Research Paper

Selection of the optimal extraction protocol to investigate the interaction between trace elements and environmental plastic

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HIGHLIGHTS

- Different extractions are tested on environmental and pristine plastic samples.
- Sequential extractions are valid tools to investigate plastic-element interaction.
- Ageing affects elemental speciation on plastic surface, regardless of polymer type.
- The next steps for a tailored plastic-element extraction protocol are suggested.

GRAPHICAL ABSTRACT
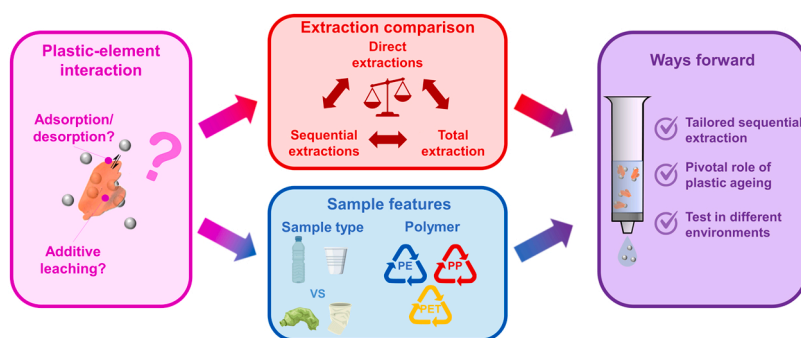
ARTICLE INFO

ABSTRACT

The interaction between environmental plastic and trace elements is an issue of concern. Understanding their interaction mechanisms is key to evaluate the potential threats for the environment. To this regard, consolidating confidence in extraction protocols can help in understanding the amount of different species present on plastic surface, as well as the potential mobility of trace elements present inside the plastic matrix (e.g., additives). Here we tested the efficacy of different reagents to mimic the elemental phases bonded to meso- and microplastic in the environment, in relation to the grade of ageing and the polymer composition. Results showed that a relatively high portion of trace elements is bonded in a weak phase and that other phases abundant in other matrices (e.g., oxides and bonded to organic matter) are only present to a limited degree in the plastic samples. The comparison of different sample types highlighted the important role of plastic ageing in governing interactions with trace elements, while the polymer composition has a limited influence on this process. Finally, the future steps toward a tailored extraction scheme for environmental plastic are proposed.

1. Introduction

The interaction between environmental plastics and trace elements in water has gained the attention of researchers. Plastics are known to impact freshwater and marine environments [34,39,46,6,7] and have been observed to effectively adsorb metal ions (including highly toxic species) in laboratory and mesocosms experiments [9,12]. Moreover, plastic can contain diverse mixtures of metal-containing additives [26, 33,62], which can be released to water after plastic dispersion and ageing [43]. The pressure posed upon aquatic ecosystems is of concern, especially in heavily polluted environments. Effects may include: changes in the environmental fate of toxic metals [12], alteration of natural biogeochemical cycling of micronutrients [58] and "vector" effects whereby microplastics ingested by biota enhance internal exposure to conveyed toxic metals [11].

Plastic degradation and biofouling are observed to promote both element adsorption from the environment [10,35,66] and releases from the plastic matrix, with exchange rates enhanced by up to one order of magnitude [22,43]. Water chemistry (i.e., pH, salinity and dissolved organic matter) also influence the adsorption/desorption processes of trace elements on plastic [12,38]. The interplay of these mechanisms on the interaction between elements-plastic and the consequences for ecosystem exposure are still insufficiently understood [9].

Validated analytical methods to analyze elements-plastic interactions are necessary to solve this gap. To this end, the development of a reliable extraction protocol is key [30,59]. Available studies typically utilize single-step extraction procedures [13,23,37], while the application of the Community Bureau of Reference (BCR) sequential extraction protocol was firstly applied on microplastic samples only in two recent studies [14,70].

Sequential extraction protocols present several advantages in comparison to other analytical methods, such as their easiness of application, cost efficiency and the availability of reagents and protocols already validated for other environmental matrices [50,52,9]. Their application on environmental plastic samples may however require additional optimization due to a series of challenges: i) the potentially low amount of available samples especially when focusing on meso- and microplastics; ii) the high heterogeneity of plastics found in the environment; iii) the expected relatively low concentration of extractable elements bound at the interface between water and plastics; iv) the lack of protocol specificity for the plastic matrix and v) difficulties related to sample handling which can result in artifact or sample losses (especially considering the separation of plastic from the extracting solution). Still, an evaluation of sequential extraction reliability to test the interaction of plastic with trace elements is not available in literature yet.

In this study, therefore, we evaluated and compared single and sequential extraction protocols to environmental plastic for the first time. Then, we tested these extractions on several samples to understand how plastic features (e.g., environmental ageing and polymer type) affect the fractionation of extracted elements. Through this study, we provide recommendations for the best extraction methodology, as well as knowledge and guidance on the applicability of sequential extractions in environmental plastic research.

