Marine Pollution Bulletin 217 (2025) 118080

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Release of heavy metals during in vitro fish gastrointestinal digestion from microplastics collected at Calabrian coasts

Eduardo Bolea [a], Luana S. Brunetti [a,b], Isabel Abad-Alvaro [a], Emilio Cellini [c], Silvestro A. Ruffolo [b,*], Mauro F. La Russa [b], Francisco Laborda [a]

[a] Group of Analytical Spectroscopy and Sensors (GEAS), Institute of Environmental Sciences (IUCA), University of Zaragoza, Pedro Cerbuna, 12, 50009 Zaragoza, Spain
[b] Department of Biology, Ecology and Earth Sciences (DIBEST), University of Calabria, Pietro Bucci, 87036 Arcavacata di Rende, Cosenza, Italy
[c] Regional Agency for the Environment – Calabria (ARPACAL), Regional Marine Strategy Center (CRSM), Catanzaro, Italy

ARTICLE INFO

Keywords:
Heavy metals
Microplastics
ICP-MS
Mediterranean Sea

ABSTRACT

Migration of different environmentally relevant elements (Pb, Cd, Cr, As, Sb, Sn, Zn, and Hg) from microplastics collected at different points on the Calabrian coast (areas of both Tyrrhenian and Ionian seas) during simulated fish digestion processes has been studied. The effect of particle size and polymer composition on migration processes has been studied using three different polymers (low density polyethylene (LDPE), polypropylene (PP), and polyvinyl chloride (PVC)) as models. In vitro fish digestion simulation consists of two different phases: gastric (simulated gastric fluid (SGF)) and intestinal (simulated intestinal fluid (SIF)). In general, larger percentages of released metal were found during the gastric phase with respect to the intestinal, likely due to the more acidic conditions along the gastric phase. The total amount of migrated metals after the whole process (SGF + SIF) was also measured, being lower than the initially migrated during the gastric step. In comparison, the amounts of metals migrated during the intestinal phase were not significant for most of the metals studied, diluting consequently the concentration of the metals at the end of the process. Reduction of the polymeric material size (from diameters of several mm (pellets) to 300–500 μm in average (milled)) leads to higher concentrations released during both digestion phases for most of the metals studied. Plastics collected on the Calabrian coast also show metal migration during digestion simulations, being significant for chromium, lead, cadmium and zinc. Particulates containing lead were also detected by single particle ICP-MS, which may correspond to solid deposits on plastic surfaces released during digestion simulations.

1. Introduction

The issue of plastics in the seas represents one of the main problems of the 21st century (Bettencourt et al., 2021). About 80 % of the plastic present in the oceans derives from terrestrial activities (urban landfills, malfunctioning sewage systems, industries) that are transported to the seas thanks to rivers; while the remaining 20 % derives from maritime activities (cruises, recreational and commercial fishing, aquaculture) (Castro-Jimenez et al., 2019; Lechner et al., 2014). To date, microplastics have been observed everywhere, from the poles to the equator; in fact, they have been reported in considerable concentrations even in remote sites such as Antarctica and on the seabed of the Mariana Trench (Barnes et al., 2009; Browne et al., 2011). This happens because, once introduced into the sea, they can become part of the gyres. These natural phenomena act in a similar way to vortices, dragging debris (including plastic) towards their center. Due to these movements, waste tends to accumulate in five "ocean garbage patches" located in the North Atlantic, South Atlantic, North Pacific, South Pacific, and Indian Oceans. The largest of these areas is the Great Pacific Garbage Patch, located between Hawaii and California (Lebreton et al., 2018). The Mediterranean Sea is indicated as the sixth-largest accumulation area of floating marine plastic waste, due to its hydrodynamics. This Sea is a semi-closed convective basin and this structure determines, not only the maintenance of local plastic pollution but also the entry of floating waste from the Atlantic Ocean (Béranger et al., 2010; Soto-Navarro et al., 2010). Once in the sea, plastics undergo a degradation process over time determined by chemical, physical, and biological actions that lead to the formation of very small fragments, of millimeters and/or micrometers

* Corresponding author.
E-mail addresses: edbolea@unizar.es (E. Bolea), iabad@unizar.es (I. Abad-Alvaro), e.cellini@arpacal.it (E. Cellini), silvestro.ruffolo@unical.it (S.A. Ruffolo), mauro.larussa@unical.it (M.F. La Russa), flaborda@unizar.es (F. Laborda).

https://doi.org/10.1016/j.marpolbul.2025.118080
Received 19 November 2024; Received in revised form 28 April 2025; Accepted 29 April 2025
Available online 1 May 2025

sizes, defined as Microplastics (MPs) (Arp et al., 2021). As regards chemical-physical actions, oxygen present in the air is one of the most important, interacting with polymers and creating free radicals that cause breaks in the polymer chains. UV solar radiation also contributes to the formation of these free radicals inducing hydrolysis reactions. The breaking of the chains causes the phenomenon of chemo-crystallization, creating crystalline spherules with a volumetric contraction, producing increasingly smaller fragments (Schmid et al., 2021). Biological degradation is also produced and includes mechanisms that involve extracellular enzymes produced by environmental bacteria and other microorganisms that break the chemical bonds that bind the plastic molecules (Zettler et al., 2013; Shah et al., 2008). It has been reported that different types of plastic polymers have variable sensitivity to the action of enzymes and UV rays (Lambert et al., 2014). Some characteristics of plastic products can influence the speed of degradation processes, both biotic and abiotic, including the complex polymer structure and the components of the materials. It has been highlighted that MPs have been found in many commercially important fish species (Barboza et al., 2018). Essentially, when microplastics enter the seas, they can be mistaken for food due to their small size and be ingested by fish, thus entering the food chain. Generally, fish species consumed whole (such as mollusks and crustaceans, anchovies, and sardines) pose a greater threat than gutted fish or peeled shrimp. However, the presence of microplastics has recently been detected in the muscle of some fish species such as Ephinephelus coiodes, *Platycephalus indicus*, *Sphyraena jello*, *Saurida tumbil*, *Sillago sihama*, and in crustaceans such as Peneaus Semisulcatus (Akhbarizadeh et al., 2018; Abbasi et al., 2018). These observations raise concerns about what could be the potential effects on humans, considering the presence of metals that the sea microplastics could contain. In fact, the bioaccessibility of heavy metals through the ingestion of tire particles has been already demonstrated (Chen et al., 2022; Masset et al., 2021). Metals may remain in plastics as catalytic or reaction residues, although their principal primary source is functional additives. Some metal-based additives are insoluble inorganic compounds, partially soluble organic compounds, or organometallic liquids or salts (Turner and Filella, 2021). These metal-based additives have a wide range of functions in plastics, mainly as fillers or pigments for color, but they can also act as flame retardants, biocides, antimicrobial agents, or lubricants. Due to concerns about the environmental impact of those inorganic and organometallic compounds, because of their non-biodegradable nature, leading to bioaccumulation in the food chain (Catrouillet et al., 2021), there has been a gradual shift towards organic compounds or non-metal-based alternatives. Although hazardous metal-based additives are no longer intentionally incorporated into contemporary plastics, at least in Europe, it is still possible that such additives would be still employed in certain consumer goods available in the EU (Turner and Filella, 2021). In any case, all these plastics containing metal-based additives still persist in soils, sediments, and waters.

