PSD of weathered PET samples determined by NTA

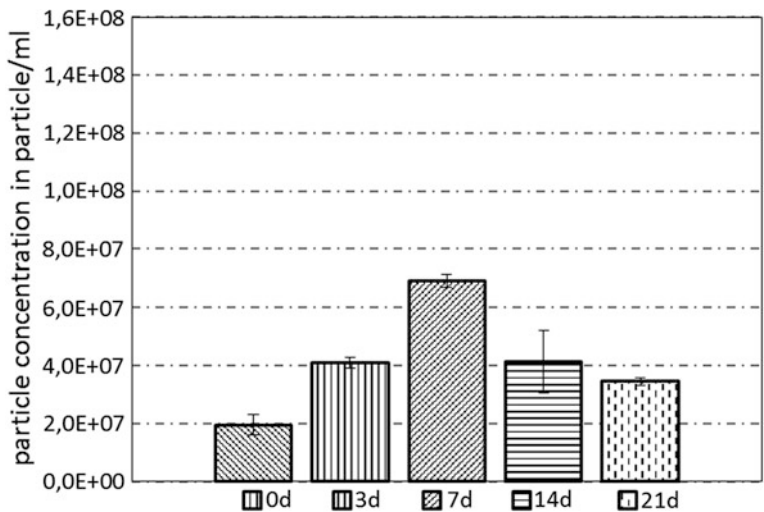


Fig. 6 Particle concentration of weathered PET samples detected by NTA

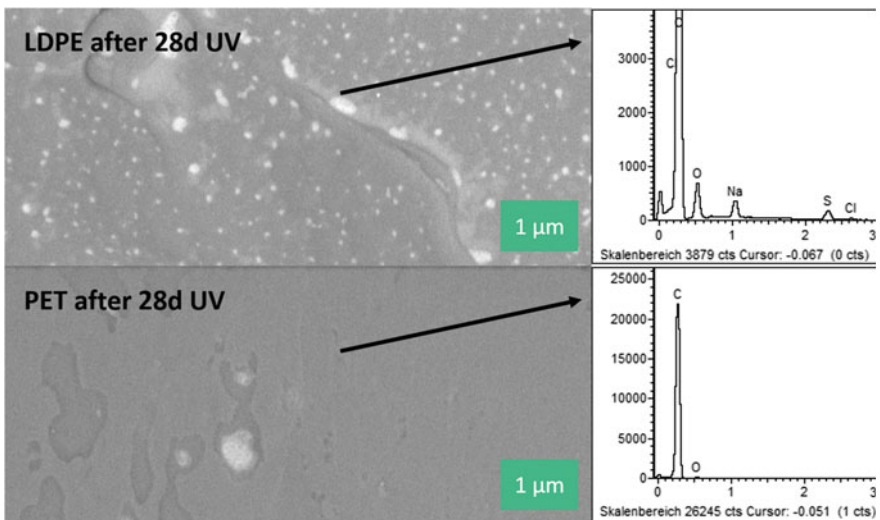
### 3.2 Discussion

The chemical structure of the materials, as well as their density, is decisive for the course of the weathering process of polymers in the environment. The chemical structure determines which chemical reactions are relevant for the degradation, whereas the density strongly influences the distribution of the polymer particles in

the water column. For the study, two polymers are chosen that differ significantly in their chemical structure and density. LDPE has a very simple chemical structure based on single bonds between carbon and hydrogen atoms and is degradable by photo-induced oxidation. PET has a more complex structure with double bonds between the atoms and an additional element in the structure: oxygen. The degradation of PET is driven by photo-induced oxidation and hydrolytic reactions. LDPE has a lower density ( $0.92 \text{ g/cm}^3$ ) than saltwater ( $\sim 1 \text{ g/cm}^3$ ); therefore, the particles float at the water surface and are directly exposed to UV light. In contrast, PET ( $1.43 \text{ g/cm}^3$ ) has a higher density than water; the particles sink to the ground and are, therefore, protected from the UV light by the water layer above them. Hence, a more pronounced effect of UV light is expected on LDPE particles.

While no changes in the density of the polymers are detected during the weathering process, an increase in crystallinity is measured. One reason could be the leaching of additives out of the polymer structure. With the loss of the leaching products, the ratio between the amorphous and crystalline phases in the polymer is shifted toward the crystalline phase.

The analysis of polymer surfaces with a scanning electron microscope equipped with a detector for material contrasts visualizes differences between non-weathered and weathered particles. While non-weathered particles show a homogenous colored surface, white spots occur on particle surfaces that are weathered for 28 days under UV light, as shown in Fig. 7. The LDPE surface is characterized by a high number of white spots in contrast to PET. It can be assumed that the different surface properties of the polymers are related to the different UV light intensities the



**Fig. 7** Particle surface of LDPE and PET particle weathered for 28 days under UV light in artificial seawater and examined by SEM with a detector for material contrasts. On the right side is the EDX spectra of the white spots (next to LDPE) and the gray background (next to PET) shown

materials were exposed to during the weathering process. As discussed in Gewert 2015 [9], LDPE might be almost immune to photo-initiated degradation due to the absence of double bonds in the polymer backbone. The altered surface may not be caused by the release of very fine LDPE fragments, but by the leaching of additives that leave small defects in the particle surface. The white spots form on the resulting surface defects. The element analysis of the white spots with EDX (energy dispersive X-ray spectroscopy) reveals that they consist of sodium, chlorine, and sulfur, which are the main components of the artificial seawater. The gray background represents the polymer itself, where only carbon and oxygen are detected. The existence of the white spots on weathered particle surfaces is an indication for changed particle surface properties due to the weathering process.

The analysis of the data from the dynamic image analyzes shows no changes in the PSD or the particle shape distribution during the weathering of the polymers. It is possible that no changes occur during the relatively short period of time or that the changes are minor and therefore not detectable by the measuring system. For that reason, special attention is paid to the analysis of small particles. The evaluation of the data measured with NTA reveals for LDPE a decrease in particle size, when comparing the starting (0d) and endpoint (21d) of the weathering process. The additional increase in particle concentration within the measurement period seems to confirm either the fragmentation of LDPE particle or the release of leaching products caused by UV light exposition. In contrast to LDPE, the PET PSDs of 0, 3, 7, and 21 days are similar to each other, which implies that no fragmentation occurs. Also, the particle concentration after 3, 14, and 21 days is nearly equal. Compared to LDPE, the changes that are detected for PET are minor. The data seems to support the hypothesis that the water layer extenuates the intensity of the UV light and therefore protects the particles from weathering.

## 4 Conclusions

The weathering study confirms that changes in material properties like crystallinity and surface properties take place, indicating the influence of UV light on polymers. The more pronounced changes of the LDPE properties also endorse that materials floating on the water surface are more affected by UV light than sinking materials. If the fragmentation of LDPE particles or the release of leaching products leads to the decrease in particle size and the increase in particle concentration should be further investigated. Therefore, measurements concerning the chemical composition of particles  $<1.2 \mu\text{m}$  are interesting. To better understand the weathering process, it would be reasonable to close the particle size gap between the two applied measuring methods. A particle counter (FAS 362, Topas GmbH, Germany) could be useful since it determines PSDs and particle concentrations in a size range from one to  $200 \mu\text{m}$ . The combination of the applied analytical techniques allows it to follow the fragmentation process over a wide range of particle sizes, generating information about particle sizes and concentrations. The changes in both material

properties help to understand how the fragmentation process proceeds. In the environment, the weathering of the polymers is not only caused by UV light. Additionally, turbulences and abrasive particles will intensify the weathering process. Dynamic imaging is a suitable method to evaluate the influence of abrasive particles on the weathering of polymers.

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# Microbial Degradation of HDPE Secondary Microplastics: Preliminary Results

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Martina Franchini and Nicolas Kalogerakis

## 1 Introduction

The presence of plastic debris in the marine environment has been overlooked for years, and only recently, it was recognized as an emerging threat for the wellbeing of the planet [1]. While the origins of plastic pollution have been thoroughly researched and recognized as mostly land-based [2], the fate of plastics following their release in the environment has just begun to be studied [3]. The interactions of plastics with the marine environment can be of physical, chemical, or biological nature. Plastic debris undergoes transportation, sedimentation, accumulation, or degradation can absorb and adsorb pollutants such as Persistent Organic Pollutants [POPs] or metals and negatively affect marine organisms, through entanglement, ingestion, or the enabling of species migrations [4, 5]. Almost 270,000 t of plastics have been estimated to float in the world's oceans, with current convergence areas, such as the ocean gyres, and enclosed waterbodies, namely the Mediterranean Sea, being most heavily affected [6, 7].

Plastic items with a size smaller than 5 mm are called microplastics [8]. Virgin polymer pellets and micro-beads used in personal care products are characterized as primary microplastics [9]. At the same time, mechanical stress, solar radiation, heat, hydrolysis, and the enzymatic processes of microorganisms affect plastics during their residence in the marine environment and can cause the polymeric chains to degrade and the plastic items to fragment into smaller ones called secondary microplastics [10]. The fragmentation of plastics has been studied in marine and land conditions, although limitedly, due to the complexity of interactions among a large number of factors, such as material and matrix properties, temperature, radiation, oxygen levels, and mechanical stress, that are hard to be simulated effectively. Even in the few existing studies, the results are varying and in many

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cases contradictory. Changes in structure and mechanical properties have been repeatedly reported [11–16].

Polyethylene (PE), in its two basic forms (Low-Density Polyethylene—LDPE and High-Density Polyethylene—HDPE), is one of the most commonly used type of synthetic polymer and can cover a number of needs, including carrier bags, which are the most ubiquitous pollutant of the European marine environment [17, 18]. PE accounts for almost 80% of microplastics, having undergone weathering in the marine environment [19, 20]. It is, therefore, crucial to develop a successful method of degradation. PE degradation by marine microorganisms has yielded encouraging results, with reported weight losses up to 7.5% for LDPE [21] and up to 24% for LDPE with pro-oxidants [22], emphasizing the need for more research.

Recognizing the research gap, the present work studies the potential for biodegradation of two indigenous marine communities on microplastics produced by the combined effect of artificial ultraviolet (UV) radiation and mild mechanical stress on thin HDPE films. Weight loss measurements were used for the determination of polymer degradation, while the colonization process was monitored through EPS (protein and carbohydrates) measurements, viable cell concentration, and abundance of *alkB* gene.

## 2 Experimental

### 2.1 Materials

HDPE films with dimensions 10 cm × 1 cm and a thickness of 0.1 mm were exposed to artificial UV radiation in a closed sand system, until the point when slight mechanical stress would cause fragmentation. The temperature of the system and the amount of radiation were monitored using two Onset HOBO Temperature Light 3.500 DP Loggers.

### 2.2 Methods

#### 2.2.1 Preparation of Samples

Sufficient fragmentation was presented in HDPE films after 4 months of irradiation, while complete fragmentation was achieved by the application of mild mechanical stress [16]. The plastic fragments went through a size exclusion process, employing two sieves with opening sizes of 2 mm and 250 μm. Fragments outside the diameter area between 2 mm and 250 μm were collected and stored separately, while those of the desired size were further weighed and separated in 50 ± 2 mg groups, sprayed with 70% ethanol for sterilization and left to dry in an incubator at 37 °C for 3 days.

### 2.2.2 Consortia Development

An acclimated consortium capable of degrading PE films developed in our laboratory (*Agios* consortium) and indigenous marine community from the area of Souda bay in Chania (*Souda* consortium) were exploited for the degradation experiment. A 200  $\mu\text{m}$  filter was used in filtering the seawater collected from Souda bay and 150 mL of this filtrate was incubated with approximately 1 g PE for 7 days at 25 °C, for acclimatization. Subsequently, each consortium was cultured until the late lag phase in rich medium.

### 2.2.3 Experimental Setup

The biodegradation experiment was conducted in triplicates. In each microcosm, 50 ml sterile filtered seawater, 50 mg sterile PE fragments and the consortia (starting concentration:  $10^5$  cells  $\text{mL}^{-1}$ ) were added. Batch reactor containing only 50 mL seawater and 50 mg HDPE was used as the abiotic control, for monitoring the extent of abiotic degradation. Sampling occurred at the end of each month by permanently removing three batch reactors per treatment. The experiment was conducted in 25 °C in darkness, under continuous shaking at 120 rpm and lasted for 4 months.

### 2.2.4 Weight Loss Measurements

Pieces of 250  $\mu\text{m}$  plankton mesh were dried at 37 °C for 5 days, weighted and subsequently used for the removal of the plastic fragments. Washing with 2% Sodium Dodecyl Sulfate (SDS) ensured the removal of biofilm on the surface of microplastics. The collected fragments were dried at 37 °C for 5 days and weighted, using a 5-decimal-place scale. The difference between the final elevated and the initial mesh weight was considered to be the weight of the plastics contained in each flask. This weight was then subtracted from the initial weight of the plastics giving an estimation of the biodegradation.

### 2.2.5 Viable Cell Concentration, Extracellular Polymeric Substances Measurements

The filtrate was serially diluted and spread on rich medium plates in order to estimate the cell concentration, along with protein and carbohydrates measurements. Concentration of proteins and carbohydrates was monitored using the Lowry and anthrone protocol, respectively. The remaining liquid was collected and stored at -20 °C for further analysis.



### 2.2.6 DNA Extraction and QPCR

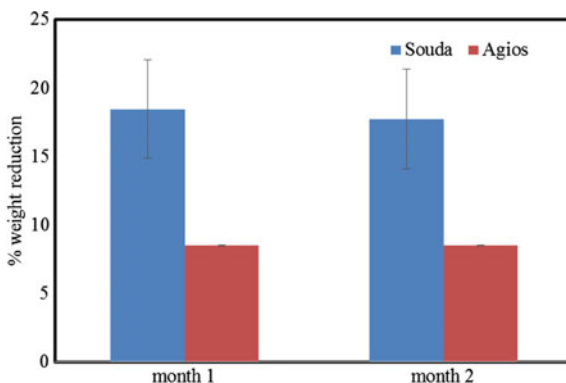
DNA was isolated from the filtrate and extracted according to the Hexadecyltrimethylammonium bromide (CTAB) protocol for bacterial genomic DNA. The abundance of *alkB* gene was monitored via real-time PCR using the primer pair *alkB*-f [5'- AAYACIGCICAYGARCTIGGICAYAA -3'] and *alkB*-r [5'- GCRTGRTGRTCIGARTGICGYTG -3'] [23] and conditions as previously described in [24]. The amplification efficiency and coefficient ( $R^2$ ) was 105% and 0.98, respectively, while the detection limit was set to  $10^2$  gene copies  $\text{mL}^{-1}$ .

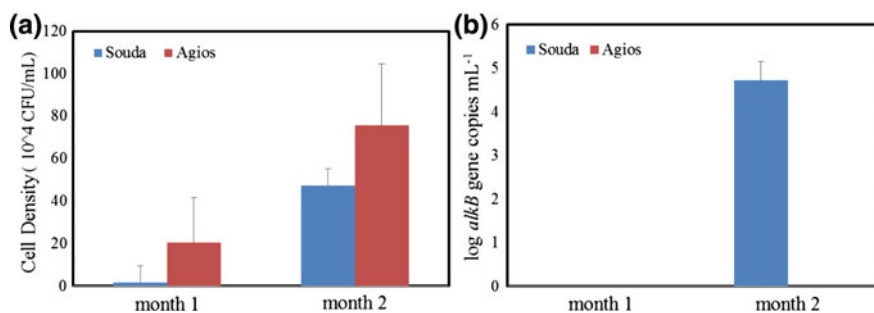
## 3 Results and Discussion

There is scarce information about the fraction of floating microplastics on the sea surface [6, 25]. Moreover, a decrease in the abundance of fragments with size less than 2 mm has been realized [25, 26] indicating the contribution of biotic, abiotic factors, or combination of them to microplastics removal from ocean's surface. To better understand the role of biotic mechanisms, a batch experiment was conducted, investigating the ability of two marine consortia to degrade secondary HDPE microplastics under simulated marine conditions.