2. Materials and methods

2.1. Experimental approach

The study design is selected to compare different elemental extraction approaches on a range of environmental and pristine plastic samples. Plastic objects included in the study were collected from beached litter. Pristine plastic materials were also collected to elucidate possible confounding factors affecting the release of elements not related to the adsorption in the environment (e.g., the release of additives). In total, 3 samples of polyethylene (PE), 3 of polypropylene (PP) and 3 of polyethylene terephthalate (PET) were tested (1 pristine and 2

environmental samples for each polymer type, details in Section 2.3.1). These polymers account for more than 44.5% of European plastic demand and are the most common polymers found in the environment [60].

All samples were ground to simulate meso-microplastic samples and subjected to a series of seven different extraction strategies, including: 4 single direct extraction (DE) approaches, 2 sequential extraction (SE) approaches and a total extraction (TE) approach. The extraction strategies were selected from available studies analyzing elements bound as specific chemical phases to particles or other solid environmental matrices. The present study focused on comparing these approaches and selecting an optimal extraction method for environmental plastics. The description of the different the extraction strategies is given in Table 1.

These extraction approaches were performed in an innovative system to avoid sample handling and loss (see Section 2.3.3). Each approach (DE1–4 and SE1–2) was compared with the TE. The latter is based on aggressive digestion with concentrated nitric acid capable of extracting the so-called residual fraction of elements, including those potentially originating from within the polymer matrix. The total mass of extracted elements was considered for ranking extraction performance and selection of the optimal method. The choice of method is also discussed in relation to the required workload and the completeness of the information yielded on the origin of the extracted elements, especially considering factors such as the polymer types and the level of environmental ageing.

2.2. Reagents and protocols

All extracting solutions were created with ultrapure water (obtained with a Sartorius Arium mini, 18.8 MΩcm resistivity). Nitric acid solutions were obtained by sub-boiling distillation of 65 wt% nitric acid (Carlo Erba reagents, Italy) using a Milestone (USA) DuoPUR system [45]. Ammonium nitrate and hydroxylamine hydrochloride solutions were prepared from analytical-grade compounds (Carlo Erba reagents, Italy). Acetic acid was obtained by dilution of glacial acetic acid (Carlo Erba reagents, Italy), while hydrogen peroxide 8.8 M was purchased from Sigma-Aldrich (USA). Standard solutions for inductively coupled plasma-mass spectrometry (ICP-MS) calibration were obtained by Sigma-Aldrich (USA) multi-elemental standard. All laboratory operations were performed under a laminar flow hood to avoid airborne contamination and all the lab containers were washed with 2% (v/v) nitric acid solution for 24 h to avoid metal contamination.

2.3. Plastic sample collection and analysis

2.3.1. Collection and pre-treatment

Environmental plastic items, including litter and mesoplastics with a visibly advanced ageing state, were collected manually on the surface of two beaches of an oligotrophic lake (Lake Como, Italy, 45°48′54′′ N – 9°04′30′′ E, [4]). Collected items were classified based on their category, color and polymer type [20]. To avoid possible elemental contamination, objects were collected and handled using nitrile gloves and stored in polyethylene bags. The pristine samples were purchased from market suppliers representing items belonging to the same categories, colors and polymeric composition as those collected from environment (Table 2).

All the specimens were rinsed with ultrapure water, ground with a commercial blender and sieved to a dimension < 2 mm. This operation yielded sufficient quantities of materials in the form of microplastics. The use of a blender with steel blades (which is likely to contaminate the samples with metals) was selected after the failure of other grinding methods (e.g., the grinding of plastic with an agate ball mill). Consequently, further control samples were added to assess the possible trace element contamination (see Section 2.3.4).

Table 1

Comparison of the different direct, sequential and total extraction protocols tested in this study, including extractable phase, method used and the rationale for the selection.

Extraction name	Focus of extraction	Method	Notes and Rationale
DE1	Extraction of physisorbed and readily soluble elements [24, 50]	Single extraction with ammonium nitrate 1 M	Ammonium nitrate was selected over e.g., sodium and calcium chloride [29,59] to avoid an excess of chloride in the final solution, known to negatively affect the quantification of elements such as Fe, Cr, V, As via ICP-MS [42]
DE2	Extraction of elements dissolving in slightly acidic condition	Single extraction with acetic acid 0.1 M	Method used in previous studies to determine the carbonate-bonded and bioavailable fraction [16,59,67]
DE3	Extraction of elements bonded to organic microlayer.	Single extraction with hydrogen peroxide 8.8 M	Method used in previous studies for the characterization of the element fraction bound to biological and organic layer surrounding plastics in the environment [55, 57,61]
DE4	Extraction of total adsorbed material	2% (v/v) nitric acid extraction after 10 min in ultrasonic bath at 120 W	This extraction method was reported to desorb all loosely bonded elements on PP and PVC after ageing in seawater [23]
SE1	Sequential extraction method (DE1-DE3 in sequence)	-	Assess the feasibility of gaining higher detail of information (compared to DE methods) on differential type of bonding for elements in the same plastic sample, without losing information due to low concentration and matrix effects of different reagents
SE2	Canonical BCR sequential extraction scheme [52]	Sequential extraction testing acetic acid 0.1 M, hydroxylamine hydrochloride 0.5 M and hydrogen peroxide 8.8 M in sequence	This is an inter-laboratory harmonized protocol for soils and sediments, and the only sequential extraction protocol tested on microplastics thus far [14,70]
TE	Total extraction	Acid digestion with 65 wt% nitric acid	Total extraction of the elements adsorbed and inside the polymer matrix, used as a reference to calculate the extraction efficiency [27,33]

Table 2

List of analyzed samples and their main features, including: the type of sample analyzed object category, the color and polymer composition.