Therefore, this study aims to clarify the role of microplastics in the bioaccessibility of metals in gastric and intestinal fluids of a model fish species by highlighting the potential for solubilization of polymer-bound elements during intestinal transit. In particular, it has been carried out in vitro simulations of the digestive process of *Oncorhynchus mykiss*, which is one of the most common cold-water fish species in aquaculture and the seventeenth most widely cultured commercially important finfish in the world and, therefore, in the human diet (Singh and Srivastava, 2021).

2. Materials and methods

2.1. Materials and samples

Three different plastic materials: Low Density Polyethylene (LDPE), Polypropylene (PP) and Polyvinyl chloride (PVC) were used as models for digestion migration assays. Certified reference material ERM-EC681m (JRC, Geel, Belgium) was used as model for LDPE. This material is commercialized as pellets of 3 × 3 mm. Fragments of plastics collected on beaches of the French Atlantic coast were analyzed and fragments of PP were isolated and subsequently used as model for PP. Finally, PVC in powder (Sigma, St. Louis, MO) was used as model for PVC.

Plastics from four environmental (marine water) samples were collected and studied. Samples from the Calabrian Ionian Coast (Southern Italy) were collected in Corace, Neto, and Crati (river mouths), whereas those from the Calabrian Tyrrhenian Coast were collected in Cetraro, Vibo Marina, and Gioia Tauro (port and tourist areas). All of them were collected during two campaigns, along the first (I) and second (II) half of 2022. Samples from each coast were combined in the experimental procedure, giving four different samples: Ionian (I and II) and Tyrrhenian (I and II). The characterization of such samples is reported elsewhere (Brunetti et al., 2025).

Plastic composition varied from one coast to another, being 77 % PE and 14 % PP on average for the Ionian coast samples and 66 % PE and 18 % PP for the Tyrrhenian coast ones. Ethylene-vinyl acetate (EVA), polystyrene (PS) and polyamide were also detected in different proportions in both types of samples.

Sodium Chloride (NaCl), Potassium Chloride (KCl), Magnesium Sulphate ($MgSO_4$), Calcium Chloride ($CaCl_2$), Pepsin, Porcine Bile Extract, Pancreatin, Sodium Hydrogen Carbonate ($NaHCO_3$), HEPES were purchased from Sigma (St. Louis, MO). All the reagents used were of analytical grade. Ultrapure water (18 MU cm of resistivity) was obtained from a Milli-Q purification device (Millipore Co., Bedford, MA, USA).

2.2. Methods

2.2.1. Size reduction of raw plastic materials used as models

A ball mill MM400 (Retsch, Haan, Germany) was used to reduce the size of LDPE, PP, and PVC materials described in Section 2.1. Two different procedures were compared: a wet milling based on the use of methanol inside the jars together with the polymer, and a cryogenic milling based on the use of a liquid nitrogen bath. As for the first protocol, to reduce 1 g of LDPE, 7 milling cycles lasting 5 min each at a frequency of 25 Hz were required, adding 2 mL of methanol in every cycle. As for the second protocol, to reduce 5 g (2.5 g in each container) of LDPE, 12 milling cycles of 2 min each at 30 Hz were required. Before each milling, containers with the samples were placed in the liquid nitrogen bath for 5 min. To reduce 5 g of PP and PVC with cryogenic milling, two cycles of 2 min each at 30 Hz were required. Frequency and times were adjusted following the recommendation of the mill manufacturer, whereas the number of cycles was optimized by visual inspection after each cycle, in terms of homogeneity and particle size.

2.2.2. Determination of size distributions by laser diffraction

A Mastersizer 3000E (Malvern Panalytical, UK) was used to characterize the size distribution of each material after milling, using both protocols described in Section 2.2.1. <1 g was used for measurements, introduced as solid dispersion by aspiration. Five replicates were made for each sample and average sizes were used along the study.

2.2.3. Characterization of plastics collected at Calabrian coasts and plastic models

2.2.3.1. Determination of metal concentration by ICP-MS. The samples were subjected to acid digestion to determine the total amount of heavy metals. The protocols used along this work were adapted from (Lehtimäki and Väisänen, 2016). A microwave-assisted digestion was performed using a One Touch MARS6 (CEM Corporation, Charlotte, NC USA) with TFM™ (modified PTFE) vessels. The operating conditions are reported in Table S1. For digestion, 50 mg of each sample were placed in Teflon vessels with 2.3 mL of HNO_3 (65 %) and 1 mL of H_2O_2 (30 %).

After the digestion program, the samples were diluted with ultrapure water, then analyzed by ICP-MS. A Perkin Elmer Elan DRC-e mass spectrometer (Toronto, Canada) was used for ICP-MS measurements. Data acquisition was performed using a dwell time of 50 ms, 20 sweeps, and 10 replicates per measurement. Instrumental parameters are described in Table S2. Instrumental limit of quantification (LQ) was calculated as 10 times of blank standard deviation divided by linear regression slope.

2.2.4. In vitro digestion

In vitro digestion protocol was adapted from (Masset et al., 2021). Basically, it consists of 3 h of digestion in a Simulated Gastric Fluid (SGF), to simulate the transit time in the fish stomach, followed by 23 h of digestion in a Simulated Intestinal Fluid (SIF), to simulate the transit time in the fish intestine. The composition of both simulated fluids and protocol are described in detail in SI.