According to the preliminary results, it seems that Souda community could reduce more efficiently the weight of secondary microplastics since it decreased approximately by 18% the initial PE weight at the end of the second month (Fig. 1) while Agios community managed to decrease by 8% the PE mass at the same sampling interval. Interestingly, the two populations achieved high weight reduction after being incubated for one month with the microplastics and no further weight decreased was observed. In general, in situ biodegradation experiments have focused on mesoplastics with promising results [21, 22]. For example, two marine bacteria have been found able to decrease by 9% the weight of thermally treated

**Fig. 1** Weight reduction in two different treatments



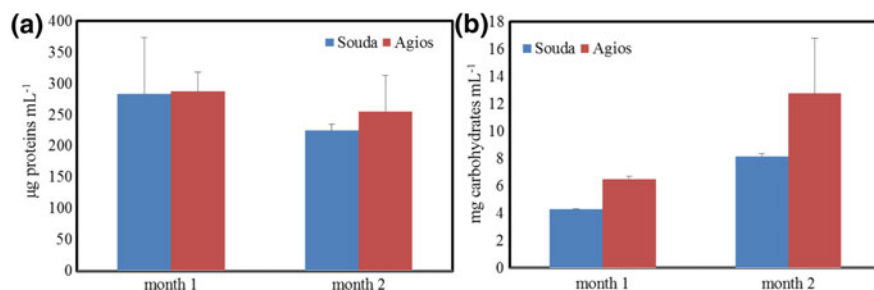


**Fig. 2** Abundances of **a** cells and **b** *alkB* gene in both treatments

HDPE films [27]. Higher HDPE removal percentages (60%) have been exhibited under thermophilic conditions [28].

Monitoring of the viable cell populations suggests that the microorganisms could grow under oligotrophic conditions where the secondary HDPE microplastics are the only available carbon source (Fig. 2a). Indeed, the cell abundance of both communities increased along time while Agios population exhibited higher abundances ( $8 \times 10^5$  cells  $\text{mL}^{-1}$ ) in comparison to Souda ( $5 \times 10^5$  cells  $\text{mL}^{-1}$ ) at the end of month 2. Considering the starting initial concentration ( $10^5$  cells  $\text{mL}^{-1}$ ), no decline was noticed implying that the marine populations could not only survive but also proliferate at these conditions. The abundances of *alkB* gene were quantified within marine communities since it has been characterized as a key gene in polyethylene degradation pathway [21]. As seen in Fig. 2b, this gene was not detected in Agios assemblages while it exhibits high concentration in Souda population. Interestingly, we can suggest that *alkB* harboring bacteria dominate Souda community since  $10^4$  gene copies  $\text{mL}^{-1}$  were enumerated while the total population is estimated to reach  $5 \times 10^5$  cells  $\text{mL}^{-1}$ . Moreover, these results comply with the high weight reduction observed in this treatment.

The carbohydrate and protein content (EPS) were estimated throughout the experimental period (Fig. 3). Concerning carbohydrates, the concentration increased from month 1 to month 2 at both treatments, while the protein content



**Fig. 3** Protein (a) and carbohydrate (b) content at both treatments

marginally decreased during this period. Polyethylene serves as a carbon source and as a substrate to microorganisms, but it is prerequisite for microorganisms to overcome the surface hydrophobicity [29]. EPS in microbial networks contain several binding sites for metals and organic pollutants while carbohydrates contain hydrophobic regions [30], hence increase in carbohydrate content suggests increase in microbial ability to adhere to microplastics. Once submerged to ocean, microplastics are subjected to fouling [31] and plastic-associated microorganisms form diverse communities, distinct from the surrounding pelagic microbiota [32]. Adherence to plastic surfaces and biofilm development is considered the first step in polyethylene degradation process [29]. Although PE degradation pathway has not been described yet, molecular weight reduction and oxidation of the polymer are the key reactions for this process [21].

## 4 Conclusions

Two indigenous marine communities were investigated in terms of degrading HDPE secondary microplastics. Souda community seemed more efficient in decreasing the HDPE weight in comparison to Agios community. The protein content marginally decreased at month 2 while the carbohydrate content increased in both treatments at the same time interval. Interestingly, cell abundances increased along time but only the abundances of *alkB* gene in Souda community increased in accordance with the high recorded weight reduction.

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# Assessing Marine Biodegradability of Plastic—Towards an Environmentally Relevant International Standard Test Scheme

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## 1 Introduction

In the process of becoming independent from fossil hydrocarbon resources bio-based plastic is one option, being also favoured by programmes of the European Commission [1]. To achieve maximum sustainability bio-based polymers that are also biodegradable are entering the market, with nationally differing regulation. It is wise to assess the risk of these new materials especially in fields where their use is intrinsically linked to a loss to the environment (e.g. by conversion to microplastics through wear), or where unintentional littering is probable. Thus, standard tests (e.g. ISO) are needed to validate the claim of a material being “biodegradable” for consumer safety and environmental impact. Here, we give an overview of current

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marine standard tests and present results from the EU-funded project Open-Bio on the development of tests of biodegradability and disintegration under marine conditions.

## 2 Experimental

We present feasible field test systems for three coastal scenarios: plastic being buried in intertidal beach sand (eulittoral test), plastic floating in the shallow water column (pelagic test) and plastic sunken to the sandy sublittoral (benthic test). The field tests were optimised to a state that the test material could be observed in situ over a time of up to 2 years without loss of larger fragments, and marine disintegration could be followed for common biodegradable polymers [2]. Degradation rates for the tested polymers are given. A set of mesocosm experiments simulating the same three habitats supported the field research in a semi-controlled setting of environmental conditions [3] and allowed to critically evaluate field and laboratory test results [4], leading to an environmentally relevant test scheme. Our outlook shows the next steps of test development needed to provide a comprehensive toolset to cover the majority of conditions in which plastic is found in the marine realm.

## 3 Discussion

Degradation is an irreversible multi-stage process which changes the polymer structure and subsequently causes a loss of properties as defined by the American Society for Testing and Materials [5]. Multiple factors may influence this process including temperature, solar radiation, mechanical forces, as well as enzyme activity and other biotic interactions [6, 7]. The degradation of an item usually leads to disintegration, i.e. the breaking-up into smaller fragments. Biodegradation is caused by organismic activity [5] leading to a complete transformation of all components into CO<sub>2</sub> or CH<sub>4</sub>, water and biomass so all elements can re-enter the ecological cycle. A variety of terms are used for different types of alternative plastic products that are descriptive for certain properties but sometimes also misleading. Therefore, we focus here on three main terms: biodegradable, bio-based, and conventional plastic. Biodegradable plastics are degradable to complete remineralisation as mentioned above, and can be fossil as well as bio-based. Bio-based plastic products consist of biogenic base materials such as starch or vegetable oils. They can be, but are not necessarily, biodegradable. As conventional plastic, we consider all synthetic products which are thought not or to be biodegradable and disintegrate mainly at very low rates.

Currently six test methods for the biodegradation in the marine environment are available: one from OECD (Organisation for Economic Co-operation and Development) [8], two from ISO (International Organisation for Standardisation)

[9, 10] and two from ASTM (American Society for Testing and Materials) [11, 12]. All of the limited number of biodegradation tests available are dedicated to the biodegradation under aerobic conditions. Another ASTM standard only addresses disintegration and is not suited to measure biodegradation directly [13]. The only standard specification that addresses disintegration, biodegradation and environmental impacts under the marine conditions of aerobic water or anaerobic sediment (ASTM D7081 [14] in combination with ASTM D6691 [11]) has been withdrawn and is currently under revision. In early 2015, the Belgian private agency Vinçotte introduced the certification scheme for the “OK biodegradation MARINE label” based on the narrow criteria of ASTM D7081.

The development of alternatives, such as the substitution of classical polymers by biodegradable plastics is increasing. However, biodegradability is still difficult to predict in the marine environment. The ability to biodegrade can vary a lot and depends on the quality of the item and on the environmental conditions of the ecosystem of interest. Since bio-based polymers are not biodegradable per se biodegradability needs to be tested for each product. A review carried out within Open-Bio gave the baseline for the practical work for how to deal with biodegradability issues of polymers under different marine environmental settings and so on the design of the test development [2]. The aim was to develop new tests and to adapt existing tests using a three-scale approach. The practical strategy adopted in the project was to simulate the biodegradation under marine conditions at three levels: laboratory, mesocosm and field.

Generally, the laboratory tests should mimic optimal conditions for biodegradation and disintegration, and thus conditions in the field may deviate from the laboratory. Complementary field and mesocosm tests provide the basis to assess and evaluate such deviations. The comparison of the results obtained in mesocosm and field tests and the results of tests carried out in the laboratory helped to come up with recommendations for setting up the standardised test set for these three marine habitats as well as scales.

In Open-Bio tests that represented a selected set of marine conditions: warm seawater with high oxygen and low nutrient levels were developed and conducted. Tests were developed for the biodegradation in the sandy eulittoral (intertidal), in the free water and at the water-seafloor interface of the sandy sublittoral zone. Novelty tests were in the intertidal sediment and at the water-sediment interface, also with high oxygen and low nutrients and organics in a temperature range of 20–25 °C. This was an extension beyond the OECD and ASTM standards and new tests were developed.

## 4 Conclusions

So far, biodegradation tests for polymers in the marine environment are very specific and standardised only to a little extent. Most of the guidelines have not been designed for the biodegradation of solid polymers, and thus have to be adapted



or developed anew. In order to better understand the great variation within the entire marine ecosystem, a set of marine habitats needs to be characterised according to their physical, chemical and biotic properties to obtain a baseline for conditions as natural as possible to be applied in standardised tests. In nature there are several more sets of conditions that are important: some coastal areas have increased nutrient and organic concentrations and high microbial activity. Along heavily urbanised coasts plastic debris is ubiquitous in shallow water ecosystems. Many marine areas are very low in oxygen (hypoxic) or free from oxygen (anoxic), vast regions are covered with very fine sediment (mud) and are cold. These sediments are mostly low in organics and therefore low in microbial activity. 70–94% of plastic waste sinks to the seafloor and the biggest sink for microplastic is the deep-sea sediment.

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# Marine Fate of Biodegradable Plastic— Substitution Potential and Ecological Impacts

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## 1 Introduction

Biodegradable plastic is gaining attention, also through market regulation by a growing number of countries. The substitution of conventional plastic by these new materials is discussed as one mitigation measure to the ever-growing global problem of marine plastic litter. Based on available market data of plastic production, littering risk estimations [1], meta-analyses of the marine litter in the global oceans [2] and field observations, we present mathematical and conceptual models to assess the substitution potential of biodegradable plastics for conventional polymers and evaluate the possible role as a mitigation strategy against marine plastic litter accumulation.

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## 2 Results and Discussion

Modelling showed that the estimated substitution potential on a global scale might be small, with a contribution of only 0.3% to the total marine debris. However, there are huge potentials for substitution on local level, and for single plastic items and applications of one single polymer. As an example, at some beaches up to 80% of the marine plastic debris consisted of 100-mL mono-dose water cups that could be specifically targeted by market regulation and eventual substitution by biodegradable materials. Another example is the composition of floating marine debris, of which 30% is polystyrol foam [2], for which also biodegradable substituents are being developed and become available.

In order to properly assess the risk of these new materials for natural systems, the importance to better understand the ecological impact of biodegradable artificial polymers when entering the marine environment is highlighted. Currently, our still limited knowledge on the fate of conventional plastic and these new materials in the ocean is based on laboratory tests, analysis of materials collected in the field, observational field data, and only a few in-situ studies so far. Functional aspects as the interaction of biofilm and fouling communities with the polymer surface and the metabolism of these biota are largely unknown [3]. Since five years we have been investigating the formation of the microhabitat of marine plastic in consecutive field experiments addressing the short-term (weeks) biofilm formation [4] and the mid-term (up to one year) fouling succession [5] in two environmental coastal settings, a sublittoral sandy seafloor and the water column in the warm-temperate climate of the Mediterranean Sea.

Eich et al. [4] could show that after one month of exposure diatom communities on starch-based biodegradable plastic foil differed significantly from the ones that had formed on conventional low-density polyethylene foil, and also between habitats. Net oxygen production rates of the biofilm were negative on all samples, indicating that the initial biofilm on marine plastic litter consumes oxygen regardless of the plastic type in both pelagic and sublittoral settings. Mechanical tests did not reveal polymer degradation within one month of exposure but SEM imaging displayed potential signs of degradation on the film surface, depending on polymer type.

Pauli et al. [5] found that the biomass of the fouling layer on a starch-based biodegradable polymer blend and conventional polyethylene (PE) film increased significantly over time and within one year of exposure each sample had become heavy enough to sink to the seafloor. The fouling communities after one year of exposure, consisting of 21 higher taxa, were distinct between habitats, but not between polymer types. In contrast to the benthic habitat, positive primary production was measured in the pelagic habitat, suggesting that large accumulations of floating plastic could be a local source of oxygen for some ecosystems, as well as a carbon sink. Contrary to PE, the biodegradable plastic showed a significant loss of tensile strength and disintegrated over time in both habitats. These data indicate that in the marine environment biodegradable polymers might disintegrate at higher

rates than conventional polymers. This should be considered for the development of new materials, environmental risk assessment and waste management strategies.

We also report the preliminary results of comparative in-situ experiments currently in progress in the tropical ocean of NE Sulawesi, Indonesia. Data of biofilm and fouling development and composition, metabolic rates and degradation rates of common biodegradable polymers and blends serve as a baseline for further studies towards a comprehensive understanding of the ecological impact of plastic in the marine environment.

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# Biodegradable Poly(Butylene Succinate)-Based Composites for Food Packaging

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## 1 Introduction

Active packaging refers to packaging systems with active functions beyond the inert passive containment and protection of the product. It is commonly used with foods, as it helps extend shelf life, improve safety or sensory properties and maintain food quality [1]. Most diffused active packaging systems include the use of oxygen scavengers, carbon dioxide or ethylene emitters and scavengers, ethanol releaser, as well as antimicrobial and antioxidant agents. The active agent may be placed in the package with the food, within a small sachet or pad of permeable material, able to release volatile antimicrobial agents without allowing a direct contact with the food product.

Incorporation of volatile active agents into polymers is a technological challenge, due to the need to avoid evaporation of the additive during melt processing of the polymer. Such challenge can be solved by incorporation of the volatile molecules within cyclodextrins (CD), as detailed in this contribution.