Label	Type of sample	Object category	Color	Polymer
PE1	Pristine	Cap/lid	Blue	PE
PE2	Environmental	Cap/lid	Blue	PE
PE3	Environmental	Other	Gray	PE
PP1	Pristine	Crate and container	Transparent	PP
PP2	Environmental	Crate and container	White	PP
PP3	Environmental	Crate and container	Transparent	PP
PET1	Pristine	Bottle	Transparent	PET
PET2	Environmental	Bottle	Transparent	PET
PET3	Environmental	Bottle	Transparent	PET

surfaces were investigated using a Philips® (the Netherlands) Field Emission Gun-Scanning Electron Microscope (FEG-SEM), with a 20 keV beam under high vacuum conditions. Prior to SEM analysis, samples were uniformly covered with a ca. 5 nm thick gold layer using a Cressington (United Kingdom) 108 auto vacuum sputter coater to make the plastic surface more conductive and improve image quality.

The TE was performed through microwave-assisted acid digestion. About 60 mg of ground sample was weighed and inserted into a Teflon vessel and 4 mL of nitric acid 65 wt% were added. The samples were then digested in a ETHOS One Milestone (USA) microwave applying a temperature ramp reaching 180 °C in 10 min with a following isotherm of 15 min. The digested solution was then left to cool at room temperature before being diluted in ultrapure water for further analysis via inductively coupled plasma-mass spectrometry (ICP-MS, see Section 2.3.3 for details).

2.3.3. Experimental system for extractions

The proposed direct and sequential extraction approaches (DE1–4 and SE1–2) were performed using a set up validated with sediment samples using the BCR-701 certified reference material ([8,16]; recoveries are listed in Table S1). This procedure and equipment were introduced to reduce the handling of the sample (and therefore potential contamination) through the sequential extractions, limiting sample losses and facilitating the separation of solution for the following analysis of trace elements.

The extraction routine is schematically depicted in Fig. S1. About 60 mg of plastic was weighed and inserted into an empty solid phase extraction (SPE) polypropylene cartridge. The system was closed with a lid and placed horizontally on an orbital shaker at 150 rpm for 24 h (sufficient for reaching equilibrium, see Sections 2.3.4 and 3.1 for details). This setup was selected to ensure a continuous interaction between plastic particles and the extracting solutions, hardly reachable in static conditions due to the high hydrophobicity and buoyancy of plastic materials.

Next, the extract was filtered through the SPE guard polypropylene disk under vacuum. The filtered extract was then collected in plastic vials and analyzed by means of Thermo Scientific™ Icap-Q ICP-MS for different micronutrients (Mn, Fe, Co, Cu, Zn, Sr) and other potentially toxic trace elements (Al, Ti, V, Cr, Ni, As, Sn, Ag, Cd, Ba, Pb and U).

Following extraction, the plastic samples were then rinsed twice with ultrapure water for 15 min in an orbital shaker. The aqueous solution was eliminated by filtration. Before proceeding with sequential extraction, the solution used in the following step was used to condition the extraction cartridge (e.g., by reducing memory effects and adsorption of elements by the container walls). For this 4 mL were added for 15 min to the cartridge containing the plastic specimen and then discharged (after verifying it did not contain measurable element levels). Then, the following extraction step was implemented following the same routine.

2.3.4. Quality assurance and control (QA/QC) protocols

Several QA/QC protocols were tested, including calculation of method detection limits (MDLs), evaluation of potential contaminations

2.3.2. Sample characterization

All plastic specimens were analyzed using a Thermo Scientific™ (USA) Nicolet™ iS™ 10 ATR-FT-IR spectroscope for the determination of the polymer and the level of surface alterations linked to polymer degradation and/or biofouling. For each plastic specimen, 32 scans were performed in the spectral range 4000 cm^{-1}–650 cm^{-1}, with a resolution of 0.482 cm^{-1}. A background spectrum was collected before the analysis of every sample. The morphological characteristics of the plastic sample

(due to matrix effects and sample processing), replicability tests and extraction kinetics assessment.

A set of quality assurance samples were included in the analysis. Method blanks for every extraction solution and acid digestion were tested to verify matrix effects. Method detection limits for every extraction step were calculated as the average concentration of the method blanks plus three times the standard deviation of a set of 10 method blanks [64]. Then, procedural blanks were analyzed to assess the entity of contamination due to the grinding of plastic samples using a commercial blender: a set of replicates (n = 3) of ultrapure water was used to rinse the blender, the water was collected in vials and analyzed to assess this issue.