2.2.5. Analysis of digestion media after in vitro assays for metal released determination

After in vitro digestion, the digestion media (SGF, SIF, and SGF + SIF) were filtered at 0.45 μm with PTFE filters (Omnipore, Merk), diluted with ultrapure water (1:20) and analyzed by ICP-MS, using the same conditions as described in Table S2. Matrix recovery assays were conducted in all media (SGF, SIF, SGF + SIF) for all metals monitored by addition of metal concentration standards and measured under the same conditions as samples. Recovery values between 85 % and 130 % were obtained for all the elements, except for As in the samples collected at sea, which were lower than 20 %, which justifies that the values obtained (below the limit of quantification of the method) were not reported.

Concentration results are expressed as the difference between metals concentration levels found in simulated fluids after digestion conditions in the presence of the corresponding plastic and in absence. Differences statistically not significant are expressed as below the limit of quantification, which corresponds to the limit calculated from the concentration of the metal in the different simulated fluids.

Samples from Ionian and Tyrrhenian Coast subjected to the in vitro assays were also measured in single particle mode (SP-ICP-MS). For this purpose, a Perkin Elmer NexION 2000B mass spectrometer was used. Following the in vitro digestion process, the SGF + SIF digestion media were filtered at 10 μm with PTFE filters (Omnipore, Merck) and subsequently diluted 10 times with ultrapure water prior to analysis by SP-ICP-MS. The sample introduction system consisted of an Asperon™ linear pass spray chamber (Perkin Elmer, Toronto, Canada), equipped with a flow focusing nebulizer (Ingeniatrics, Sevilla, Spain). A μDx Single Cell Autosampler (Elemental Scientific, Omaha, NE, USA) equipped with a syringe pump operating at 10 μL min^{-1} was used for sample introduction. ^{208}Pb was monitored at a dwell time of 100 μs for a total acquisition time of 60 s. Default instrumental parameters are listed in Table S2.

3. Results and discussion

3.1. Effect of particle size on metal released

Firstly, the effect of the size of microparticles on the amount of metal released was studied. The certified reference material ERM-EC681m (trace elements in polyethylene) was used for this assay. This material is commercialized as pellets of 3 × 3 mm made of pure LDPE and pigments. This material was reduced in size by the milling process described in Section 2.2.1. Although similar size distributions were obtained following the two different milling procedures (cryogenic and wet) (see Fig. S1), both milling conditions were used throughout this study. Size distribution obtained by laser diffraction shows a maximum at 272 μm, with 10 % of volume population below 100 μm. Raw and milled materials were conducted under the same in vitro digestion procedure described in Section 2.2.4. and results are compared in Table 1.

In general, higher concentrations were obtained for most elements determined for the milled material (cryogenic and wet) in both media, gastric and intestinal. However, the ratio between the concentrations observed varies to a large extent from one metal to another and between milling conditions. For instance, lead, antimony and cadmium show the largest difference, with ratios around 1:10 or even of 1:100, whereas chromium showed small differences, being even the opposite (smaller the concentration released from the milled material) in the case of Sn and Zn (exception made for Sn with the cryogenic milling). This last behavior is opposite to that expected since the surface of contact is much lower in the case of the raw material (pellets). The high concentration values of zinc obtained in the blank (probably due to the impurities in the different reactants used during the intestinal phase) and the large uncertainty associated with the values for the rest of the samples analyzed, make these results not significant. In any case, other variables different from particle size should be considered to explain all these results. Given that intestinal phase lasts much longer (23h) than the gastric one (3 h), it is possible that chromium, tin or zinc releases following a different kinetic than the rest of metals, similarly to that observed in (Masset et al., 2021) for zinc.

If milling conditions are compared, higher concentrations were observed with wet milling for most metals. The slight differences observed in the size distributions between these two procedures (Fig. S1) do not seem to justify such differences, although they may contribute to some extent. It is possible that the use of methanol during the milling process could modify the composition on the surface of the material, facilitating the release of metals, assuming that they are homogeneously distributed. For this reason, and considering that cryogenic milling is less aggressive to the milled material, this procedure was selected for the rest of the experiments. In any case, the trend discussed above about the effect of the total surface remains the same, with larger amounts of metals released as the particle size is reduced (larger surface area).

If the composition of the additives is considered, which is the main source of these released elements, Zn and Sn were present as sulfides, same as cadmium and mercury. Sulfides of Zn, Sn, and Cd are insoluble in water, although they can be solubilized in a small extent in acid media (pKs values around 25), whereas HgS is quite insoluble, which would explain the low values (below limit of quantification in all cases) obtained. It is possible that some adsorption processes onto the materials would play a significant role after the release of these metals, reducing the total concentration remaining in the medium in the case of the milled materials.

Precisely, the acidity of the gastric medium could also explain the higher concentrations of some metals released during this phase compared to the intestinal phase, in which pH is increased up to values around 7. This effect was also observed by other authors using the same simulated digestion (Masset et al., 2021).

3.2. Metals released from microplastics used as models during simulation of fish digestion phases

Three common polymers, LDPE, PP, and PVC were used as models for metal release in in vitro fish digestion studies. Table 2 shows the results obtained for these three materials under different conditions. Along this study, “gastric and intestinal” phase is referred to the total bio-accessible fraction of metals after digestion, whereas individual phases were also analyzed, so percentages of metals released on each phase could be compared. As previously discussed, larger concentrations of released metal were found during the gastric phase for the three materials studied.

The lower concentrations observed at the end of the process seem to correspond to the lower concentrations released and the dilution required during the intestinal phase. Comparing the different polymer materials, the lowest concentrations of metals released correspond, in

Table 1
Effect of particle size and milling conditions on metal released during in vitro digestion for LDPE material. Hg was also measured, but all values were below the limit of quantification of the method (0.1 μg L^{-1}), referred to the blank level in the corresponding simulated fluids (see Section 2.2.5). Mean ± standard deviation (n = 3).