Cyclodextrins are biocompatible, biodegradable cyclic oligosaccharides produced from a renewable natural material, starch [2]. Cyclodextrins can form inclusion complexes with various non-polar, aliphatic and aromatic compounds,

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including antibacterial molecules, whose release could enhance the quality of the packaged product and extend its shelf life [3]. In the research detailed below,  $\beta$ -CD was used as cage to include D-limonene (4-isopropenyl-1-methylcyclohexene), the major constituent in several citrus-derived essential oils, which is also a natural antimicrobial agent [4–6]. D-limonene is listed in the Code of Federal Regulations and generally recognized as safe flavoring agent and food preservative [7]. Its outstanding antimicrobial activities have already been tested with different species of food-related microorganisms, such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella enterica*, *Saccharomyces bayanus* [8, 9]. Unfortunately, D-limonene is susceptible to oxidative degradation, which directly results in its loss of activity [7, 10]. Moreover, similar to other volatile essential oils, limonene aroma could be easily released to the packaged food, thus contaminating it. In order to overcome to these drawbacks, D-limonene was included into CDs, and the inclusion complex CD-Lim thus obtained was dispersed into a biodegradable and bio-based polymer matrix, namely poly(butylene succinate) (PBS). PBS is a biodegradable and bio-based polyester, currently used for food packaging, and its use in this market area is expected to largely increase in the coming years [11].

The aim of this paper is to prepare a novel biodegradable polymeric-based system, as potential bioactive food packaging material, by dispersing CD-Lim inclusion complex into PBS polymeric matrix and to investigate the chemical-physical properties of the obtained blends.

## 2 Experimental Part

D-limonene/ $\beta$ -CD inclusion complex, containing 7% w/w of limonene [12], was melt mixed with PBS pellets in a HAAKE MiniLab extruder equipped with anti-rotating twin-screw at 135 °C for 3 min using screws rotation speed of 40 rpm. Binary composites of PBS/CD-Lim with 100/0, 95/5, 90/10, 80/20 w/w ratio were prepared. Properties of the composites were investigated via:

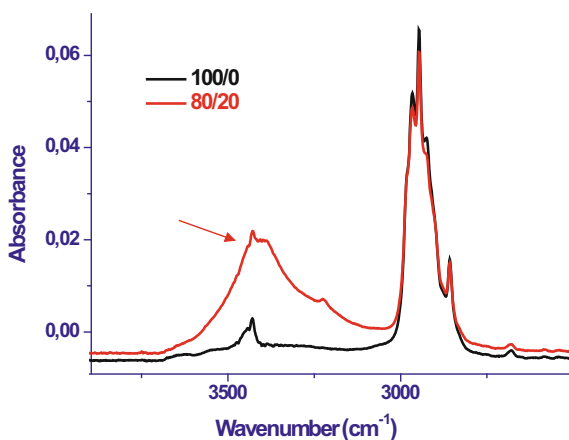
- Fourier Transform Infrared (FTIR-ATR) spectroscopy, using a Perkin-Elmer Spectrum 100 spectrometer, equipped with a Universal ATR diamond crystal sampling accessory;
- Thermogravimetric analysis (TGA), with a Mettler Thermogravimetric Analyzer Mod. TG 50;
- Optical microscopy, using a Zeiss polarizing microscope, equipped with a Linkam TMHS 600 hot stage;
- Differential scanning calorimetry (DSC), with a Q2000 Tzero DSC produced by TA Instrument, equipped with a RCS cooling accessory.

### 3 Results and Discussion

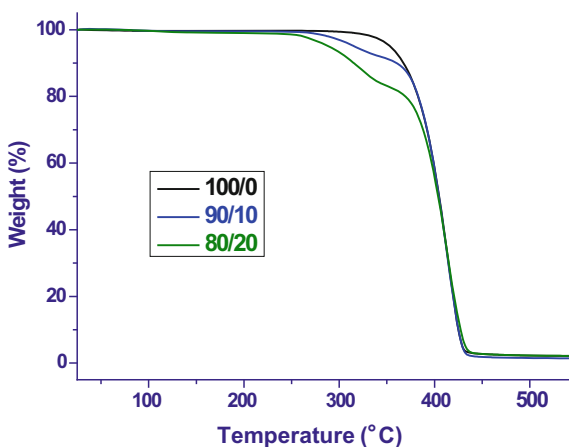
FTIR-ATR spectra of PBS/CD-Lim 100/0 and 80/20 films are presented in Fig. 1. Two main absorption bands are visible, namely  $\text{-OH}$  ( $3600\text{--}3000\text{ cm}^{-1}$ ) and  $\text{-CH-}$  ( $3000\text{--}2800\text{ cm}^{-1}$ ) bonds stretching [13]. Moreover, besides a weak absorption near  $3430\text{ cm}^{-1}$ , assigned to chain-end hydroxyl groups of PBS [14], observed in both samples, in 80/20 composition, stronger and broadened hydroxyl group vibrational absorption can be detected, as highlighted by the red arrow in Fig. 1. This proves inclusion of  $\beta\text{-CD}$  inside the polymeric matrix.

The TGA plots are reported in Fig. 2. PBS thermogram shows no significant weight loss until  $300\text{ }^\circ\text{C}$ , in agreement with literature data [15]. The major degradation step is concluded at  $440\text{ }^\circ\text{C}$ , when only 2% of the initial sample

**Fig. 1** FTIR-ATR spectra of plain PBS (100/0) and of the PBS/CD-Lim composite containing 20% of CD-Lim (80/20). Adapted from Ref. [12] with permission from Elsevier



**Fig. 2** TGA plots of PBS/CD-Lim composites upon heating at  $20\text{ }^\circ\text{C}/\text{min}$ . Adapted Ref. [12] with permission from Elsevier



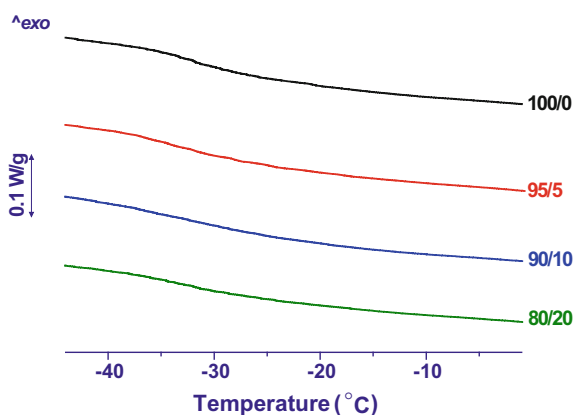


remains. The primary weight loss is caused by volatilization of small molecules, including succinic acid and butylene glycol, which is followed by major thermal degradation of PBS chains, due to random chain scission at the ester bonds, with formation of carboxylic end groups and vinyl groups [16]. In the PBS/CD-Lim composites, thermal degradation starts around about 250 °C, with a smooth weight loss followed by a rapid degradation step. The first weight loss event, from about 250 to 350 °C, is mostly due to degradation of the CD-Lim complex, followed by pyrolysis of PBS.

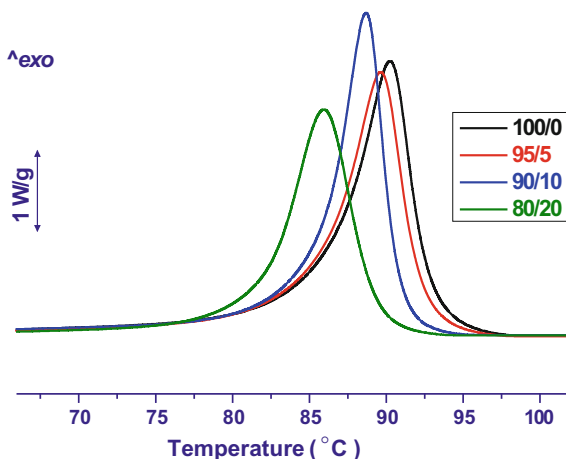
Further characterization of the composites was performed by DSC. Figure 3 illustrates the heat flow rate plots of plain PBS and the composites in the glass transition ( $T_g$ ) range.  $T_g$  of PBS is centered at  $-32$  °C, in agreement with literature data [16, 17]. Addition of CD-Lim does not shift the glass transition temperature, which remains at  $-32 \pm 2$  °C, for all the analyzed composites containing up to 20% of the filler. The invariance of  $T_g$  with filler content confirms the expected lack of miscibility of the components, and that no interaction occurs between PBS and D-limonene molecules, being the latter well trapped within  $\beta$ -CD cavities. In fact, D-limonene is known to be an efficient plasticizer for biodegradable polyesters [18], and the invariance of  $T_g$  with composition confirms that no D-limonene dissolves within the PBS matrix upon melt mixing. Moreover, the data shown in Fig. 3 display a slight broadening of  $T_g$  in the high-temperature side, linked to good interfacial adhesion between the matrix and the dispersed particles, coupled with a restriction of molecular mobility of polymeric segments near the filler surface [19].

The influence of CD-Lim complex on crystallization kinetics of PBS is illustrated in Fig. 4. Addition of CD-Lim delays the onset of crystallization of PBS, and results in a shift to lower temperatures of the whole crystallization exotherm. This demonstrates that the CD-Lim particles disturb chain ordering in the biodegradable polyester, because energy needs to be dissipated to reject, engulf or deform the dispersed particles, which causes the delayed onset and the overall shift of the phase transition to lower temperatures.

**Fig. 3** DSC plots of PBS/CD-Lim composites upon heating at 20 °C/min



**Fig. 4** DSC plots of PBS/CD-Lim composites upon cooling at 20 °C/min



## 4 Conclusions

PBS/CD-Lim composites are potentially exploitable as novel bioactive food packaging materials. The addition of CD-Lim into PBS can preserve D-limonene from evaporation during melt processing of the composites. FTIR and TGA analysis evidenced that D-limonene was efficiently encapsulated inside  $\beta$ -CD cavities, and DSC data revealed that CD-Lim complex delays crystallization of PBS.

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# Degradation of Biodegradable Plastic Buried in Sand

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## 1 Introduction

With continuous growth for more than 50 years, the world production of plastic materials in 2015 was esteemed to be 269 million tonnes. China is the largest producer of plastic materials followed by Europe and North America [1]. Plastic materials, due to their unique properties, are used for a wide range of applications [2]. The growing usage of plastics, mostly in the packaging sector, is causing serious problems concerning their disposal. Many reports highlighted that plastic litter end up in the oceans causing serious environmental problems [3]. According to a recent study, the input of plastic waste from land into the ocean results in 8 million metric tons of plastic every year [4]. Much of the litter reaches the ocean by beach-going activities, being blown into the water, or is carried by creeks, rivers, and storm drains/sewers to ocean areas.

The Mediterranean Sea has been described as one of the areas most affected by marine litter in the world. Some of the largest amounts of Municipal Solid Waste generated annually per person occur in the Mediterranean Sea (208–760 kg/Year, <http://www.atlas.d-waste.com/>). Plastic, which is the main litter component, has now become ubiquitous and may comprise up to 95% of waste accumulated on shorelines, ocean surface, or sea floor [5].

Most of the plastic waste are swirled by currents, accumulating over time in “garbage patches”, the most well known of these is the Great North Pacific Garbage Patch, discovered by Captain Charles Moore [6]. Plastic debris in the ocean has been found to have direct effects on seabirds, mammals, turtles and benthic organisms by ingestion, entanglement, and accumulation of higher density plastic debris on the seafloor, where it can inhibit gas exchange between the overlying

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waters and the pore waters of the sediments, and disrupt or smother inhabitants of the benthos [7].

In addition to large debris, there is growing concern with regards to microplastics, plastic fragments measuring less than 5 mm [3, 8, 9]. Microplastics can be distinguished in primary and secondary depending on whether they were purposely manufactured in microscopic size (primary) like in the cosmetic industry, or whether they have reached such dimension during degradation of larger plastic items (secondary) [10]. The last is the result of a combination of mechanisms, including photo, biological, mechanical and chemical degradation.

In this scenario, several attempts have been focused on the usage of biodegradable plastics representing a possible solution to these problems. Biodegradable plastics include either bio-based plastics synthesized from renewable resources, such as poly(lactic acid) (PLA) and polyhydroxyalkanoates (PHA), or plastics produced from fossil resources, including aliphatic polyesters such as polycaprolactone (PCL) and polybutylene succinate (PBS). Although these polymers are considered environmental friendly materials, they also have some drawbacks, such as their limited degradation in marine environment. In fact, recently, PCL was identified as floating particles in Mediterranean Sea [11].

The purpose of this study was to investigate the degradation of biodegradable polymers such as PLA, PCL, polyhydroxybutyrate (PHB) and polybutylene succinate adipate (PBSA) buried in sand, to verify the behaviour of these polymers in habitat where plastic waste can be stranded when carried by the sea.

## 2 Experimental

### 2.1 Burial Experiment

In order to investigate the degradation of biodegradable polymers in a marine habitat, film specimens of poly lactic acid (PLA), polycaprolactone (PCL), polyhydroxybutyrate (PHB) and polybutylene succinate adipate (PBSA) were buried in sand. For comparison HDPE films were also buried.

### 2.2 Analytical Techniques

The degradation of the selected biodegradable polymers was followed by gravimetric methods, Fourier transformed infrared spectroscopy (FTIR), thermal analyses, and scanning electron microscopy (SEM). Samples were analysed at different days of sand burial. Before the analysis, samples, after removal from the sand, were washed with distilled water and dried at ambient temperature under vacuum for 2 h.

### 3 Results and Discussion

Weight loss versus time due to degradation was evaluated on 3 specimens for each sample. The results are summarized in Fig. 1.

After 71 days of sand burial, PLA and PCL present a very low value of weight loss, about 0.15 and 1.8%, respectively. PHB and PBSA present higher values, around 15 and 6%, respectively.

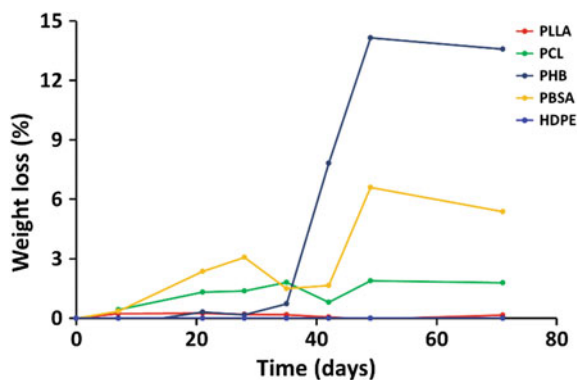
Morphological observation performed by SEM on film surface after 35 days of sand burial indicate that on all samples, except for HDPE, degradation phenomena are detectable (Fig. 2). In particular, HDPE surface is unaffected by the sand burial while starting degradation phenomena were observed on PLA surface. Formation of numerous heterogeneous pinholes, cracks and grooves were observed on PCL, PHB and PBSA surfaces, suggesting the occurrence of degradation.