Reproducibility tests and extraction kinetics to check for equilibrium during the extraction time were performed in triplicate for PP3 and PE2 using the extractions DE1, DE2 and DE3. These QA/QC protocols were applied on a reduced sub-set of samples (including the more abundant ones) due to the limited sample amount collected. The maximum relative standard deviation measured for every element in the different extraction steps was applied as a confidence interval for each individual measurement. Regarding extraction kinetics, different batches were analyzed after 1, 6, 18, 24, 48, 72 h of extraction for each of the solutions listed above.

2.4. Data analysis

During data analysis values below the MDL were replaced with half of the MDL of each individual element [28]. Data were reported as extracted concentrations of elements (in $\mu g/cm^3$ of the extracted plastic). This unit of measure was used to scale all data composed by different polymer type, considering the notable difference in density values. Density values of 0.94 g/cm^3 for PE, 0.9 g/cm^3 for PP and 1.41 g/cm^3 for PET were selected as representative values of the objects composed by these polymers [5]. It can be argued that this approach is not ideal to represent extraction of elements bound to the surface of plastic, as the shape of different plastic specimens influences these values. However, the grinding of the specimen to a homogenous granular microplastic form permit this assumption. Extraction ratios were also calculated in order to compare the available fractions (extractable from the surface) to the total elemental content in the different samples. For this the following Eq. (1) was applied:

$$Extraction\,ratio\% = 100 \frac{[E]_{ext}}{[E]_{TE}} \qquad (1)$$

where $[E]_{ext}$ is the concentration of the extracted element with a given extraction approach (Table 1) and $[E]_{TE}$ is the total concentration of the same element (analyzed through the TE, Table 1) both expressed as $\mu g/cm^3$.

All datasets were evaluated for normality using a Shapiro-Wilk test prior to further analysis. Since assumptions of normality and homogeneity of variance were violated, the non-parametric Kruskal-Wallis test was used to validate differences between environmental and pristine samples, as well as among different polymers. The relation among the partitioning of elements obtained from SE1 and 2 in environmental and pristine samples was analyzed by principal component analysis (PCA). Descriptive statistics, non-parametric tests and PCA were computed using Origin 2018 software (OriginLab Corporation, USA).

Infrared spectral data were scaled (from 0 to 1) on the maximum absorbance peak and smoothed using the Savitzky–Golay filter (applying a window of 30 points). Different indexes were then obtained to compare spectral data more quantitatively, following the Eq. (2):

$$Index_b = \frac{A_b}{A_{ref}} \qquad (2)$$

Where $Index_b$ is the index of the specific FT-IR band, applied to different surface functional groups (namely, carbonyl group: 1715 cm^{-1},

hydroxyl group: 3500 cm^{-1}, amides: 1650 cm^{-1}, and polysaccharides: 1040 cm^{-1}); A_b indicates the absorbance value obtained at the specific wavelength of the analyzed functional group, and A_{ref} represents the absorbance values at specific reference bands, depending on the sample polymer: the C-H band at 1465 cm^{-1} for PE [41], the reference peak absorbing at 1892 cm^{-1} for PP [32] and the unalterable band absorbing at 721 cm^{-1} for PET [3].

3. Results and discussion

3.1. QA/QC results

The analysis of MDLs using different extractions (Table S2) reveals results comparable with other environmental matrices (e.g., sediments, [16]), with a clear variance related to the elemental sensitivity of analytical instrumentation. Lower performances are instead observed considering the replicability in different aliquots of the same plastic samples, showing on average 15% relative standard deviation (Table S3). This issue has been already observed in assessing the total amount of trace elements in plastic samples and is due to the intrinsic inhomogeneity of plastic matrix [27]. However, this level of precision results reliable for mild extraction of metals from solid fractions [50]. The procedural blanks (i.e., water added in the blender) show concentrations below the MDL, ruling out potential contamination during the grinding of plastics (Table S4).

Analyzing instead the extraction kinetics (Fig. S2 and Table S5), most of the extractants were above 85% of the equilibrium concentration after 24 h under shaking. In some cases, a decline of about 15% in the extractant concentration was observed after the maximum at 24 h. Therefore, 24 h of shaking was selected as equilibration time for all extraction experiments.

3.2. Total and extractable abundance of elements in plastic

A summary of the concentration of individual elements measured through the different extraction approaches (all DEs and SEs), as well as their total concentrations from acid digestion (TE) are presented in Table 3. Al, Mn, Fe, Zn, Sn and Ba were the most abundant elements in the extracts of all plastic specimens, considering both TE and DEs. Eight elements (namely V, Cr, Co, As, Ag, Cd, Pb and U) were instead found below MDL in more than 50% of the samples (Table 3).

The TE values of different analyzed elements (e.g., Ba, Zn and Fe) show a wide variance, with values ranging up to 3 orders of magnitude. This is in accordance with previous literature testing the total amount of metals present in plastic litter and objects [27,33]. We observed instead lower values of different (toxic) elements such as Cd, Pb, Cr, As and Ni: this indicate that the plastic matrix can contain a variable amount of metal additives depending on the use of the final product [62].