LDPE form	Digestion phase	Pb	Cr	Cd	Sb	Sn	Zn
		Concentration (μg L^{-1})					
Pellet	Gastric	0.5 ± 0.2	163 ± 1	0.5 ± 0.2	0.05 ± 0.1	0.6 ± 0.1	15 ± 2
Wet milled		50 ± 2	227 ± 1	16 ± 0.6	10.1 ± 0.5	0.5 ± 0.1	10 ± 2
Cryo milled		24.5 ± 0.3	131 ± 2	6.0 ± 0.1	7.0 ± 0.2	6.0 ± 0.1	<0.4
Pellet	Intestinal	0.9 ± 0.3	151 ± 1	0.7 ± 0.2	0.6 ± 0.2	1.2 ± 0.1	47 ± 3
Wet milled		4.2 ± 0.4	243 ± 1	2.05 ± 0.2	6.5 ± 0.3	1.1 ± 0.1	<5
Cryo milled		1.8 ± 0.9	44 ± 3	0.5 ± 0. 1	<0.6	2.0 ± 0.1	<5

Table 2
Concentrations of metals in digestion media after in vitro assays. Mean ± standard deviation (n = 3). Concentration values expressed as the difference with respect to blank values. All Hg concentrations were lower than the limit of quantification (0.1 μg L^{-1}).

Material	Digestion phase	Pb	Cr	Cd	As	Sb	Sn	Zn
		Concentration (μg L^{-1})						
PVC	Gastric	<1	<20	<1	<2	<0.6	<2	<0.4
PP		75 ± 1	27.9 ± 0.1	6.8 ± 0.1	6.0 ± 0.1	1.01 ± 0.01	<2	<0.4
LDPE		24 ± 1	54.1 ± 0.2	6.4 ± 0.4	7 ± 1	5.7 ± 0.4	<2	<0.4
PVC	Intestinal	<1	<20	<0.3	<2	<1	<1	<5
PP		2.32 ± 0.02	<20	1.0 ± 0.1	<2	<1	<1	<5
LDPE		1.84 ± 0.04	<20	0.4 ± 0.1	<2	1.96 ± 0.01	<1	<5
PVC	Gastric and intestinal	<1	<20	<1	<2	<0.6	<1	<5
PP		22.2 ± 0.5	24 ± 1	6.0 ± 0.4	<2	1.5 ± 0.4	<1	<5
LDPE		10.8 ± 0.5	56.3 ± 0.1	4.0 ± 0.1	<2	6.7 ± 0.1	<1	<5

general, to the PVC. However, this comparison should be made in relative terms, considering the total metal concentrations in the bulk materials (see Table S3), so results can be expressed as percentages of metal released with respect to the total metal content in the solid, as shown in Fig. 1.

Percentages were calculated after the gastric phase and at the end of the digestive process for the three microplastics considered. In general, percentages lower than 15 % were obtained for most of the metals determined, except for Sb in the case of PP. In these cases, the high uncertainty values observed, due to the low concentration found in the PP for Sb (see Table S3), make these results less significant. In general, PVC shows no significant migration in comparison to the other two materials (LDPE is a reference material that contains several pigments, whereas PP is a fraction of microplastics collected in the environment). Despite these differences, chromium is released in a significant percentage in the rest of cases, especially from LDPE. This material contains chromium as $CaCrO_4$, which may explain its solubility in acidic media, remaining in the final medium after the intestinal phase. Lead and

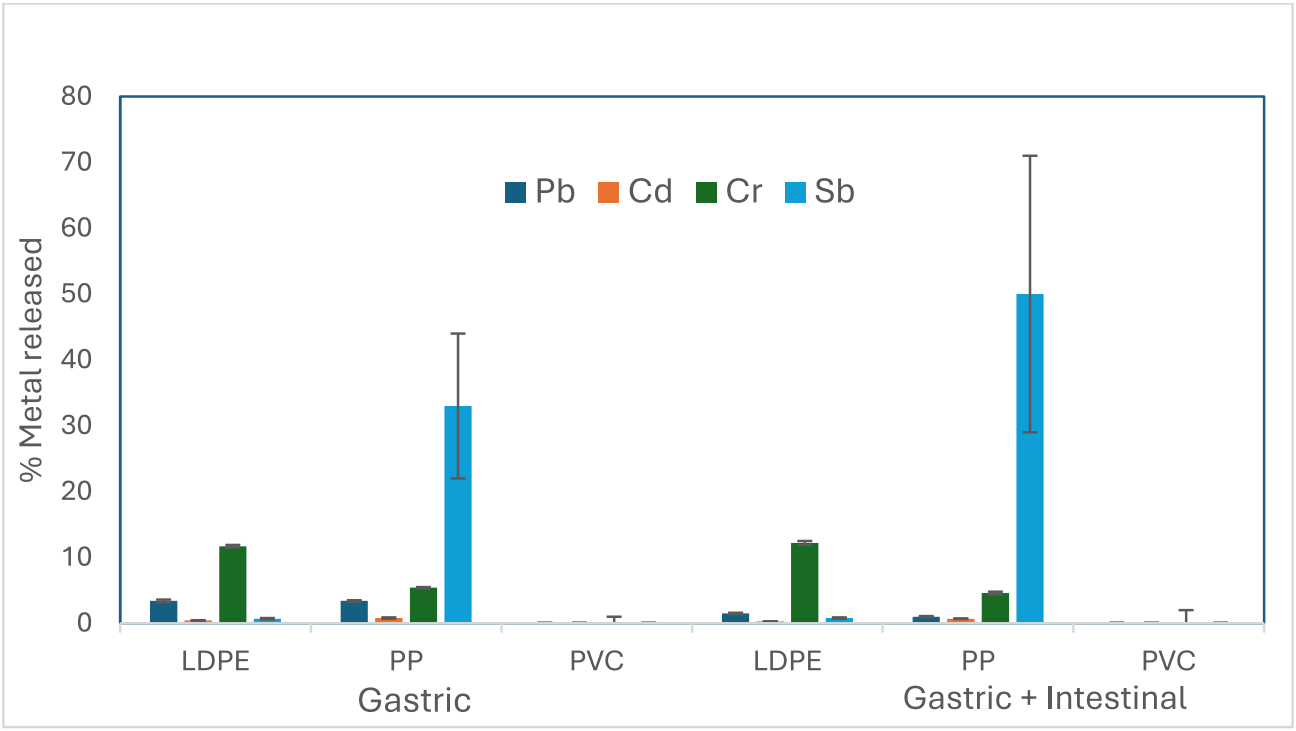

Fig. 1. Percentage of metal released during gastric phase and at the end of the digestion (after both gastric and intestinal phases).

cadmium show a similar behavior, with larger percentages during the gastric phase.