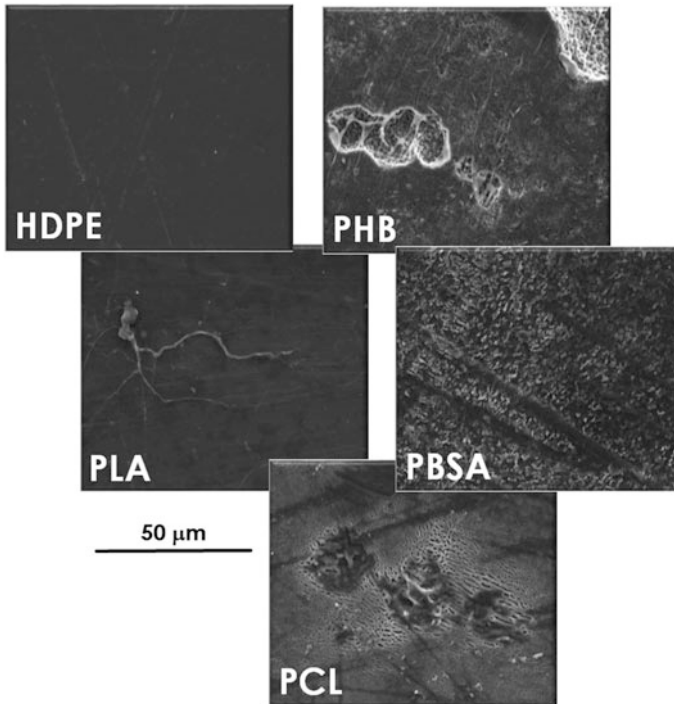
The influence of sand burial on thermal transitions ( $T_g$ ,  $T_c$  and  $T_m$ ) as well as on the thermal stability of the samples was analyzed. For all the samples a shift to higher temperature of the glass transition as well as of the crystallization temperature was observed. No effect was observed on the melting temperature. A reduction in the thermal stability as function of the burial time was detected, thus confirming the occurrence of ageing phenomena.

Chemical changes of film samples during sand burial were monitored by FTIR-ATR spectroscopy.

Polyesters are susceptible to hydrolysis. Preliminary results did not show the evidence of pronounced degradation phenomena, even if a slight broadening of the absorption band assigned to the C=O stretching was observed for PLA.

**Fig. 1** Weight loss versus time of sand burial





**Fig. 2** SEM images of film surface after 35 days of sand burial

## 4 Conclusions

The comparison reported in this work among the effects of sand burial and the degradation of different biodegradable polymers allows to conclude that the analysed biodegradable materials are susceptible to degradation, in the used experimental condition, with different rates. Even if some biodegradable polymers are more susceptible to degradation, all the analyzed samples show initial degradation signals. Further analysis at longer sand burial times will be needed to evaluate the effect of the polymer structure on the degradation kinetics of the selected biodegradable materials in the experimental conditions.

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# Non-conventional Ultrasound-Assisted Extraction of Alginates from *Sargassum* Seaweed: From Coastal Waste to a Novel Polysaccharide Source

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## 1 Introduction

*Sargassum* algae are brown free-floating seaweed found worldwide in temperate and tropical regions and provide shelter and food for many animal species. In recent years, their wide-spread presence has gone out of control, leaving dense clumps of rotting weeds and toxic waste along urban beaches [1]. Nevertheless, this harmful brown seaweed is a valid source of sodium alginate (SA), a well-known biodegradable and biocompatible polysaccharide, widely used in food, pharmaceutical and biomedical applications due to its stabilizing and gelling properties [2]. In this work, new eco-cost-effective extraction methods have been used to obtain alginate from *Sargassum* raw seaweed waste with the aim of producing a polymeric matrix of mulching films for agricultural applications [3, 4]. Generally, these compounds show similar or better yields than those obtained from conventional extraction processes, using less energy, time and solvent volume, thus making them greener processes. In this work, an eco-sustainable, ultrasound (US) technique-based extraction method has been used to obtain SA from *Sargassum* seaweed wastes. The novel material has been subsequently used as a polymeric matrix for agricultural mulching films [5, 6].

US can dramatically affect biomass structure by maximizing mass transfer in liquid suspensions and inducing cell wall rupture [7, 8]. The US-assisted extraction of *Sargassum Algae* has been compared to the conventional protocol, and huge

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reductions of time and volumes were observed. A one-step extraction protocol may cut down on waste and chemical disposal.

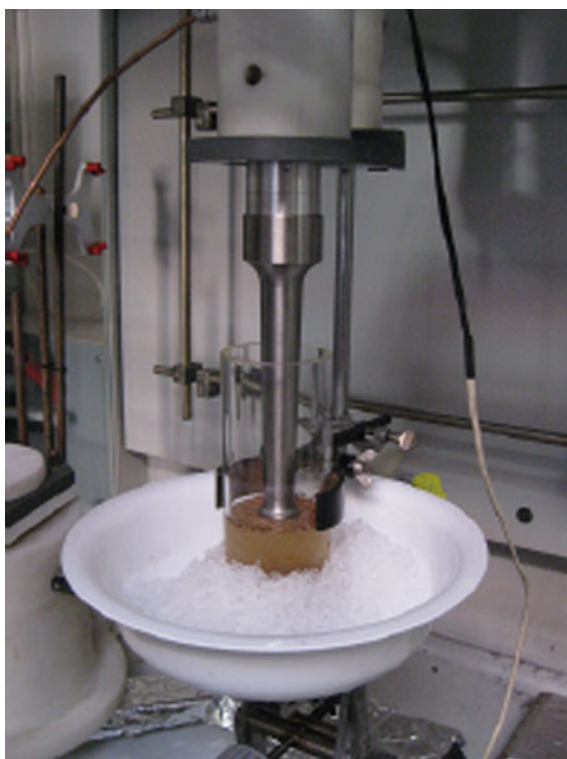
Isolated SA has been structurally and thermally characterized by means of thermogravimetric (TGA), spectroscopic (FTIR-ATR) and morphological (SEM) analyses, and the results have been compared with the commercial product. Finally, a cast film of the raw polymer was obtained.

In conclusion, this work proposes a new and sustainable waste valorisation protocol, starting from a negative-cost biomass and leading to the production of an exploitable bio-derived polymer.

## 2 Experimental Part

Dried and chopped *Sargassum* seaweed particles, recovered from the wastes of beach-dumped raw algae, were used as source to extract SA by means of sustainable methods. Extraction under low-frequency US induces seaweed cell wall disruption, dramatically improving SA yield. A high-power 20.5 kHz US probe with a titanium horn (Danacamerini sas, Turin) was inserted into the extraction media in an ice-cooled Pyrex vessel (Fig. 1).

**Fig. 1** US-assisted extraction equipment with a titanium horn



**Table 1** Experimental set-up SA extraction

Sample	Power (W)	Time (min)	Step 1 (HCl 0.2 N)	Step 2 (Na <sub>2</sub> CO <sub>3</sub> 3% w/v)
Alg1	200	30	Yes	Yes
Alg2	100	60	Yes	Yes
Alg3	200	30	No	Yes

All the extraction parameters are summarized in Table 1. The first two tests (Alg1 and Alg2) implemented the US technology in a conventional acid/basic extraction, [9] dramatically reducing treatment time and temperature. The third test used cavitation to remove the first acid step and directly applies the basic treatment (Alg3).

All the films were investigated by means of:

- Fourier transform infrared (FTIR-ATR) spectroscopy, using a Perkin-Elmer Spectrum 100 spectrometer, equipped with a universal ATR diamond crystal sampling accessory;
- Thermogravimetric analysis (TGA), with a Mettler thermogravimetric analyzer Mod. TG 50;
- Gel permeation chromatography using a GPC Malvern equipped with a Viscotek TDA apparatus with three detectors (refractive index, right angle laser light scattering (90°), low angle laser light scattering (9°) and a viscometer). The refractive index detector has been used to discuss GPC results.

Commercial alginate from Sigma-Aldrich was used as the control.

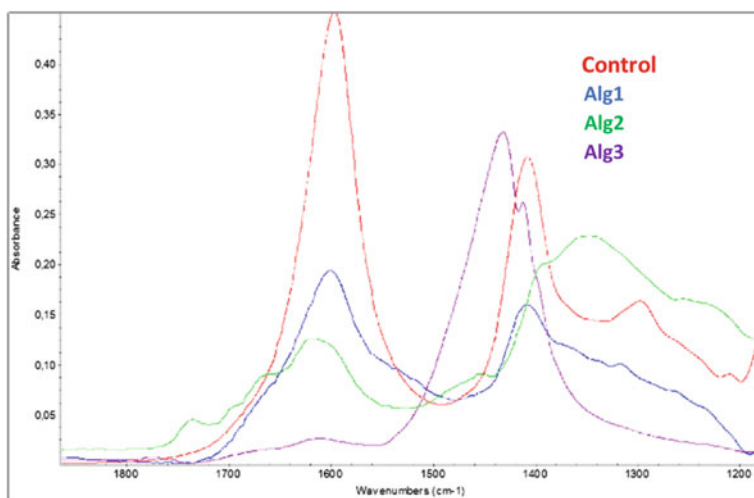
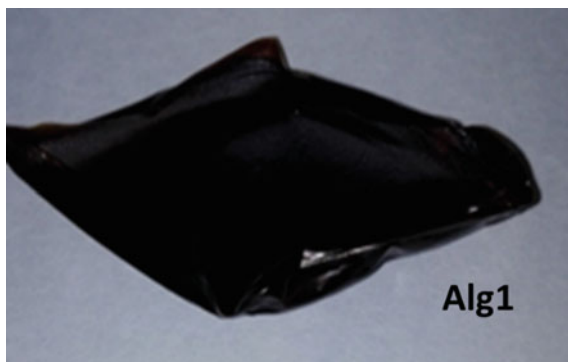
### 3 Results and Discussion

All the products were solubilized in water, casted on a glass surface and allowed to dry at room temperature under a ventilated hood with the aim of evaluating sample filmability. All analysed samples showed film forming properties, proving that SA extraction from the algae matrix is effective. Figure 2 depicts an alginate film; the dark colour is due to co-extracted pigments and polyphenols present in the seaweed cell walls, which are useful for agricultural mulching activity.

The main FTIR-ATR vibrational modes of alginate films fall in the carbonyl region with both asymmetric (around 1600 cm<sup>-1</sup>) and symmetric (around 1400 cm<sup>-1</sup>) COO<sup>-</sup> stretching (see Fig. 3) [10].

Thermogravimetric (TGA) and derivative (DTG) curves of all the samples are reported in Fig. 4a, b. It is possible to observe a different pattern of weight loss in the TGA curves. In particular, the water release from the polymeric matrix at 150 °C is about 12% for Alg2 and Control samples and 8 and 2% for Alg3 and Alg1, respectively. As a matter of fact, it is difficult to assess thermal film behaviour at this

**Fig. 2** SA film extracted under US treatment



**Fig. 3** FTIR-ATR carbonyl region spectra of SA samples

step of polymer extraction. Nevertheless, it can be hypothesized that some protein fractions are included in the polymeric mass, inducing the formation of hydrogen bonds between the polar groups of polysaccharide, protein and water, reducing the water loss percentage. DTG curves suggest that the thermal degradation of all the films occurs around 200 °C, except for Alg1. The degradative profile of this sample is more complex, probably due to the presence of some low molecular weight segments, which explain the early degradation.

The most important result of the thermogravimetric analysis is the presence of the typical degradation temperature of pure alginate in all the samples (see Fig. 4b), thus confirming the presence of the polymer inside the films.

GPC analyses of the samples are detailed in Fig. 5 and Table 2 reported below.

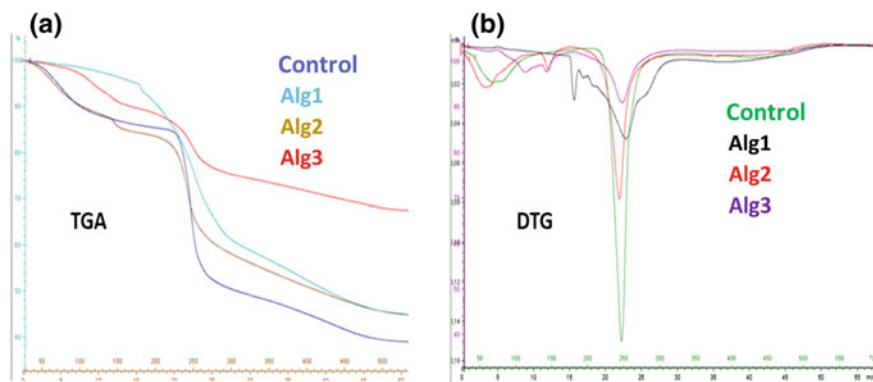


Fig. 4 a TGA and b DTG thermograms of alginate-based films

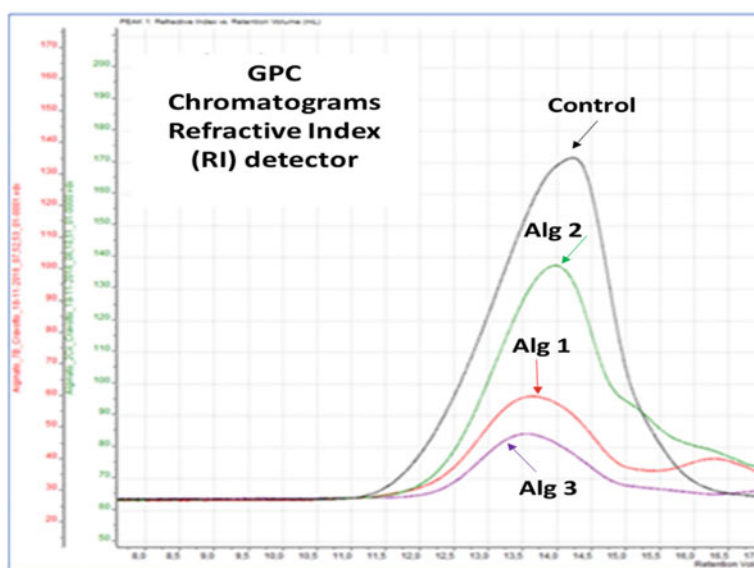


Fig. 5 GPC chromatograms of extracted samples compared with the control

Table 2 GPC data of all the tested samples

Sample	Mn	Mw	DP
Alg1	240,000	438,000	1.8
Alg2	142,000	312,000	2.19
Alg3	141,000	262,000	1.9
Control	27,000	59,000	2.1

The GPC chromatograms display that the highest peak intensity belongs to commercial alginate, as expected because of its purity. Of the extracted samples, Alg2 shows the highest SA concentration; the experimental set-up selected for this sample is responsible for enhanced and more selective SA expression, reducing the degradation of macromolecules. On the other hand, the real concentration of the polymer is very low in the case of Alg1 and Alg2, probably due to the presence of some soluble polyphenols and protein, lowering the relative content of SA.

The analysis of the data reported in Table 2 is promising nonetheless, as the extracted samples generally provide the highest molecular weights, as compared to the control. When application as mulch is the aim, the presence of high molecular weights assures better film performance once applied to the soil as a mulching coating.

## 4 Conclusion

In this work, SA extraction under US was investigated and the film forming ability of a variety of freeze-dried samples was assessed. Structural analyses (GPC and FTIR-ATR) confirm that the US technique may be a valid method with which to obtain sodium alginate in a cost-effective and environmentally friendly manner. Moreover, TGA analyses establish that all SA films obtained are thermally stable.

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# Eco-Sustainable Finishing Treatment of Polyamide Fabrics to Reduce the Release of Microplastics During Washing Processes

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## 1 Introduction

The global production of plastic is estimated to be 300 million tons per year, with a significant quantity that ends up as waste in marine ecosystems [1]. The entity of such major source of marine pollution involves not only the macroscale but also the microdimensions, with the presence of microplastics. Microplastics are defined as plastic fragments smaller than 5 mm, distinct in primary and secondary depending on whether they were purposely manufactured in microscopic size (primary) like in the cosmetic industry, or whether they have reached such dimension during degradation of larger plastic items (secondary) [2].