From now on, the discussion of protocols tested and differences among samples will be focused on the more abundant elements (namely Al, Mn, Fe, Zn, Sn and Ba) to more specifically observe the main differences observed in the samples. Incidentally, some of these elements present important function as micronutrients (such as Mn, Fe and Zn), while the others (especially Sn) can present several toxic effects at increasing concentration. Most of the other elements show instead a good portion of samples close to or below the MDLs, negatively affecting the measurement accuracy and the statistical evaluation of results.

3.3. Comparison among extractions

3.3.1. Extraction ratio comparison

The comparison of extraction ratios obtained from different DEs and SEs is given in Fig. 1. This analysis provides insights on the potential differences in the bonding strength of elements on plastic. Different DE methods yielded extraction ratios with a similar median (ranging 2 –

Table 3
Descriptive statistics (25th–75th percentiles, median and maximum) for extractable metals (from the different DEs and SEs) and total concentration (from TE) in all samples (in μg/cm^3). Raw data are instead listed in Table S6.

Element	Extraction	25th percentile	Median	75th percentile	Maximum
Ag	TE	0.1	0.21	0.71	3.42
	DE	<MDL	<MDL	0.002	0.03
Al	TE	12.58	74.42	195.81	250.32
	DE	0.06	0.38	2.36	5.18
As	TE	<MDL	0.02	0.07	0.21
	DE	<MDL	<MDL	0.03	0.13
Ba	TE	<MDL	1.18	1.88	1124.88
	DE	0.16	0.52	32.52
Cd	TE	<MDL	0.02	0.06	0.12
	DE	<MDL	<MDL	0.01	0.03
Co	TE	<MDL	0.09	0.17	0.68
	DE	<MDL	<MDL	0.01	0.03
Cr	TE	0.26	0.51	1.12	1.62
	DE	<MDL	<MDL	0.06	2.14
Cu	TE	0.42	0.93	7.78	30.42
	DE	<MDL	0.06	0.2	0.9
Fe	TE	30.16	71.71	104.08	195.18
	DE	0.22	0.98	4.3	15.16
Mn	TE	0.54	1	5.41	9.49
	DE	0.07	0.15	1.75	6.3
Ni	TE	0.48	0.76	1.01	1.42
	DE	<MDL	0.12	0.21	0.78
Pb	TE	0.09	0.16	0.45	0.83
	DE	<MDL	<MDL	0.09	0.39
Sn	TE	13.82	33.9	53.43	77.95
	DE	0.18	0.63	3.34	53.77
Sr	TE	0.06	0.17	0.78	19.86
	DE	<MDL	0.03	0.18	0.85
Ti	TE	1.12	2.56	9.85	27
	DE	<MDL	<MDL	0.02	0.12
U	TE	0.003	0.006	0.008	0.012
	DE	<MDL	<MDL	<MDL	0.004
V	TE	<MDL	0.04	0.08	0.18
	DE	<MDL	<MDL	0.01	0.01
Zn	TE	3.95	4.66	72.49	609.94
	DE	<MDL	0.66	0.96	4
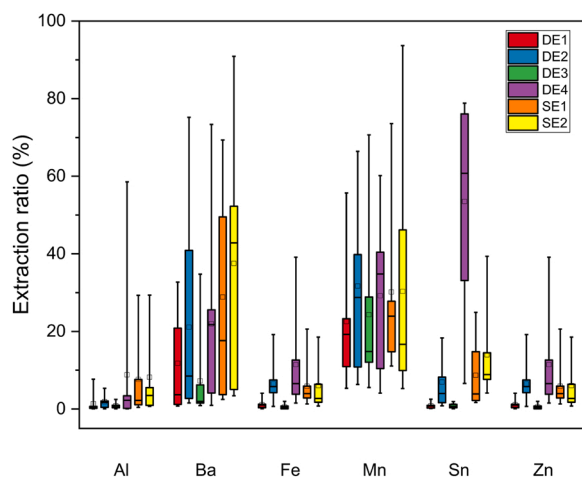
Fig. 1. Box and whisker plot of the extraction ratios obtained with DE1–4 and SE1–2 in all analyzed samples (both pristine and environmental). Data for DE1–4 indicate the values obtained in different samples, while SE1–2 include the sum of the three different extraction steps. Boxes indicate the 25th–75th percentile range, whiskers indicate the minimum–maximum range, squares indicate the mean value and horizontal lines the median.

10%), with the exception of Sn, Ba and partly Fe with DE4 and DE2. These elements seem more prone to be released in an acid environment. The generally low median value of extraction ratios indicates that DE approaches can extract mainly metals associated to plastic surface through weak bonds.

As expected, the two tested SEs extracted a higher fraction of the total elements present in plastic for most of the cases. There are few exceptions related mostly with DE4 (the extraction with 2% nitric acid), which extracted slightly higher amounts of Sn, Fe, Al and Zn. This can be due to a partial digestion of the polymer matrix by nitric acid. This reagent can in fact degrade the surface of (especially aged) polymers [10, 65], possibly dissolving elements contained in the polymeric amorphous phase of plastic objects.