Therefore, it is not possible to establish a general pattern to explain the differences observed between the different materials and metals studied. Some elements, such as Sb, seems to be released significantly during the intestinal phase from PP, which suggest the presence of metal forms accessible by complexation with the proteins added during this phase, although the large uncertainty associated with these data should also considered. C. Catrouillet et al. (Catrouillet et al., 2021) found that Zn, together with some other elements, such as Al or Mn, progressively released from the plastic surface to the external environment by the analysis of acidic leaching and acid digestion with plastics collected in the environment. By contrast, metals such as Cu, Pb, Cr, and Cd, were adsorbed in the altered plastic surface, being released afterwards during the acidic leaching assay. As previously discussed, Masset et al. (Masset et al., 2021) also observed an increment on the percentage of total Zn solubilized during the intestinal phase, whereas no significant desorption occurred in this phase for other metals such as Fe, Pb or Co, although the total concentration of these metals in the original material was also lower. Considering all these potential effects, it may be justified by the different behavior observed between the three different plastics models used. In the case of PP, environmentally aged plastic, adsorption processes suffered during this time on its altered surface can make some metals more accessible respect to those present in the LDPE, which corresponds to an unaltered plastic, so released metals during the digestion simulation come from the known additives it contains. Even though, dynamic sorption processes may also occur during the digestion, modifying the amount of metal finally bio-accessible.

3.3. *Metals released from samples collected at Calabrian Coasts. Comparison with microplastic models. Effect of particle size*

Total metal concentrations in these samples were determined following the procedure described in Section 2.2.3 and results shown in Table S3. These concentrations are in good agreement with those found by other authors in environmentally aged ocean plastic fragments (Baalousha et al., 2023; El Hadri et al., 2020; Cheng et al., 2010). In general, metals in environmental plastics can be found as adsorbed species, which depends on the surface plastic composition and may increase its reactivity towards the metals present in the environment, or as additives (El Hadri et al., 2020) as previously discussed. Some strategies have been developed to distinguish between these two forms based on the metal concentration profiles in microplastics surface. This study (El Hadri et al., 2020) revealed that there was a large variety of profiles depending on the origin of these microplastics, although some elements, such as As and Zn, showed larger concentrations at the subsurface layers, whereas other elements, such as Pb or Sb present constant distributions, which suggests they were additives in these plastics. It is evident that metal release processes during digestion will be affected by the forms (sorbed vs. additives) these metals are present in plastics, as observed with the models already studied.

Plastics collected from different areas of Calabrian coast (see details in Section 2.1), were subjected to the same digestion procedure as plastics models and results are shown in Table 3.

Since microplastics used along this study were not classified by color or composition, the results correspond to the bulk material, trying to simulate a real scenario of microplastics ingested by fishes. Pb and Zn are the metals released to a larger extent, although concentrations are lower than those observed with the microplastics models. In general, the values of these two metals released during the gastric phase are higher than those obtained at the end of the process. An unexpected relatively low value was obtained in the case of the Ionian II sample for the gastric and intestinal phase, which may be considered as anomalous. If samples from the two coasts are compared, no significant differences are observed. Similar discussion as in Section 3.2 about the influence of chemical forms of these metals in plastics on the release process can be done here. Plastics collected were observed by SEM and elemental composition analyzed by EDS, as described in (Brunetti et al., 2025). Results showed that, while in the case of raw plastics used in that work, metals were distributed uniformly all over the surface analyzed, the plastics collected at sea showed some spots containing metals (Si, Na, K or Al were the most frequent, but Cr, Ti, Pb or Zn were also detected), which suggests that these metals may be present on the plastic surfaces as deposits sorbed onto their surface. When these samples were analyzed by single particle inductively coupled plasma mass spectrometry (SP-ICP-MS), which allows the simultaneous discrimination between dissolved species and particles (Laborda et al., 2014), some signals corresponding to particles containing Pb were detected. Fig. 2 shows the time scans obtained for the four different samples from Calabrian coast when monitoring Pb. As in the case of Table 3, these samples had previously been subjected to the whole digestion process (gastric and intestinal phase). Time scans for ultrapure water and digestion medium blank are also shown in Fig. 2 for comparative purposes. As can be observed, micro/nanoparticles containing Pb were detected for all Tyrrhenian and Ionian Sea samples, being this value higher in the case of Ionian II sample, which is in agreement with lead concentrations released to media shown in Table 3. These particles containing Pb could be related to lead released from the microplastics themselves as insoluble forms (Pb_3O_4, $PbCrO_4$), although the formation of insoluble hydroxides in the medium itself once the Pb^{2+} is released cannot be discarded. Therefore, it is possible that metals deposit on microplastic surfaces in the environment, facilitating their release during digestion processes as insoluble forms (particulates) afterwards. In any case, further experiments should be carried out with more samples and monitoring more elements to confirm these results.

If percentages of metal released with respect to the amount of metal present in the samples are directly compared (see Fig. S2), it makes evident the differences between the environmental samples and those selected as models, being much larger in the latter case (except for Zn, although large uncertainties were observed).

That would lead to the wrong impression that the amount of each metal released from these samples is less significant than those observed for those plastics used as models. However, the effect of the surface area should be considered, given its effect on this kind of processes, as

Table 3
Concentrations of metals in digestion media after in vitro assays. Mean ± standard deviation (n = 3). Concentration values expressed as the difference with respect to blank values.