In the last years, some studies have connected the proportion of synthetic fibres found in marine sediments to that used in clothing [3, 4]. During washing processes, synthetic fabrics are damaged and release microfibrils, which remain in the wastewater reaching in this way marine ecosystems. Different researches have investigated the potential harmful effect of textile microfibrils on the marine fauna and their presence in fish sold for human consumption [5, 6]. A possible mitigation strategy of this environmental problem could be the development of textile finishing treatments able to protect the fabric during washing processes, in order to prevent such source of pollution at its very beginning.

The work here presented proposes this type of approach by using a natural polysaccharide and pectin, as main material for the finishing treatment of polyamide

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fabrics. Pectin is a component of cell walls of plants, with the function of hydrating agent and cementing material for the cellulosic network. Commercial pectin is mostly extracted from fruit waste such as citrus and apple peels, and it is widely used in the food industry as gelling and stabilizing agent [7]. Its chemical structure is mainly composed by D-galacturonic acid monomer, occasionally alternated to L-rhamnose units or other monomers [8]. Such heterogeneous and complex chemical structure, rich in esters, carboxyl and hydroxyl groups, is responsible for the peculiar high reactivity of pectin, making it sensitive to plenty of common reagents. Nevertheless, the greatest disadvantage for the finishing application of pectin is its solubility in water, a problem that could be solved by masking the most polar groups through their conversion into esters.

In the present work, pectin was chemically modified by reaction with glycidyl methacrylate (GMA). Then, the new obtained product pectin-GMA was grafted on the surface of polyamide exploiting the functionalities of GMA. Finally, the finishing treatment was tested in wash trials to assess its action in preventing the shedding of microfibrils from the polyamide fabric.

## 2 Experimental

### 2.1 *Finishing Treatment of Polyamide Fabric*

The procedure developed to functionalize polyamide 6.6 fabric comprised two steps.

In the first step, pectin was dissolved in distilled water and subsequently stirred with GMA for 24 h under nitrogen atmosphere at 50 °C, in order to synthesize pectin-GMA.

Then, the second step focused on the grafting of pectin-GMA on polyamide, by dipping the fabric in the pectin-GMA solution in the presence of sodium persulfate as catalyst.

### 2.2 *Washing Test*

Treated and untreated fabrics underwent laboratory simulations of domestic washing processes by using the “Linitest” apparatus. Wash trials were performed using a commercial detergent in the dosage recommended by the manufacturer. The washing effluents coming from each test were filtered through PVDF filters with a pore size of 5 µm. Then, such filters were observed by a field-emission scanning electron microscope (SEM) for the evaluation of the number of microfibrils released during the washing.

### 2.3 Characterization Techniques

The pectin-GMA-polyamide textiles were characterized by SEM, attenuated total reflectance (ATR) Fourier transform infrared (FT-IR) spectroscopy and solid-state  $^{13}\text{C}$  nuclear magnetic resonance (NMR) spectroscopy.

## 3 Results and Discussion

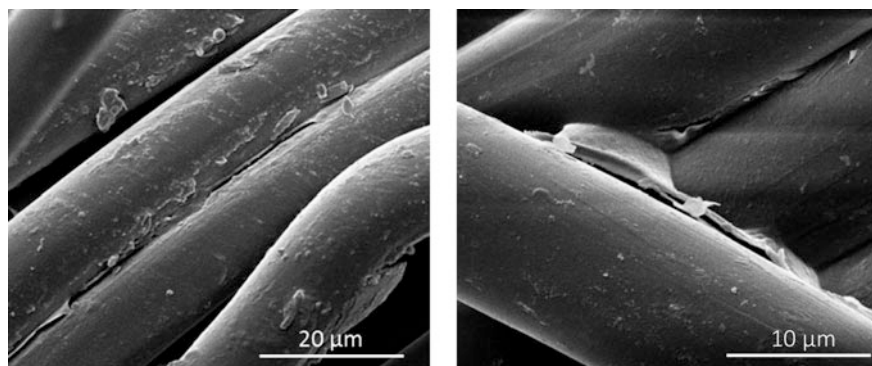
The chemical modification of pectin with GMA was essential to reduce the solubility of pectin in water and to graft the polysaccharide on polyamide 6.6 fabrics. The reaction was in charge of the epoxy ring of GMA whose opening allowed the esterification of carboxyl groups of the pectin [8]. GMA also introduced vinyl groups that were exploited to attach pectin-GMA on the polyamide main chain, through free radical polymerization using sodium persulfate as radical initiator [9].

SEM analysis of the pectin-treated fabrics revealed the presence of a thin film covering contiguous fibres of the textile (Fig. 1). Moreover, ATR FT-IR and solid-state NMR investigations assessed the presence of the pectin-GMA coating on the fabric. In particular, the FT-IR spectrum of the coated fabric showed the presence of absorbance bands attributed to the vibrations of functional groups of pectin-GMA, while the NMR spectrum-recorded signals corresponded to the carbons of the main chain of pectin.

The effectiveness of the finishing treatment in preventing the release of microfibrils was assessed through washing tests performed on coated and uncoated polyamide fabric samples. The results showed an impressive reduction of the number of microplastics released, of more than 80%. SEM and ATR FT-IR analyses of the washed pectin-GMA-coated fabrics confirmed the presence of the coating after the washing process, even if it needs further improvements.

## 4 Conclusions

A natural polymer, pectin, was successfully used as finishing material for an innovative treatment of polyamide fabrics. Different characterization techniques confirmed the presence of a homogeneous coating of pectin-GMA on the surface of the textile. Moreover, the results of the washing tests performed on both neat and treated polyamide showed that the coating was able to reduce shedding of fibres from the main yarn of the fabric. Such developed procedure paves the way to a novel approach in the mitigation of microplastic pollution caused by washing processes of synthetic clothes.



**Fig. 1** SEM micrographs of polyamide treated with pectin-GMA

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# Mitigation of the Impact Caused by Microfibers Released During Washings by Implementing New Chitosan Finishing Treatments

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## 1 Introduction

Until today, many studies [1, 2] were focused on the origin or the formation mechanisms of microplastics. Microplastics comprise a very heterogeneous group of fragments that vary in size, shape, color, specific density, chemical composition, and other characteristics.

Microplastics deriving from textiles washing effluents are expected to be constituted by synthetic fibers, widely produced by textile industry, that are mainly polyester, acrylic, polypropylene, polyethylene, polyamide.

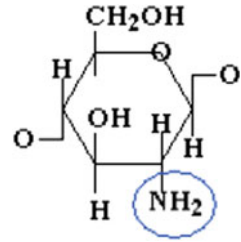
Very few works have been carried out to find possible solutions to this emerging environmental problem. For what concerns microplastics deriving from washing processes of textile based on synthetic fibers, a common approach is to study filtering systems to be applied to laundry machines to block the particles contained in the effluents. Instead, in this work, solutions from the point of view of textile finishing processes were taken into account to reduce the tendency to microplastics release during washing cycles. In particular, a biopolymer, chitosan, already used in textile field as sizing agent or flocculant, that is a sub-product of food industry, was tested as coating to be applied to fabrics with the aim of decreasing the microplastics detachment from the textile surfaces.

Chitosan is obtained from deacetylation of chitin, a structural polysaccharide found in nature as a principal component of exoskeleton of insects and marine invertebrates (such as shrimps). Chitosan shows interesting characteristics such as antibacterial, anti-shrinking, and anti-felting properties. Moreover, it is non-toxic, biocompatible, and completely biodegradable with a chemical structure similar to

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**Fig. 1** Chemical structure of chitosan repeating unit



the cellulose. In Fig. 1, the chemical structure of the chitosan repeating unit is shown.

In nearly all cases, the chemical finish is a solution or emulsion of the active chemical on water. Chemicals that do not have affinity for fibers are applied by means of a variety of continuous processes that involve either immersion of the textile in the solution or the use of mechanical methods. After the application, the fabric must be dried and, if necessary, the finish must be fixed to fiber surface, usually by heating in a curing step.

Chitosan treatments are established processes only for textiles based on natural fibers, especially cotton; the novelty of the study consists also in the treatment of synthetic fabrics; 2-hydroxyethyl methacrylate (HEMA), an acrylic monomer with low environmental impact, was used in the formulations to ensure the permanence of chitosan on textile fabrics. HEMA polymerizes with thermal or UV curing [3, 4], forming poly(hydroxyethylmethacrylate) (PHEMA), generating a dense net in which chitosan is embedded and giving yarn flexibility and softness.

## 2 Experimental

### 2.1 Materials

Chitosan (PM = 150.000 Da), citric acid, HEMA, and APS (ammonium persulfate) were purchased from Sigma-Aldrich (Italy). Standard Fabrics of woven polyester (PET) were purchased from Ausiliari Tessili (Italy).

### 2.2 Methods

Impregnating solutions were prepared as follows: 1, 2, 3% w/v of citric acid aqueous solutions were prepared adding the same w/v percentages of chitosan. The solution was stirred at 50 °C for 4 h. HEMA was added to the solution in the same quantity of chitosan (1, 2, 3% w/v). APS was used as initiator in the same ratio of dry chitosan [2]. The fabrics (20 × 20 cm) were impregnated using a padding

laboratory equipment and squeezed between two rollers with an established pressure (4 bar) and speed (4 m/min). After the deposition of the chitosan-based solution, the fabrics were thermal cured at 150 °C for two minutes in order to ensure HEMA polymerization. Finally, the weight gain was determined by dry weight. Washings were performed according to UNI EN ISO 105-C06, using a nonionic surfactant (Triton, Sigma-Aldrich) and a Linitest laboratory equipment. After washing, water effluents were filtered on polyvinylidene fluoride (PVDF) filters (inserted in a XX4304700 Millipore in-line filter holder) with a porosity of 5 µm by means of a peristaltic pump.

### 2.2.1 Analytical Techniques

The yield of the impregnation process was determined by weight. The presence of the finishing on the surface before and after washing processes was confirmed by a colorimetric method using Red Remazol (Sigma-Aldrich, 1.2% owf (Over the Weight of Fabric), bath ratio 1:50, 60 °C for 30 min). Scanning electron microscopy (SEM) was used to confirm the presence of chitosan finishing before and after washing processes. The PVDF filters were analyzed by SEM, and microplastics fragments released were counted with an internal statistical method.

## 3 Results and Discussion

### 3.1 Weight Increase

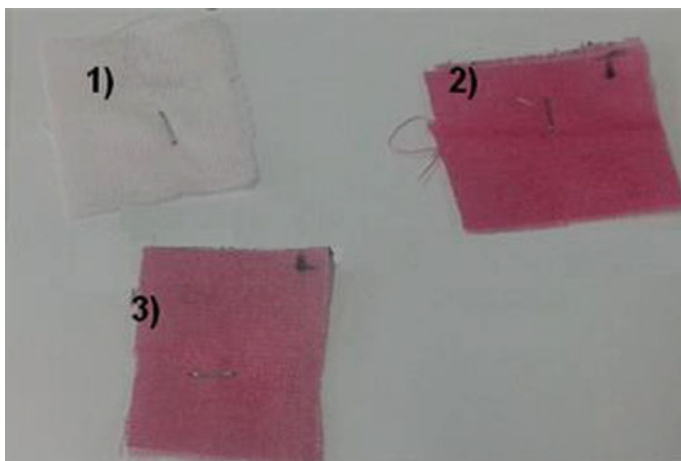
As Table 1 shows, there was an increase of the fabric weight, especially for those treated with 2 and 3% chitosan containing solutions.

### 3.2 Staining Test Before and After Washing

To determine the presence of chitosan on the fabrics surface, it was used a staining test with a reactive dye (Red Remazol). Chitosan has good affinity for this kind of dye because of its chemical composition (similar to cellulose), while polyester and other synthetic fibers are not dyed. If chitosan is present on the surface, it assumes a

**Table 1** Fabrics weight increase after padding and thermal curing

Chitosan % (w/v) in the solution	Fabric weight increase (%)
1	0.33
2	2.58
3	4.06



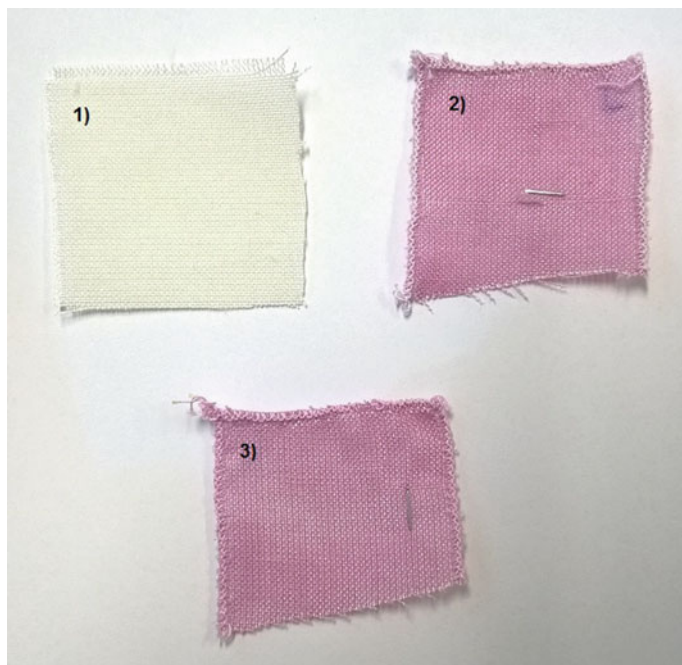
**Fig. 2** Untreated polyester standard fabric (1), finished polyester fabric (3% chitosan solution) (2), finished polyester fabric after washing (3)

pink coloration. The test was repeated also after washing test to determine the resistance of the finishing. The washing test was carried out according to UNI EN ISO 105-C06 that indicates the following conditions: 150 ml bath volume, 10 stainless steel balls, temperature 40 °C, and a time of treatment of 30 min. As detergent, a nonionic surfactant was chosen (Triton).

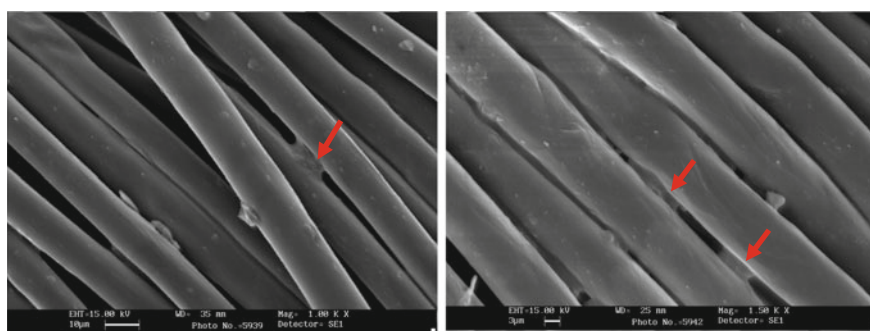
After the staining test (Fig. 2) with Red Remazol, the standard polyester fabric is not affected and remains white, while the treated fabric assumes a dark pink coloration. After washing test, polyester-treated fabric is characterized by a slight decoloration and turns into light pink. This indicates that part of the finishing treatment in excess is removed from the surface, but chitosan is still present on it. The same behavior was observed for 2% chitosan containing solution, while fabric treated with 1% chitosan padding solution (Fig. 3) showed a less significative decoloration process after washing, indicating a better efficiency of the treatment.

### **3.3 SEM Analysis Before and After Washing**

The presence of the finishing treatment was also confirmed by SEM investigations. In Fig. 4, the SEM images of the polyester treated with the chitosan containing solution are shown; the presence of the treatment can be assessed by the formation of bridges between fibers, visible also after the standard washing test.