Fig. 1 clusters data from all polymer types and plastic specimens (including both pristine and environmental samples); hence, the large variance in extraction ratio results should not surprise. The data breakdown for environmental and pristine plastic shows a slightly lower variance when comparing pristine and environmental plastic only (Fig. S3). This indicate that also plastic matrix can release trace elements heterogeneously, as a further confirmation of its complex structure.

3.3.2. Comparison of sequential extractions

The elements extracted through SE1 and 2 approaches (Fig. 1) present higher extraction ratios than DE1-DE3 methods and a comparable percentage with DE4 in most of the cases. It can be argued that SEs adequately represent the bulk of readily exchangeable pool of elements bounded to the plastic surface.

The two tested SEs highlighted relevant trends when observing their average partition in the different extraction steps (Fig. 2), further supporting their use to gain insights in plastic-trace element interactions. In SE1 the first extraction step (ammonium nitrate 1 M) alone yields 25% of the total extractable Mn, as well as more than 5% of Zn and about 10% of Ba. Moreover, the combination of step 1 (ammonium nitrate 1 M) and step 2 (acetic acid 0.1 M) reaches ratios comparable to the to the efficiency of the first step of BCR protocol (SE2), which is a direct extraction with Acetic acid 0.1 M (as the second step of SE1, Fig. 2). This suggests a good replicability of the 2 different SE methods analyzed here. Acetic acid extraction with SE2, moreover, counts for more than a half of the portion extracted with the whole protocol. This is in accordance with the limited results recently published applying BCR extraction protocols to plastic samples [14,70]. These observations highlight that weak interaction of plastic with elements can be expected, especially in environmental samples, and reagents extracting these phases play a pivotal role towards a plastic-specific extraction protocol.

Lower portions of most analyzed elements are instead observed in the hydroxylamine hydrochloride 0.5 M extraction step of SE2, compared with previous studies [14,36]. This step extracts mainly Ba, Fe and Sn only, while for other elements it represents the least abundant among the tested reagents in SE2 (Fig. 2b), as an index of limited oxide species present on the surface of the analyzed samples. Finally, there are relatively low extraction rates obtained by the hydrogen peroxide 8.8 M step in both extracting protocols, suggesting that a low portion of elements is strongly bonded to organic matter. This is unexpected considering the pivotal role played by biofouling of plastic in regulating adsorption of ionic compounds and metals from the surrounding environment [1,54,9]. However, it can indicate that biofilms on plastics yields loosely adsorbed trace elements, such as those complexed by extracellular polymeric substances and not stored in the biofilm matrix. Previous work confirm the dissolution of micronutrients in slightly acidic conditions from biofilm samples [31].

3.4. Role of plastic polymer and ageing in the speciation of elements

3.4.1. Differences in extractable elements

Fig. 3a shows that environmental plastic samples generally yield significantly ($p < 0.05$) higher loads of most elements measured in the
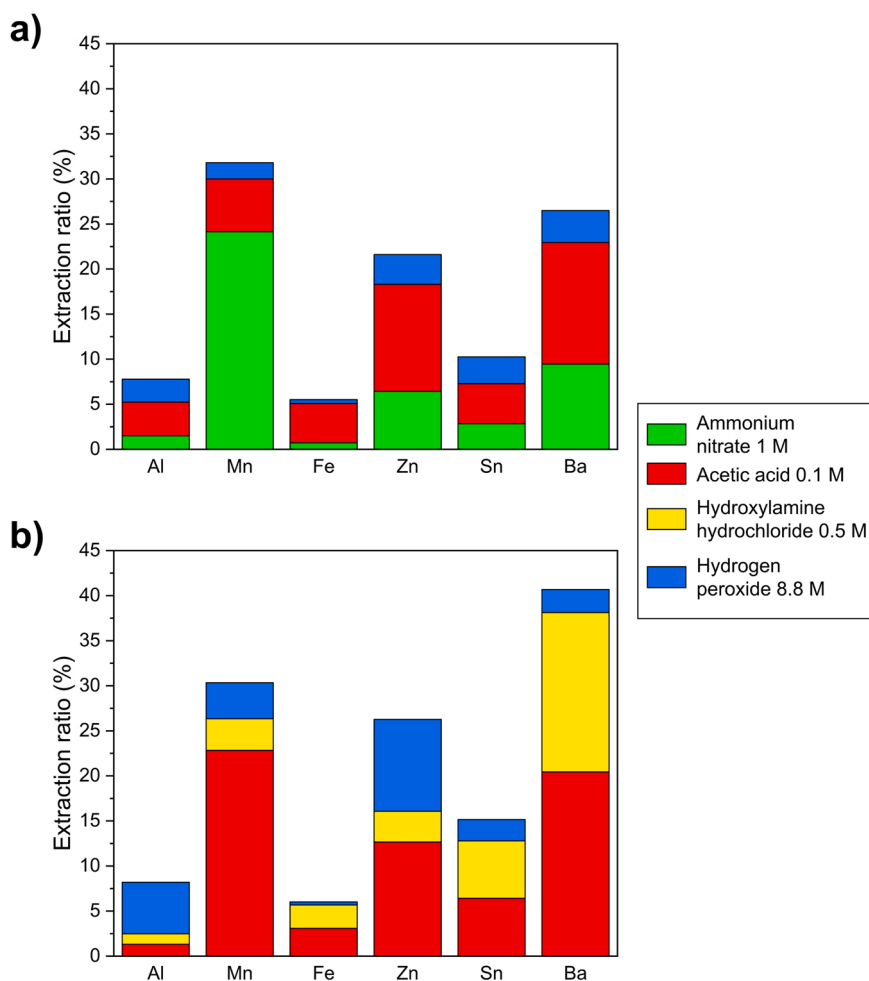
Fig. 2. Percentage of extracted elements (average of all analyzed samples) in every step of SE1 (panel a) and SE2 (panel b), color-coded with the different reagent used.