Sample	Digestion phase	Pb	Cr	Cd	Sb	Sn	Zn	Hg
		Concentration (μg L^{-1})						
Ionian I	Gastric	6.4 ± 0.1	<20	1.0 ± 0.1	<0.01	<2	103 ± 9	<0.1
Tyrrhenian I		5 ± 1	<20	0.20 ± 0.02	<0.01	<2	<0.4	<0.1
Ionian II		23 ± 8	<20	0.8 ± 0.1	<0.01	<2	51.4 ± 0.1	<0.1
Tyrrhenian II		<1	<20	0.8 ± 0.1	0.5 ± 0.3	<2	102 ± 44	<0.1
Ionian I	Gastric and intestinal	1.3 ± 0.4	<20	1.0 ± 0.1	<0.01	<1	88 ± 30	<0.1
Tyrrhenian I		<1	28 ± 8	<0.5	0.3 ± 0.2	<1	<5	0.40 ± 0.04
Ionian II		4 ± 2	<20	0.6 ± 0.1	<0.01	<1	<5	0.2 ± 0.1
Tyrrhenian II		<1	<20	<0.5	0.6 ± 0.3	<1	63 ± 25	0.2 ± 0.1

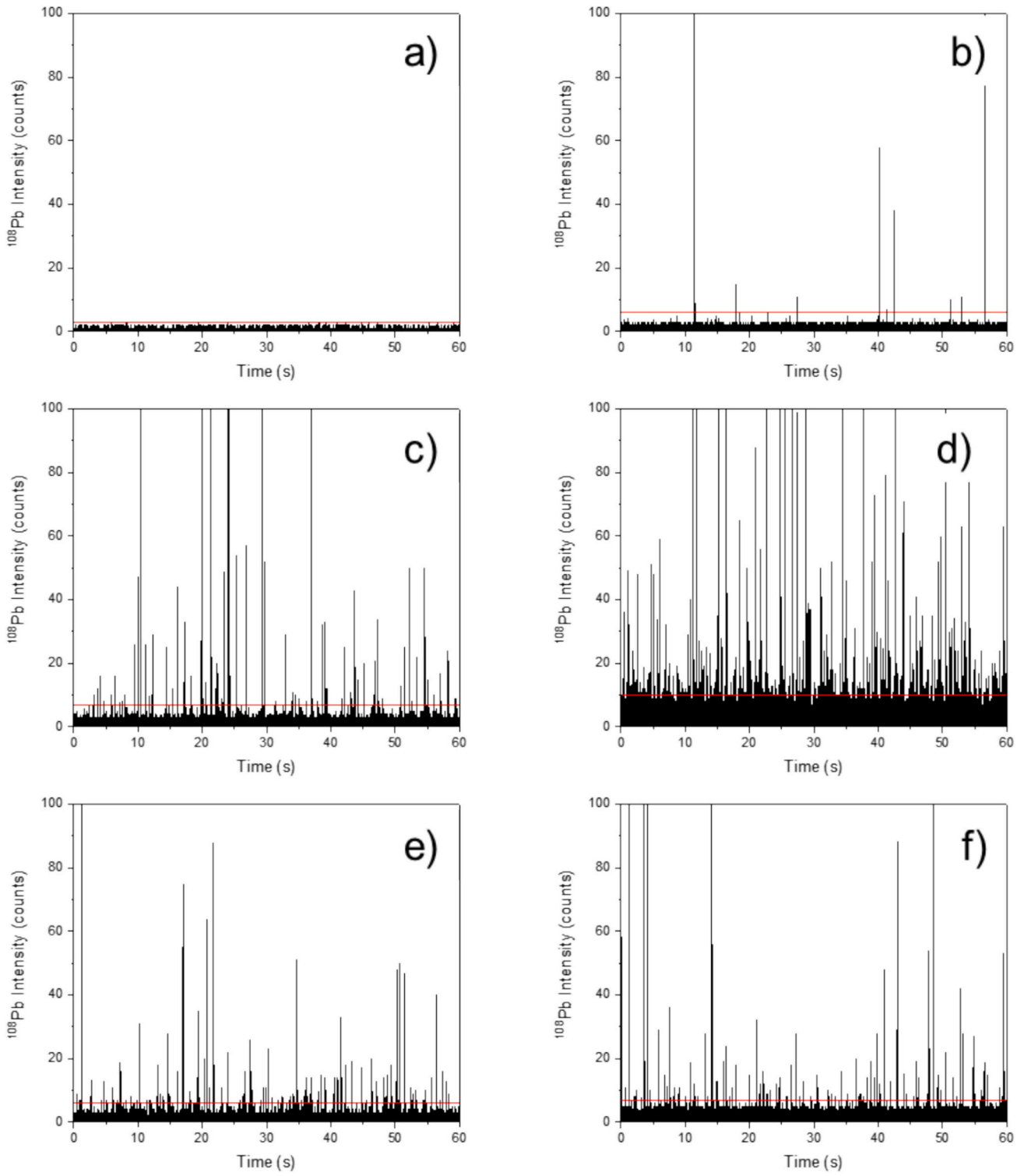

Fig. 2. ^{108}Pb SP-ICP-MS time scans for a) ultrapure water b) Blank (SGF + SFI); c) Ionian I; d) Ionian II; e) Tyrrhenian I; f) Tyrrhenian II. Red line: critical value for identification of particle events. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

previously discussed in Section 3.1. Fig. 3 shows the percentage of metal released per surface area unit (the total surface area of the mass of microplastic added to the synthetic digestion media was estimated considering the average size and density for each sample). In general, for most metals, the percentages in environmental samples are comparable to those obtained with the microplastics models, which suggests that surface area plays a relevant role on the release of metals during digestion process. In fact, predictions of the complete release of metals or metallic compounds from very small particles of nanometer to micrometer dimensions estimate that it could take hours to days, being nanoplastics exposed to the environment rapidly depleted of metal additives (Turner and Filella, 2021).