**Fig. 3** Untreated polyester standard fabric (1), finished polyester fabric (1% chitosan solution) (2), finished polyester fabric after washing (3)



**Fig. 4** Treated polyester fabric before (left) and after (right) washing test

### 3.4 SEM Microplastics Counting

After filtration, the filter was cut into eight circular sections and for the readings, four of them were chosen. Twenty fields at 250X for each section were observed with a total number of eighty fields in the whole filter area. The counting method





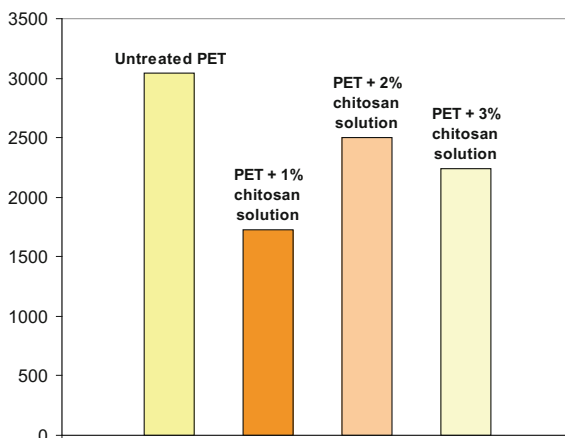
**Fig. 5** Microplastic (on PVDF filter) obtained from filtration of washing effluents

was adapted from an analytical method used for the analysis of asbestos fibers (Italian Law 6-9-1994). The PET microplastics fragments deriving from the textile substrate showed the same morphology of the starting fibers (Fig. 5).

After the microscopic observations, the data were collected and elaborated to provide an estimation of the release of microplastics during washing. At least, two replicates of each sample were analyzed. The average data (Fig. 6) were expressed in terms of number of fibers per gram of fabric tested. As a reference, the release of microplastics from untreated polyester, washed with the detergent, was taken.

Figure 6 shows that all the treated and washed fabrics are characterized by a lower release compared to the untreated PET. The decrease ranges from 18 to 43%.

**Fig. 6** Microplastics counting



The best results were obtained with the padding process using the 1% chitosan containing solution. This confirms the observation done with the staining test: Probably, this percentage of chitosan is sufficient to resist to washing stresses and at the same time lower the microplastics release.

## 4 Conclusions

An innovative textile finishing treatment was tested on PET standard fabrics with the aim of find new solutions to the microplastics environmental problem. The fabrics were impregnated with a padding system with solutions prepared with a 1:1:1 ratio of citric acid, chitosan and HEMA. Then, the monomer was thermally cured using APS as initiator of the polymerization to obtain a dense net in which chitosan is embedded. Microplastics release was determined after standard washing tests using SEM microscopy. The results achieved indicated a good behavior of chitosan treatments to reduce the microplastics release from polyester fabrics. This achievement, obtained at laboratory scale, needs further studies to obtain a scale-up of the treatment at industrial level.

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# MWCNT/Polyaniline Nanocomposites Used for pH Nanosensors of Marine Waters

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## 1 Introduction

One of the biggest environmental problems related to the quality of the marine waters is ocean acidification. Acidification and pH changes can affect an enormous number of chemical reactions and equilibria among organisms in solution, with unpredictable effects on the marine ecosystem [1]. Because of that, during the last decades, many scientific reports have discussed the problems related to the measurement of pH of marine water and the main techniques used to this aim [1, 2].

Besides the traditional methods (optical, electrical, polymer-based) for pH measurements, one of the most promising new techniques is application of nanocomposites as a pH nanosensors. Namely, the use of nanoscaled materials, both organic and inorganic, in the realization of sensing devices has resulted to very interesting improvements in sensor performances [3]. It was found that the application of polymer matrix in the nanocomposites for sensors has offered many advantages over the traditional, inorganic materials. Polymers are cheap, tailorable, easy to process, different from metal oxides exploited for sensing. Especially, electrodeposited polymers were considered good candidates for the realization of pH sensors, providing that the electropolymerization provides a strong bond between the polymer and the electrode surface. The modified electrodes obtained in this way can be assembled into potentiometric pH sensors exploiting the fact that the protonation of heteroatoms (nitrogen, oxygen) contained into the conducting polymer as a function of pH can affect their electric properties. Among various

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organic polymers, Polyaniline (PANI), apart from the interesting electrical properties, has shown excellent optical pH sensing properties and it has been proposed as the most suitable for pH sensing in aqueous media. The use of PANI has received attention due to its high sensitivity, ease of synthesis, and its stability under ambient conditions. Huang [4] studied in detail the effect of pH on conducting PANI and showed that the pH influences the redox processes of PANI in aqueous electrolytes. This principle was subsequently generalized and used to develop a variety of sensors. As a rule of thumb, it was generally observed that conductivity of conjugated polymers decreased in contact with nucleophilic (electron donors) such as methanol and ammonia, while it increases after contact with electrophilic substances like  $\text{NO}_x$  and  $\text{SO}_2$  [5]. Sensors based on PANI film deposited onto ITO glass electrodes employing constant potential can measure pH in the range from 2.15 to 12.54 [6].

The continuous need for higher sensitivity, fast response, flexibility, and cost-effectiveness is the driving forces for the research of new solutions and materials for sensing. In particular, carbon-based nanostructures such as nanotubes (CNTs) are among the most investigated and most promising nanomaterials due to their chemical structure and unique electric, mechanical, chemical properties. Both materials show very high conductivity, high mechanical, and chemical stability, electrocatalytic properties, and high surface area: Those characteristics led to an increasing interest in their use for sensing applications [3, 7]. The properties of carbon nanomaterials arise from their structure, based on a network of carbon atoms in  $\text{sp}^2$  hybridization (like in benzene ring). The presence of defects and the synthetic methods used for fabrication also contribute to define material properties, like the various kinds of surface functionalization that can be realized. The development of functionalization methods aims not only at the tuning of sensing properties but also at the modification of surface chemistry to enhance the interactions of CNTs with polymers in the realization of functional nanocomposites. CNTs show the highest elastic modulus and tensile strength ever recorded and also very high electrical and thermal conductivity, but their properties are strongly dependent on the structure. PANI/CNTs composites can be prepared using direct mixing methods, chemical and electrochemical polymerization [8, 9]. In direct mixing methods, PANI and MWCNTs powders are mixed by mechanical blending, mechanical stirring, or sonication. In the chemical and electrochemical approaches, PANI composites are produced by polymerization of aniline, mostly in acid solution. Chemical polymerization is used when large quantity of polymer is requested, while electropolymerization is a proper way for the obtainment of polymer films [10]. In fact, electrochemical polymerization is an appropriate and flexible method for controlling the thickness and conductivity of the produced PANI or PANI composite film. To improve interfacial interactions between PANI and CNTs, MWCNTs should be previously functionalized in acid medium [11], in which carboxylic acid groups are produced at the defect sites of carbon nanostructures, thus improving the CNTs dispersion. Very interesting results have been obtained by combining COOH functionalized CNTs with a conductive polymer, PANI, produced by in situ polymerization in presence of sulfonic acid [12]. In the framework

of FP7 project COMMON SENSE (OCEAN 2013.2-614155), screen-printed electrodes as a pH nanosensors based on nanocomposites of conductive polymer matrix-PANI and MWCNT were prepared by electrochemical polymerization and tested in wide range of pH.

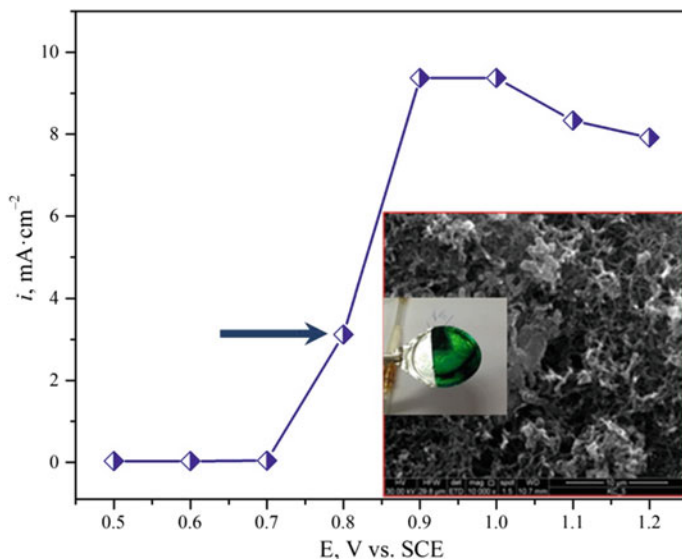
## 2 Experimental

PANI/MWCNTs nanocomposites were obtained by electrochemical polymerization of aniline in the presence of MWCNTs as received from JRC-ISPRA. Electrochemical cell with three electrode system was used. Platinum tiles were used as working and counter electrodes, while saturated calomel electrode (SCE) was used as reference electrode. The electrolyte contained 0.1 M aniline and 0.5 M  $\text{H}_2\text{SO}_4$ . MWCNTs were dispersed into the electrolyte by sonication (30 min) in an ultrasonic bath. Steady-state polarization measurements were performed in order to determine working conditions of electrochemical synthesis of PANI nanocomposites. Electropolymerization was performed at constant potential of working electrode of +0.75 V versus SCE. Different PANI/MWCNTs nanocomposite samples were prepared varying the content of the MWCNTs (1, 2, and 3 wt%) and the time of electropolymerization (40 and 60 min). The produced nanocomposites were doped in 0.1 M HCl for 24 h. Nanosensor was prepared by direct electropolymerization on the gold wires of the screen-printed electrode.

Morphology of the nanocomposites was analyzed in high vacuum mode by means of a scanning electron microscope (SEM)—FEI Quanta 2000 using a secondary electron detector and acceleration voltage of 30 kV. Structural characteristics of the obtained nanocomposites samples were studied by Raman spectroscopy. Non-polarized Raman spectra were recorded by a confocal Raman spectrometer (Laboratory Ram ARAMIS, Horiba Jobin Yvon) operating with a laser excitation source emitting at 532 nm. Thermal stability of the materials was studied by means of thermogravimetric analysis (TGA/DTA). For TGA/DTA tests, a Perkin Elmer Pyris Diamond Thermogravimetric/differential thermal analyzer was used. Using conventional resistivity meter, pH measurements were performed in laboratory as well as field testing in the marine water.

## 3 Results and Discussion

Electropolymerization of PANI or PANI/MWCNTs composite were performed at potential on which partially oxidized emeraldine is produced, i.e., to avoid formation of fully oxidized PANI—pernigraniline. Namely, partially oxidized emeraldine with green color is a desired conductive form of PANI which has variety of application. In order to determine the working potential more exactly, the steady-state polarization measurement was performed. The obtained polarization



**Fig. 1** Steady-state polarization curve of electropolymerization of PANI (electrolyte composition: 0.1 M aniline + 0.5 M H<sub>2</sub>SO<sub>4</sub>)

curve is shown in Fig. 1. Thus, as a working potential for electropolymerization of PANI and PANI/MWCNT composite, 0.75 V versus SCE was selected.

Furthermore, for production of pH nanosensor, direct electropolymerization of PANI/MWCNT nanocomposite on the gold wires of SPE was performed in the same conditions (Fig. 2).

The morphology of PANI/MWCNTs composite was studied by SEM (Fig. 3a, b). Figure 3a shows the morphology of the nanocomposite powder obtained on the working electrode (Pt-tiles). Fibrous and porous structure of PANI can be seen with carbon nanotubes observed on the surface of PANI fibers, in some cases partially embedded in them, thus indicating strong polymer/nanofiller interactions. Figure 3b shows the morphology of the nanocomposite obtained on the surface of the gold wires of SPE. In this case, PANI has formed grain morphology with MWCNTs fibrils embedded, again.

Structural changes in the obtained PANI/MWCNTs nanocomposites were studied by Raman spectroscopy, and the obtained Raman spectra are shown in Fig. 4. Raman spectra of MWCNTs consist of a band at  $\sim 1335\text{ cm}^{-1}$  (D-band), and a complex band whose components are centered at about  $1565\text{ cm}^{-1}$  (G-band) and  $1610\text{ cm}^{-1}$  (D'-band). Characteristic Raman absorption bands of PANI emeraldine salt are centered at  $1330\text{--}1380\text{ cm}^{-1}$ , attributed to protonated structures and about  $1580\text{ cm}^{-1}$ , attributed to C=C and C-C stretching.

As shown in Fig. 4, MWCNTs induced a significant shifting of both these bands, almost irrespectively of the CNTs content. Actually, the Raman spectra of PANI/MWCNTs nanocomposites showed two main complex bands centered at

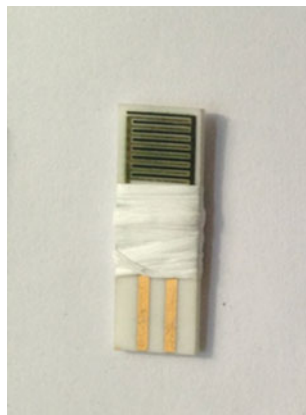


Fig. 2 PANI/MWCNTs nanosensor

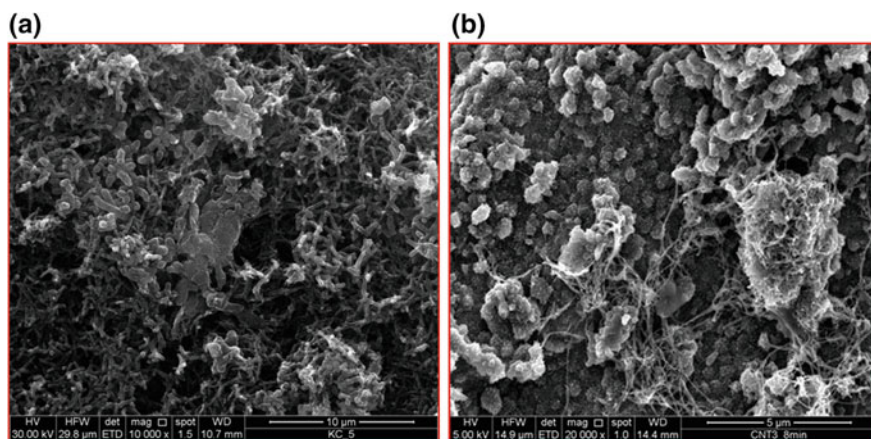
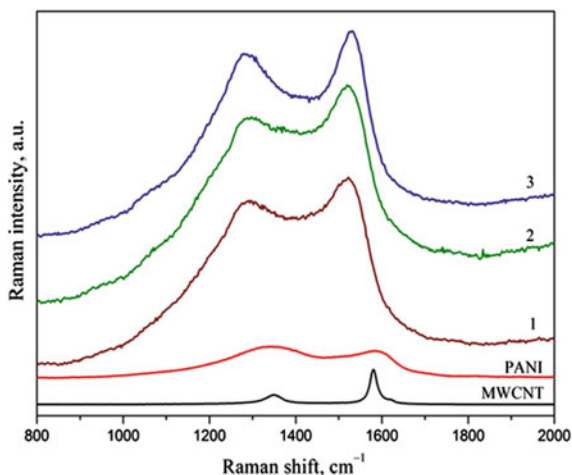


Fig. 3 SEM images of the studied PANI/MWCNTs composites **a** nanopowder on the Pt-tiles and **b** nanocomposite on the surface of SPE gold wire

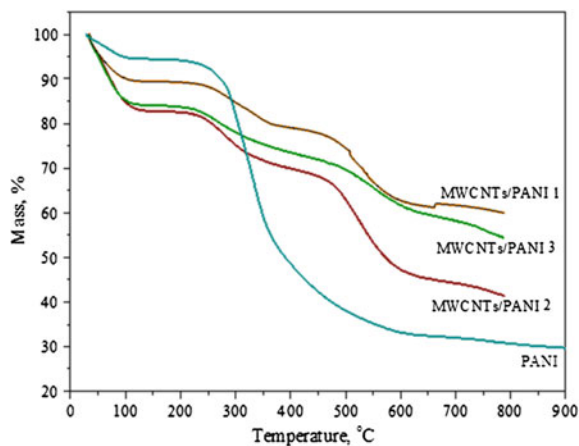
about at  $1290$  and at  $1530\text{ cm}^{-1}$ , and the recorded shifting was indicative of high polymer/nanotubes interactions.