extracts. Elements are up to ten times more concentrated in the extracts from environmental plastics than from pristine materials. This is in accordance with previous studies that analyzed the total amount of metals in plastic, whereby ageing in the environments resulted in enrichment [69]. Tin and Ba showed a different behavior compared to other measured elements, with a more skewed distribution. Because of this the differences between pristine and environmental plastic for these elements were not significant.

Materials made of different polymers (considering both environmental and pristine samples) instead displayed different patterns for different elements (Fig. 3b). The concentration of Al, Mn and Zn were lower in the extracts from the PET specimens, while non-significant differences were observed in PP and PE. Barium had anomalously high concentrations in PP specimens compared to all other samples (Figs. 3b and S4b). These values are mostly related to sample PP2 (Table S6), a white plastic object (Table 2). This color is often obtained in plastics by the addition of barium sulfate [63]. This suggests that part of Ba present in the polymer matrix can migrate to the surface and be extracted through mild extraction processes. The high Ba concentration observed in this sample also by TE (Table 3 and Fig. S4) corroborates this hypothesis. Finally, Sn and Fe are relatively uniform across all polymer types. Tin was abundant in the extract of all polymer types, as well as in their TE values (Fig. S4). Likely this element is contained in the pool of additives present in all analyzed specimens. Organometallic tin is in fact abundantly added in plastic objects as a heat stabilizer and biocide [26, 33,62].

PCA allows patterns associated to polymer and sample origin (environmental or pristine, Fig. 4) to be explored. The PCA was fed with data on extracted elements by SE1 and SE2. Principal components 1 and 2 (cumulatively explaining 59% of the total variance) evidence increasing concentrations of all elements along component 1 (with loading values ranging from 0.23 to 0.51). The environmental samples are more scattered and have a higher enrichment of extractable elements, as showed by a range of score values for component 1 between − 1.3 and 8.2. Pristine samples show instead a range between − 1.31 and 0.8. Element distribution among different polymer types again shows a less clear-cut trend, indicating a weaker influence of polymer type in defining elemental speciation on the plastic surface. As a further note, PCA does not show specific clustering of samples in relation of the different extraction reagent tested (Fig. S5). This indicates that plastic-trace element interaction is driven by complex processes which are sample specific and likely related to different environmental conditions.