4. Conclusions

Microplastics collected in areas of the Calabrian coasts carry heavy

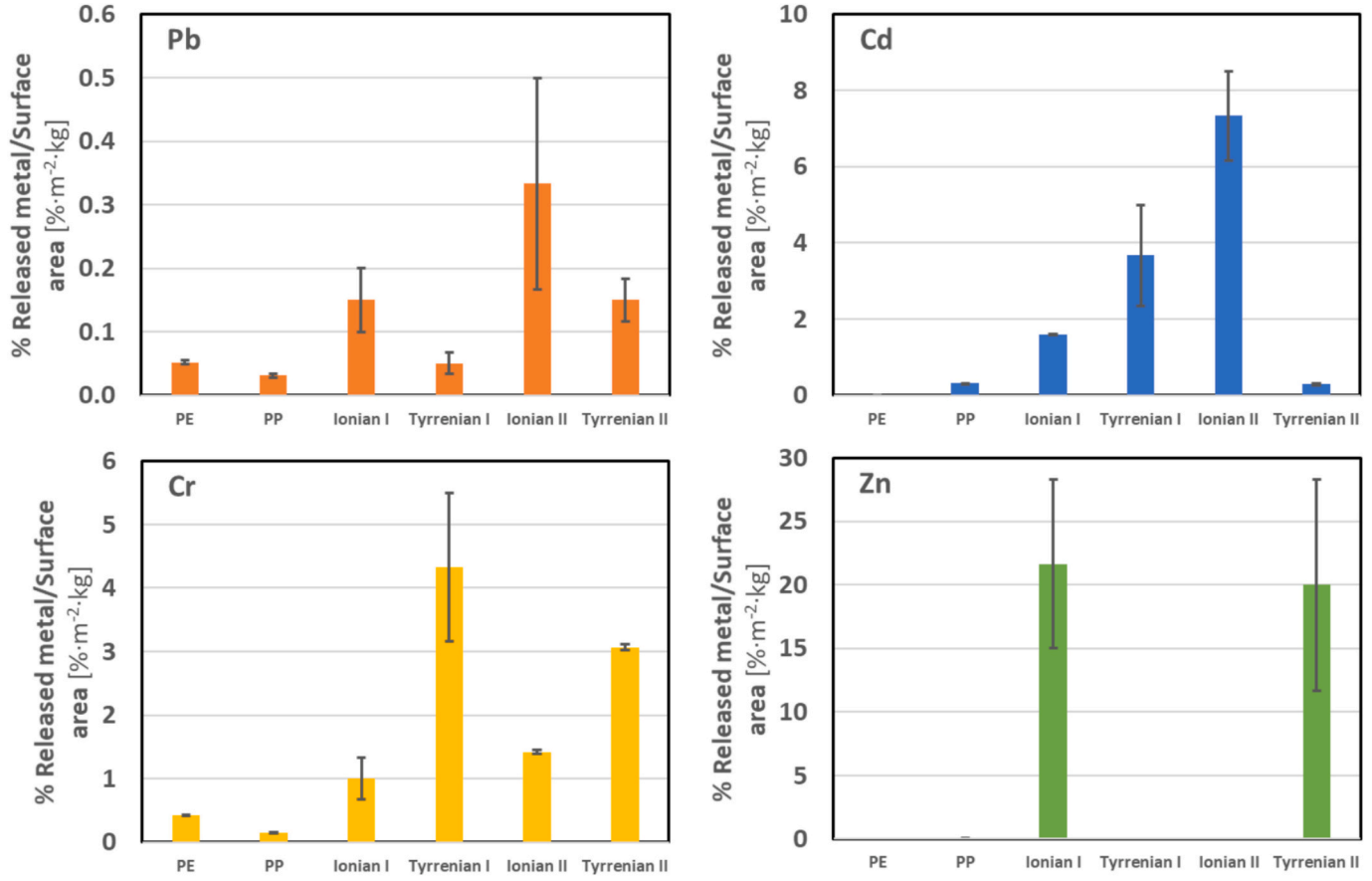

Fig. 3. Percentage of metal released at the end of the digestion (after both gastric and intestinal phases) normalized to the surface area.

metals which, through the digestion process of marine organisms, can reach humans via the food chain. Procedures based on gastric simulation in fishes represent a valuable tool for understanding these processes. Release of Pb, Cd, Cr or Zn during simulation digestion in fish has been demonstrated. Percentages of metal released may become relevant as microplastic sizes reduce, as suggested by the results obtained when the surface area is also considered. However, the chemical forms of these metals are also relevant to explain the release processes involved during digestive simulations. As expected, the use of additives based on metals as insoluble forms make them less bio-accessible, but the acidic medium during gastric phase or the presence of complexing agents during intestinal phase may favour these processes. In addition, the potential adsorption of metals on the microplastic surface once they are incorporated into the environmental systems, may increase the bioaccessibility of these metals, acting as vectors for these contaminants (having a Trojan horse effect). The release of metals presents either as additives or deposited on the microplastics surface, as particles, has been detected in the case of lead by SP-ICP-MS. The nature of these particles detected containing lead could not be determined, and they could correspond to lead released in its original form as additive, or even as part of a small fragment of microplastic released, although the formation of insoluble forms during digestion cannot be discarded. Obviously, the chemical form of the released metal will have a different impact on its bioavailability and potential toxicity. Further speciation studies are required to determine the true nature of these species and their actual impact on environmental systems and its bioaccessibility. Heavy metals, after being released from microplastics, accumulate in the tissues of living beings through biomagnification processes (Danovaro et al., 2023; Jara-Marini et al., 2020). After they enter into cells, these substances can interact with the cysteine residues of proteins, forming metal-thiol bonds, exploiting the thiol group present on the side chain of cysteine (Brunetti et al., 2024), altering the structure and functionality of proteins in cells. The main targets of heavy metals are membrane proteins, considered first-level targets due to their localization on the cell membrane (Scalise et al., 2019). In particular, the membrane transporter SLC22a4, also known as OCTN1, is an excellent target for heavy metals since it hosts, in its structure, 7 cysteine residues, four of which are located on the extracellular side and, therefore, easily reachable by xenobiotics. It is a protein involved in drug absorption processes but also in anti-inflammatory processes, and it has been shown that heavy metals can inhibit the functionality of this protein, seriously damaging the entire organism (Brunetti et al., 2024). In this regard, it is important to highlight that SLC22a4 has several orthologues in many vertebrates, including fish, including *Oncorhynchus mykiss*, whose digestive process has been used as an example in this work (Kitsanayanyong et al., 2021).

CRediT authorship contribution statement

Eduardo Bolea: Writing – review & editing, Writing – original draft, Funding acquisition, Formal analysis, Data curation. **Luana S. Brunetti:** Writing – original draft, Investigation, Formal analysis. **Isabel Abad-Alvaro:** Writing – review & editing, Software, Methodology, Investigation, Formal analysis, Data curation. **Emilio Cellini:** Writing – review & editing, Methodology, Conceptualization. **Silvestro A. Ruffolo:** Writing – review & editing, Methodology, Conceptualization. **Mauro F. La Russa:** Methodology, Conceptualization. **Francisco Laborda:** Writing – review & editing, Funding acquisition.