Characteristic TGA curves of PANI/MWCNTs nanocomposites are shown in Fig. 5. The first inflection of the curves for all samples is around  $100\text{ }^{\circ}\text{C}$  and corresponds to moisture evaporation. Further, three characteristic temperatures were detected. The first one ( $T_{\text{onset}}$ ) corresponds to the beginning of the thermal decomposition of the composites and was registered at the point of 2% weight loss of the sample (after complete water removal). The second one ( $T_{\text{d1}}$ ) corresponds to the weight loss of the doped acid. The third temperature ( $T_{\text{d2}}$ ) corresponds to the

**Fig. 4** Raman spectra of PANI/MWCNTs nanocomposites, all deposited for 40 min. (1) PANI/1 wt% MWCNTs, (2) PANI/2 wt% MWCNTs, (3) PANI/3 wt% MWCNTs



**Fig. 5** TGA curves of PANI/MWCNTs nanocomposites, all deposited for 40 min. (1) PANI/1 wt%MWCNTs, (2) PANI/2 wt% MWCNTs, (3) PANI/3 wt% MWCNTs



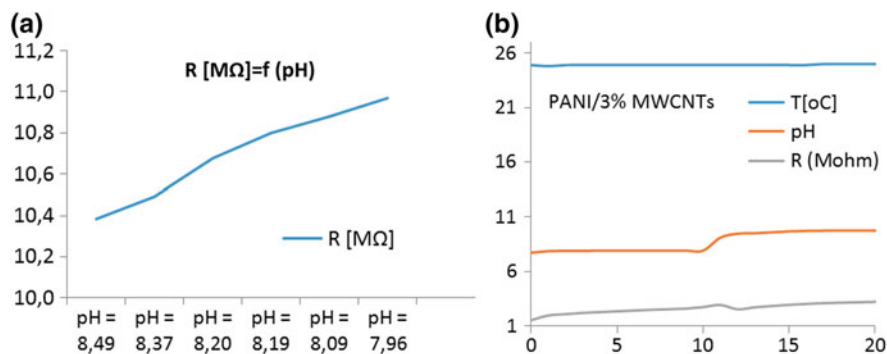
**Table 1** Characteristic TGA temperatures for different PANI/MWCNTs composites

Sample	$T_{\text{onset}}$ , °C	$T_{\text{d1}}$ , °C	$T_{\text{d2}}$ , °C
PANI/1 wt%, MWCNTs	264	332.9	507.7
PANI/2 wt%, MWCNTs	252	273.3	528.1
PANI/3 wt%, MWCNTs	251	278.5	527.3

polymer backbone degradation of the composites and indicates their thermal stability. The registered temperatures are summarized in Table 1.

It can be seen that the composite with 1 wt% MWCNTs starts to degrade at higher temperature (264 °C), while the degradation of the composites with 2 and 3 wt% starts at almost the same temperature. Variations in the  $T_{\text{d1}}$  could be attributed to the different dopant interactions with the material. Increasing the carbon





**Fig. 6** Resistivity changes of PANI/MWCNTs SPE nanosensor. **a** Validation curve in closed pH range; **b** field testing curve

nano-content,  $T_{d1}$  decreased due to the smaller interactions among the dopant and the polymer matrix. According to the values of  $T_{d2}$  as indicator of thermal stability, it can be concluded that the first composite with 1 wt% MWCNTs is the least stable (507.7 °C).

The pH sensitivity of the obtained PANI/MWCNTs SPE nanosensor was tested in laboratory (in different aqueous buffers at various pH) as well as the field testing in the marine water of Napoli, Oristano, and Barcelona bays. Resistivity (i.e., conductivity) changes were measured at different simulated pH as well as in different marine regions. The obtained curves have shown that the PANI/MWCNTs nanocomposite exhibited high resistivity changes, and characteristic curves are shown in Fig. 6. Parallel with the resistivity changes, the temperature and pH were followed with conventional temperature and pH meters.

The effect of pH variations on the electrical conductivity of PANI/MWCNTs nanocomposites was explained on the basis of different degree of protonation of the imine nitrogen atoms of the polymer chain in presence of nanofillers. Further, research activities are in progress in order to optimize the composition and the response of the SPE nanocomposite electrodes.

## 4 Conclusions

An attempt was made to produce pH sensor by direct electropolymerization of PANI/MWCNTs nanocomposites on the gold wires of SPE. Characterization of the obtained nanocomposites was done by SEM, Raman, and TGA/DTA. Electrical resistivity of the SPE nanocomposites was evaluated as a function of the pH of aqueous buffer and field testing in marine waters.

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# Removal of Heavy Metal Ions from Wastewater Using Bio- and Nanosorbents

Anita Grozdanov, Katerina Atkovska, Kiril Lisickov, Gordana Ruseska and Aleksandar T. Dimitrov

## 1 Introduction

Clean drinking water is essential to human health, and also so-called technical water is critical feedstock in a variety of key industries including electronics, pharmaceuticals, and food processing. Taking into considerations that available supplies of freshwaters are limited (due to the population growth, extended deficiency, stringent health regulations, and competing demands from a variety of consumers), the world is facing with challenges to satisfy demands on high water quality standards and quantities (volumes).

According to NEWT (USA), the Nanotechnology-Enabled Water Treatment Centre, 800 million people worldwide lack access to safe water. Global market for drinking water is  $\sim$  \$700 billion, as well as larger market for industrial wastewater reuse. The portion of total run-off which constitutes stable run-off flow is considered as the freshwater resource upon which humans depend. This stable freshwater flow has been estimated at 12,500–15,000 km<sup>3</sup> per year, from which 4000 km<sup>3</sup> per year is considered to be the total freshwater for irrigation, industry, and domestic purposes, and which is estimated to increase to a range of 4300–5000 km<sup>3</sup> per year in 2025. Alternatively, only accessible freshwater is 0.5% of the world 1.4 billion km<sup>3</sup> of water which is furthermore poorly distributed across the globe. Therefore, the current water treatment systems need to improve kinetics, efficiency, capacity, and cost.

The traditional materials and treatment technologies such as activated carbon, oxidation, activated sludge, nanofiltration (NF), and reverse osmosis (RO) membranes are not effective to treat complex and complicated polluted waters comprising pharmaceuticals, personal care products, surfactants, various industrial additives,

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and numerous chemicals reported. The removal of the toxic metal ions from water is very difficult task due to the high cost of treatment methods. Some of the most widely used methods for heavy metal removal are adsorption, ion exchange, solvent extraction, electrolysis, chemical precipitation, membrane processes [1, 2]. These processes have significant disadvantages, such as very high energy requirements, small effectivity of heavy metal ions removal in a large water amounts, production of toxic sludge. Among all of them, adsorption is the most utilized technique in wastewater purification. There are a wide list of materials that can be used as an adsorbent for heavy metals elimination from wastewaters [3–5].

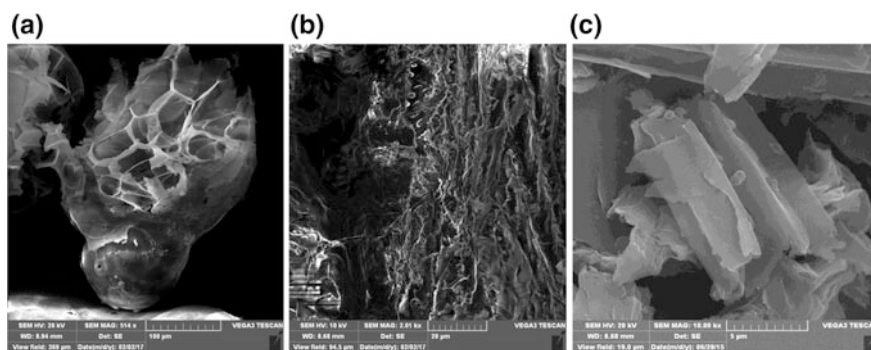
## 2 Experimental

The adsorption abilities of natural and nanosorbents, particularly of natural peanut husks, expanded perlite and graphene, to remove Ni(II), Pb(II), and Fe(II) ions from water systems, were investigated.

The influence of the pH (4–8) of the solution, the amount of adsorbent (0,5–5,5 g/l), the initial metal ion concentration (0,5–2,0 mg/l), and the contact time (5–180 min.) on the efficiency of removal of metal ions, was investigated. Thus, the optimal conditions for achieving maximal effectiveness for heavy metals removal were determined. Characterization of the sorbents was performed utilizing the following techniques: SEM, TGA, Raman spectroscopy.

## 3 Results and Discussion

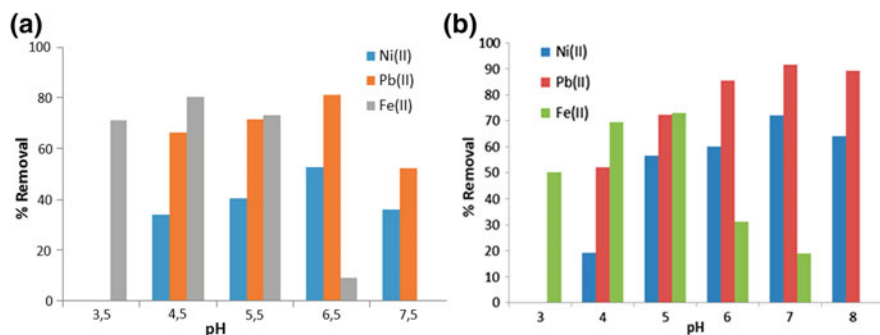
Surface morphology is very important qualitative parameter for absorption ability of the sorbent. Characteristic images of the morphology of the studied sorbents are shown in Fig. 1. Typical porous structure was found for the expanded perlite and



**Fig. 1** SEM images of studied sorbents: **a** perlite, **b** peanuts **c** graphene

**Table 1** TGA data of studied natural and nanosorbent

Sorbent	$T_{d1}$ [°C]	$T_{d2}$ [°C]	$T_{d3}$ [°C]
Expanded perlite	143.9	/	/
Peanut husks	66.5	261.3	417.1
Graphene	150.5	295.9	680.0

**Fig. 2** **a** Effect of pH on adsorption of Ni(II) on natural peanut husks; **b** effect of pH on adsorption of Ni(II) on expanded perlite

fibrilized-porous morphology for the natural peanuts husks. For the graphene, typical flakes and graphene sheets were observed.

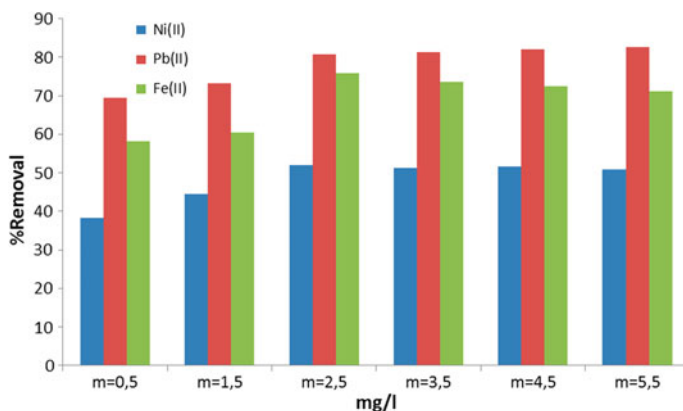
TGA data of the studied sorbents are presented in Table 1. As it was expected, the highest thermal stability, i.e., the highest  $T_{d3}$  was obtained for the graphene.

The influence of the initial pH of the solution (Figs. 2a, b) on the adsorption of Ni(II), Pb(II), and Fe(II) ions were investigated.

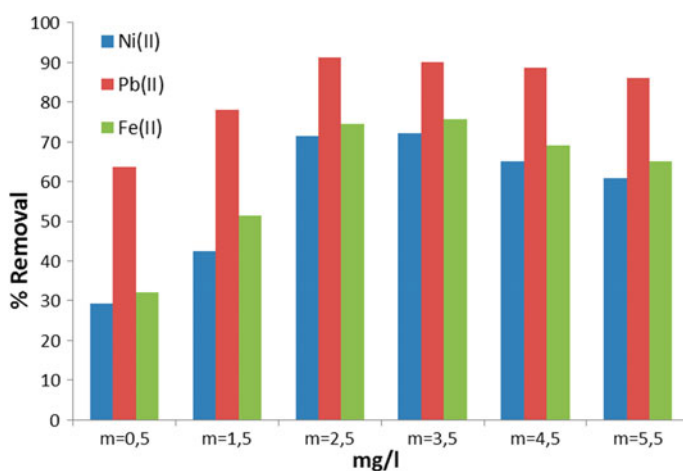
pH influence on the adsorption of heavy metal ions onto natural peanut husks was examined at pH 4.5, 5.5, 6.5, and 7.5, conducting the experiment at room temperature, with constant value of initial metal concentration of 0.5 mg/l, constant amount of expanded perlite, and peanut husks of 2.5 g/l, at 400 rpm. It was found that the % of removal increases with increase in pH value up to 6.5 and then decreased at pH 7.5. Another important parameter is the amount of biosorbent used for the adsorption studies that determine the potential of biosorbent to remove metal ions. The effect of this parameter is shown for the amount of natural peanut husks (Fig. 3) and for the amount of expanded perlite (Fig. 4). Generally, it was found out that increasing the adsorbent dose provides greater surface area and availability of more active sites, thus leading to the enhancement of metal ions uptake [3, 5].

Next parameter that was studied was initial metal concentration. Variations and their effects on the % of removal for natural peanut husks (Fig. 5), (the same found also for the perlite and graphene), show that the % removal decreases with the increase in initial Ni(II) ions concentration.