Funding

This work was funded by the Spanish Ministry of Science and

Innovation (MCIN/AEI/10.13039/501100011033), project PID2021-123203OB-I00, "ERDF A way of making Europe" and the Government of Aragon, project E29_23R.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Francisco Laborda reports financial support was provided by University of Zaragoza. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2025.118080.

Data availability

Data will be made available on request.

References

Abbasi, S., et al., 2018. Microplastics in different tissues of fish and prawn from the Musa Estuary, Persian Gulf. Chemosphere 205, 80–87.
Akhbarizadeh, R., Moore, F., Keshavarzi, B., 2018. Investigating a probable relationship between microplastics and potentially toxic elements in fish muscles from northeast of Persian Gulf. Environ. Pollut. 232, 154–163.
Arp, H.P.H., et al., 2021. Weathering plastics as a planetary boundary threat: exposure, fate, and hazards. Environ. Sci. Technol. 55 (11), 7246–7255.
Baalousha, M., et al., 2023. The elemental fingerprint as a potential tool for tracking the fate of real-life model nanoplastics generated from plastic consumer products in environmental systems. Environ. Sci. Nano 11, 373–388.
Barboza, L.G.A., et al., 2018. Marine microplastic debris: an emerging issue for food security, food safety and human health. Mar. Pollut. Bull. 133, 336–348.
Barnes, D.K., et al., 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 364 (1526), 1985–1998.
Béranger, et al., 2010. Impact of the spatial distribution of the atmospheric forcing on water mass formation in the Mediterranean Sea. J. Geophys. Res. 115.
Bettencourt, S., Costa, S., Caeiro, S., 2021. Marine litter: a review of educative interventions. Mar. Pollut. Bull. 168, 112446.
Browne, M.A., et al., 2011. Accumulation of microplastic on shorelines woldwide: sources and sinks. Environ. Sci. Technol. 45 (21), 9175–9179.
Brunetti, L.S., Piersante, C., La Russa, M.F., Cellini, E., Bolea, E., Laborda, F., Ruffolo, S. A., 2025. Examining Microplastics Along the Calabrian Coastline: Analysis of Key Characteristics and Metal Contamination. Environments - MDPI 12 (1) art. no. 4.
Brunetti, L.S., et al., 2024. OCTN1 (SLC22A4) as a target of heavy metals: its possible role in microplastic threats. Int. J. Mol. Sci. 25 (23).
Castro-Jimenez, J., et al., 2019. Macro-litter in surface waters from the Rhone River: plastic pollution and loading to the NW Mediterranean Sea. Mar. Pollut. Bull. 146, 60–66.
Catrouillet, C., et al., 2021. Metals in microplastics: determining which are additive, adsorbed, and bioavailable. Environ Sci Process Impacts 23 (4), 553–558.
Chen, X.J., et al., 2022. Bioaccessibility of microplastic-associated heavy metals using an in vitro digestion model and its implications for human health risk assessment. Environ. Sci. Pollut. Res. Int. 29 (51), 76983–76991.
Cheng, X., et al., 2010. Assessment of metal contaminations leaching out from recycling plastic bottles upon treatments. Environ. Sci. Pollut. Res. Int. 17 (7), 1323–1330.
Danovaro, R., et al., 2023. Bioaccumulation and biomagnification of heavy metals in marine micro-predators. Commun Biol 6 (1), 1206.
El Hadri, H., et al., 2020. Trace element distribution in marine microplastics using laser ablation-ICP-MS. Mar. Pollut. Bull. 160, 111716.
Jara-Marini, M.E., et al., 2020. Trace metal trophic transference and biomagnification in a semiarid coastal lagoon impacted by agriculture and shrimp aquaculture. Environ. Sci. Pollut. Res. Int. 27 (5), 5323–5336.
Kitsanayanyong, L., et al., 2021. Functional identification of ergothioneine transporter in rainbow trout (Oncorhynchus mykiss). Comp. Biochem. Physiol. B Biochem. Mol. Biol. 256, 110631.
Laborda, F., Bolea, E., Jimenez-Lamana, J., 2014. Single particle inductively coupled plasma mass spectrometry: a powerful tool for nanoanalysis. Anal. Chem. 86 (5), 2270–2278.
Lambert, S., Sinclair, C., Boxall, A., 2014. Occurrence, degradation, and effect of polymer-based materials in the environment. Rev. Environ. Contam. Toxicol. 227, 1–53.
Lebreton, L., et al., 2018. Evidence that the Great Pacific Garbage Patch is rapidly accumulating plastic. Sci. Rep. 8 (1), 4666.
Lechner, A., et al., 2014. The Danube so colourful: a potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. Environ. Pollut. 188 (100), 177–181.
Lehtimäki, E., Väisänen, A., 2016. Determination of metal concentrations in certified plastic reference materials after small-size autoclave and microwave-assisted digestion followed with inductively coupled plasma optical emission spectrometry. Spectrochim. Acta B At. Spectrosc. 127.
Masset, T., et al., 2021. In vitro digestion of tire particles in a fish model (Oncorhynchus mykiss): solubilization kinetics of heavy metals and effects of food coingestion. Environ. Sci. Technol. 55 (23), 15788–15796.
Scalise, M., et al., 2019. Exploiting cysteine residues of SLC membrane transporters as targets for drugs. SLAS Discov 24 (9), 867–881.
Schmid, C., Cozzarini, L., Zambello, E., 2021. Microplastic's story. Mar. Pollut. Bull. 162, 111820.
Shah, A.A., et al., 2008. Biological degradation of plastics: a comprehensive review. Biotechnol. Adv. 26 (3), 246–265.
Singh, A.K., Srivastava, S.C., 2021. Improved feeding strategy to optimize growth and biomass for up-scaling rainbow trout Oncorhynchus mykiss (Walbaum 1792) farming in Himalayan region. Aquaculture 542, 736851.
Soto-Navarro, J., et al., 2010. Estimation of the Atlantic Inflow Through the Strait of Gibraltar From Climatological and In-situ ADCP Data.
Turner, A., Filella, M., 2021. Hazardous metal additives in plastics and their environmental impacts. Environ. Int. 156, 106622.
Zettler, E.R., Mincer, T.J., Amaral-Zettler, L.A., 2013. Life in the "plastisphere": microbial communities on plastic marine debris. Environ. Sci. Technol. 47 (13), 7137–7146.