The effect of contact time of adsorption was studied also. Typical curves of these relations for all studied sorbents for various initial concentrations of metal ions are



**Fig. 3** Effect of adsorbent dose on adsorption of Ni(II), Pb(II), and Fe(II) on natural peanut husks

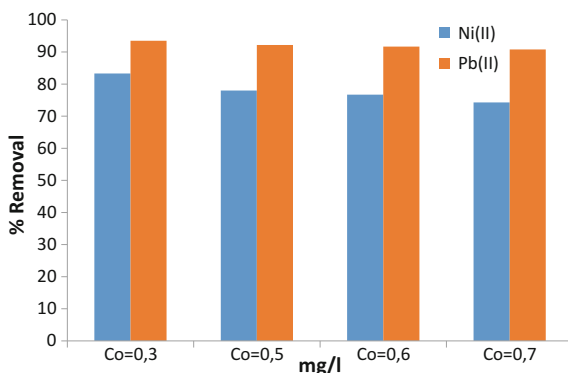


**Fig. 4** Effect of adsorbent dose on adsorption of Ni(II), Pb(II), and Fe(II) on expanded perlite

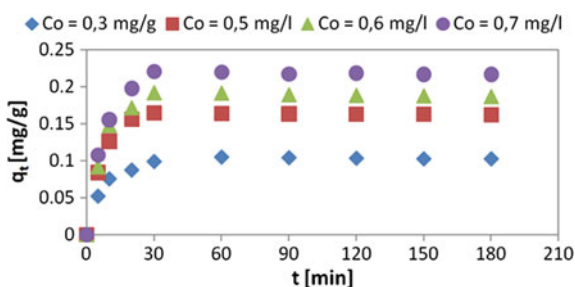
shown in Fig. 6. The experiment was conducted using 2.5 g/l sorbent, at 2 l of metal ion solutions with different initial concentration, at pH 6.5, room temperature and 400 rpm. The samples were taken at particular time (5, 10, 20, 30, 60, 90, 120, 150, and 180 min), and filtered and filtrates were collected for analysis, and the remaining metal ion concentration was determined. As shown in figures, biosorption of Ni(II) ions on the adsorbents increased with an increase of contact time. It can be seen that the adsorption equilibriums were attained after 60–90 min.

Adsorption efficiency of the graphene and its activated form for the studied heavy metal ions was shown on the Fig. 7. It was found that there is no positive effect of the graphene acid activation. For Pb(II) and Fe(II) ions, higher adsorption efficiency was obtained using the origin (non activated) graphene.

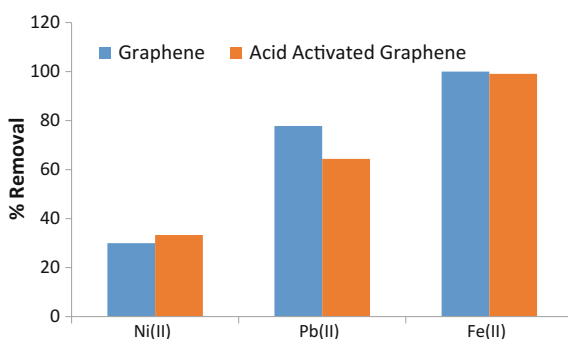
**Fig. 5** Effect of initial metal ion concentration on adsorption of Ni(II) and Pb(II) on natural peanut husks



**Fig. 6** Adsorbed amount of Pb(II) from time of adsorption for natural peanut husks



**Fig. 7** Adsorption efficiency of the graphene



In order to complete adsorption study, adsorption isotherms were used to analyze and describe the equilibrium that gives the capacity of the adsorbent and the equilibrium relationships between adsorbent and adsorbate. Experimental data were processed by the four most commonly used isotherms: Langmuir Freundlich, Langmuir–Freundlich, and Redlich–Peterson, by using the MATLAB/ Curve Fitting Toolbox. For all studied sorbents (perlite, natural peanut husks, and graphene), very high values for the correlation coefficient were obtained. Characteristic parameters that were obtained are presented in Table 2 (for natural peanut husks as a sorbent).

**Table 2** Parameters and correlation coefficients of equilibrium isotherm models for Ni(II) adsorption on natural and modified peanut husks

Isotherm	Parameters and correlation coefficients	Biosorbent	
		NPH	MPH
Langmuir	$q_m$ [mg/g]	0.1273	0.1729
	$K_L$ [l/mg]	8.483	17.58
	$R^2$	0.9984	0.9921
Feundlich	$K_F$ [l/g]	0.1369	0.2026
	$n$	2.88	3.69
	$R^2$	0.9974	0.9846
Langmuir–Freundlich	$q_m$ [mg/g]	0.1287	0.1473
	$K_C$ [l/mg]	7.954	519.5
	$n$	1.02	0.46
	$R^2$	0.9984	0.9961
Redlich–Peterson	$K_{RP}$ [l/g]	1.161	1.970
	$A$ [l/mg]	8.982	14.540
	$\beta$	0.9635	1.282
	$R^2$	0.9984	0.9947

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# Banning Microplastics in Cosmetic Products in Europe: Legal Challenges

Esther Kentin

## 1 Introduction

The sources of microplastics in the oceans—and in the Mediterranean Sea—are multiple. The first campaigns raising awareness on microplastics were focussing on microbeads in cosmetic and personal care products (I will now use the terms cosmetics and cosmetic products for both categories). Microbeads were defined as solid plastic particles, and the most frequent use was for exfoliating and scrubbing purposes. Today, microplastics are also added for other reasons such as emulsifying, film forming, binding, skin conditioning and glittering. Although representing a small part of microplastics in the environment, banning microplastics in cosmetics products seemed to be a relative demarcated exercise for three reasons. First, cosmetics are a clear defined product group in most legislations. Second, it seems that microplastics can be relatively easily replaced by alternatives, although they may be more expensive. And third, only a few types of microplastics are used in cosmetic products, polyethylene particles representing 93%, although the use of liquid synthetic polymers and new types of microplastics is increasing.

Banning microplastics from the source is more or less the only option to prevent and reduce pollution by microplastics: measures such as collection, recycling and clean up are not possible to prevent or remove microplastics from the environment. Awareness raising and education to promote the use of products without microplastics can be effective; see for example the campaign of Beat the Microbead app. But the abundance of cosmetics with microplastics and the relatively small number of products available without microplastics makes it difficult to make a significant change in real numbers. Furthermore, the majority of consumers has not

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been reached yet. Many consumers are still unaware that microplastics are added into cosmetics and trust that regulation would prevent any harmful substance in such a product. This is especially true for EU consumers with cosmetics being regulated by the Cosmetics Regulation. Despite the Beat the Microbead campaign and other awareness initiatives, the amount of microplastics in cosmetic products is still increasing.

## **2 Legal Strategies to Ban Microplastics in Cosmetic Products**

### ***2.1 National Regulation***

The United States (US) is the first country to ban microplastics from rinse-off cosmetic products by its Microbead-Free Waters Act [1]. The ban was concluded in 2015 and foresees a phase-out of microbeads in the coming years. A microbead is defined as ‘any solid plastic particle that is less than five millimetres in size and is intended to be used to exfoliate or cleanse the human body or any part thereof’. By 1 July 2018, the manufacturing of rinse-off products with microbeads is prohibited, while the sale of those products with microbeads will be abandoned by 1 July 2019. France is the second country that has adopted a ban on microplastics in rinse-off products, following the same definition of microbeads in the US Microbead-Free Waters Act. In Canada, UK, Taiwan, South Korea and Sweden, similar legislation is proposed, waiting for approval and adoption. It seems that the Microbead-Free Waters Act has set the standard for defining and prohibiting microplastics as all proposed bans use more or less the same definition of microbeads, while being applicable only to rinse-off cosmetic products.

Since October 2016, six countries—South Korea being the first on 6 October 2016, followed by Taiwan, Canada, France, New Zealand and Sweden—have notified the WTO TBT Committee of their proposed bans on microplastics in rinse-off cosmetics. The WTO TBT Committee was set up by the Agreement on Technical Barriers to Trade to monitor measures that set standards to product for public policy reasons, such as human health, environment, consumer information and quality. These measures are allowed under the TBT Agreement only if they are non-discriminatory and do not create any ‘unnecessary obstacles to international trade’. National technical standards should follow international standards as much as possible, but if these are not available, countries may set their own standards. Countries should notify the other WTO members via the Secretariat of proposed measures and provide a reasonable time for reactions and comments. No comments of other countries have been received, but it may be too early to draw any conclusions.

## 2.2 *EU Regulation*

Several EU regulations have been suggested to suit a ban on microplastics in cosmetics [2]. The Marine Strategy Framework Directive [3] is often cited as regulation that provides an obligation to prevent marine litter. The directive obliges EU Member States to develop a strategy for its marine waters and leaves it up to the Member States to adopt tools and measures. Regarding microplastics, first baselines have to be established before targets can be set [4]. France considers its proposed ban on microplastics in cosmetic products as an implementation measure under the directive. So, although the Marine Strategy Framework Directive could facilitate national measures on microplastics, it cannot provide for an EU wide ban on microplastics.

The Cosmetics Regulation [5] regulates the ingredients of products and may restrict certain substances in certain product categories. Since the regulation is based on the working of the internal market and protection of human health, a prohibition of microplastics in cosmetic products for environmental reasons would depart from its legal basis. For restricting substances for environmental considerations, the Cosmetics Regulation refers to the REACH Regulation. However, if the normal use of products with microplastics can be proven to be harmful for human health, those products can be restricted by including microplastics on the list of restricted substances by amendment.

Under the REACH Regulation [6], chemical substances are regulated to ensure a high level of protection of human health and the environment. Any new substance must be registered and evaluated and then can be authorised or restricted. However, polymers do not have to be registered under the REACH at this moment [7]<sup>1</sup>; so any existing and also new polymers are not registered and evaluated, let alone restricted. To include polymers in the REACH Regulation, the European Commission has to review the exemption using strict criteria: (1) only if a practicable and cost-efficient way is available, (2) based on sound technical and valid scientific criteria, (3) after reporting on (a) risks posed by polymers in comparison with other substances and (b) taking into account competitiveness and innovation on the one hand and the protection of human health and the environment on the other.

The Ecodesign Directive [8] is also referred to in regulating microplastics in cosmetic products, but would only be possible if it is first amended to include standards on resource efficiency. Such amendment should restrict the use of plastics or other persistent substances to minimise waste and pollution. Since the Ecodesign Directive sets standards for very specific product categories, banning microplastics in cosmetics would require multiple procedures and implementing measures. Despite these legislative hurdles, this type of regulation could force manufacturers of products containing or made of plastic to consider waste and disposal issues in the design of products.

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<sup>1</sup>REACH Regulation, recital 41 and art. 3 and 138.

### 2.3 *Voluntary Agreements*

On both national levels and regional levels, voluntary agreements have been discussed and are still being discussed. In 2015, Cosmetics Europe has advised its members ‘to discontinue the use of synthetic, solid plastic particles used for exfoliating and cleansing that are non-biodegradable in the marine environment in wash-off cosmetic products placed on the market as of 2020’.<sup>2</sup> Unfortunately, this advice does not include stay-on products and liquid synthetic polymers, while a phase out of more than five years is provided. Besides, it is only an advice, and no pledge or commitment is made. The Dutch branch organisation of cosmetics manufacturers has notified that it will not actively support a voluntary agreement between manufacturers to ban microplastics as it may be in violation with European competition law.

European competition law forbids agreements between manufacturers and other market players that would restrict competition. Exceptions to this rule are possible, but only if it would improve the production of goods or lead to technical or economic progress and consumers would benefit from these improvements. The requirement that consumers have to benefit from these agreements has been interpreted strictly by the European Commission, meaning that consumers have to benefit financially. The Commission has actually been warning that even if national authorities are encouraging companies to enter into voluntary agreements for public purpose objectives, such as environmental protection, it does not mean that these agreements are permissible under EU competition law. Therefore, a voluntary standard-setting agreement between manufacturers of cosmetic products banning microplastics has to comply with strict requirements [9]. First of all, participation to the standard-setting agreements should be open to all and transparent, while compliance should be strictly voluntary. If the agreement would lead to an increase of prices, the agreement may fall under the exceptions, but it is essential that the agreement would lead to efficiency gains—read economic gains—for the consumer. Agreements regarding prices, either to stabilise or to increase, between manufacturers will violate the law and may be punished with high fines. Due to the strict interpretation of the Commission, manufacturers and trade organisations are hesitant to enter into any agreement. Therefore, it will not be easy to conclude such an agreement with manufacturers of cosmetics.

### 2.4 *Labelling*

There are several initiatives on labelling in Europe, either from the cosmetic industry, such as NATRUE and COSMOS, or NGOs and private parties, such as Beat the Microbead and CodeCheck. Some labels of the cosmetic industry do not

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<sup>2</sup>Cosmetics Europe, recommendation on solid plastic particles. <https://www.cosmeticseurope.eu/how-we-take-action/driving-sustainable-development>. Accessed 5 July 2017.

state explicitly whether microplastics are permitted under their standards. For example, COSMOS permits synthetic substances within agro-ingredients up to 3%. Although labelling will raise awareness and gives consumers a choice, the impact of current labels is relatively small. The market is dominated by only five conglomerates owning 127 brands, L'Oréal being the leader with 39 brands.<sup>3</sup> Another 15 companies divide more or less the rest of the market, making up a total of roughly 70% market share of the global market for only 20 companies.<sup>4</sup> Since there is hardly any commitment from the branch organisations to remove microplastics from products, voluntary labelling of products concerning microplastics is not expected.

The Cosmetics Regulation requires manufacturers to place a list of ingredients on the packaging of cosmetic products.<sup>5</sup> Microplastics could thus be identified if it would be clear—also for consumers—which substances are considered as a plastic. For nanomaterials, the term 'nano' is required to be indicated on the packaging. Such requirement could equally be proposed for plastics. However, it has to be seen whether such amendment would lead to a reduction of microplastics in cosmetic products. On national level, a mandatory labelling initiative for microplastics is pending in Italy.

### 3 Conclusions and Challenges

- The present national bans only restrict the use of microplastics in rinse-off cosmetic products, while leave-on products such as sunscreen and creams pose an equal threat to the environment.
- A limited definition of microplastics is used in national bans as well as in labelling initiatives: only solids, while liquid synthetic polymers are increasingly used.
- National bans but also EU regulation may restrict more stringent regulation. The US Microbead-Free Waters Act prohibits federal states to ban microplastics in other cosmetic products, such as leave-on, and also other phases of microplastics.
- The WTO TBT Agreement is applicable to national and regional bans of microplastics, as the restriction is a 'technical barrier to trade'. Justification has to

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<sup>3</sup>Willett, M. and Gould, S.: These seven companies control almost every single beauty product you buy. Insider 18 May 2017. <http://www.businessinsider.com/companies-beauty-brands-connected-2017-5>. Accessed 5 July 2017. The five biggest companies are as follows: L'Oréal \$27.6 billion, Unilever \$22.3 billion, Procter & Gamble \$18 billion, Estée Lauder \$11.3 billion, Colgate-Palmolive \$10.7 billion (Beauty sales 2015).

<sup>4</sup>Value of the cosmetics market worldwide in 2015 was €221 billion. <https://www.statista.com/statistics/585522/global-value-cosmetics-market/>. Accessed 5 July 2017. TOP 20 Global Beauty Companies: All Over the Map. <http://www.beautypackaging.com/heaps/view/2941/1/232950>. Accessed 5 July 2017.

<sup>5</sup>Cosmetics Regulation, art. 19.

- be sought in environmental protection. International standards may be used as guidelines, so setting the ‘right’ standards, also in voluntary agreements, is crucial.
- Banning microplastics as substances in products in the EU requires amendment of the REACH Regulation to firstly include polymers in the regulation. The European Commission should take the initiative to review the exemption of polymers in REACH and take steps accordingly to include polymers in registration and evaluation procedures.
  - Voluntary agreements are restricted by EU Competition rules, and can only be concluded if they contain no obligation to comply, if they are accessible for all manufacturers, if the procedure is transparent and if products will not be more expensive for the consumer.
  - Although the gains of labelling could be small, informing consumers about microplastics in cosmetic products remains essential as long as there is no ban on microplastics.

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