3.4.2. Surface characterization of samples: the effects of plastic ageing

FT-IR and SEM analysis help to characterize the ageing of environmental plastics at the surface of the specimens [10,15], which may affect element adsorption. Starting with the FT-IR results, peaks in the -OH (3500 cm^{-1}) and -CO (1715 cm^{-1}) region appear in environmental plastic samples, while they are absent in the spectra of pristine plastic (Fig. S6). These indicate varying levels of surface oxidation induced by plastic ageing [41]. Some of the analyzed environmental samples (namely PE2 and PP3) show bands typical of biofouling coverage,
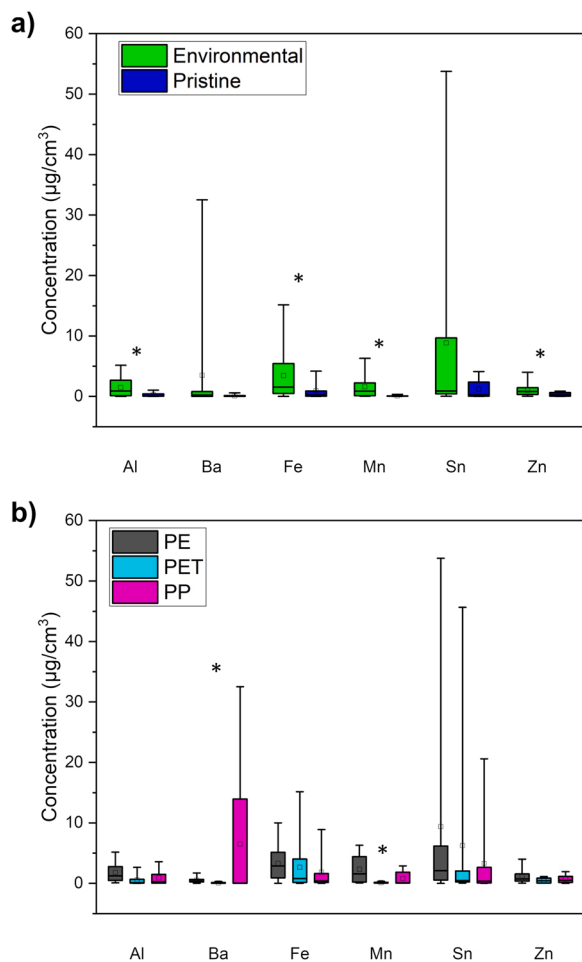
Fig. 3. Box and whisker plot of all DEs, aggregated for environmental samples and pristine ones (panel a), and aggregated for different composing polymer (panel b). Boxes indicate the 25th–75th percentile range, whiskers indicate the minimum–maximum range, squares indicate the mean value and horizontal lines the median. Significantly different data ($p < 0.05$) after Kruskal-Wallis test are indicated by asterisk.
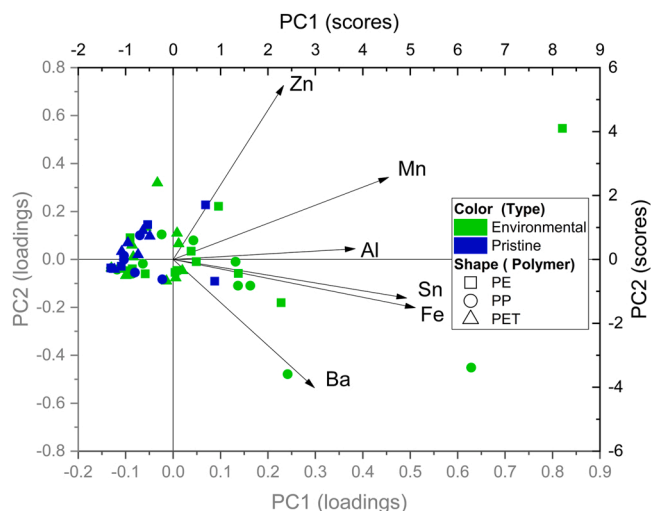
Fig. 4. PCA biplot of principal component (PC) 1 and 2 (explaining 39% and 20% of the total variance, respectively). Scores of extracted elements with the sequential extractions are reported and labeled with different shapes considering the samples polymer and with different colors considering environmental and pristine samples.

including the C-O band of polysaccharides at 1040 cm^{-1} and the bands typical of primary (1650 cm^{-1}) and secondary (1550 cm^{-1}) amides [51] which are obviously also absent in pristine plastic samples. These effects are evident comparing the index values of different bands in environmental and pristine samples composed by PP and PE (Fig. S7). The bands are instead less pronounced in the PET samples (Fig. S7c). Since PET specimens also present lower concentrations of extractable trace elements (Fig. 3b), we postulate that changes in surface functional groups of polymers after ageing affects the adsorption of elements. This is in line with results from earlier studies [35,36].

The SEM micrographs of environmental and pristine samples show several similar modifications of surface morphology after ageing in the environment, regardless of the polymer type (Figs. 5 and S8). All environmental samples presented a rougher and more complex nanostructure of the surface compared to pristine plastic, with more abundant cracks and pits which can likely enhance the reactive surface area at the polymer-water interface. Filamentous-shaped structures are also often visible on the surface of environmental plastics, which are ascribable to biofilm residuals [19]. As a further confirmation, the residual of diatom frustules deposed on the surface of plastic was observed in two environmental samples (Figs. 5d and S8a).

The differences between pristine and environmental samples, as well as their surface characterization results, confirm that environmental ageing processes are key features for plastic interaction with trace elements. This can apply also to other organic compounds which may interact with plastics in the environment [17]. It is recommended to consider surface characteristics and the level of weathering and oxidation as potentially relevant factors during assessments of ecological and human risk from microplastic exposure. This obviously calls for the need of adequate reference materials to feed these assessments. Both induced changes in surface charge, oxidation and wettability changes following polymer ageing facilitate the adsorption of dissolved ions by plastic particles [44,48,53]. These processes also facilitate the release of additives [40] and can explain the different quantity of elements observed between the environmental and pristine specimen analyzed here. Biofouling processes also play a pivotal role for the enrichment of elements on the plastic surface [25,54,56]: biofilms on plastics were observed to enhance the adsorption of micronutrients and trace elements in both mesocosm and environmental contexts [18,47,54,68].

Our results support that environmental ageing is a key process in the interaction between microplastics and trace elements in water. The increase of surface area induced by fragmentation, the increase of oxygen-containing functional groups and the appearance of new surface groups after biofouling (i.e., polysaccharides and amides) are the driving processes in the increased adsorption of elements from the water column.

3.5. Future steps toward a harmonized extraction scheme

The results described above indicate that the study of elemental speciation on environmental plastic can help to better evaluate adsorption-desorption equilibria of trace elements and help addressing possible environmental sources vs endogenous (e.g., originated from the polymer matrix) sources.

Similar to previous studies [14,70], we observed here that a large proportion of the elements associated with plastics are loosely bonded and acid-dissolvable species. However, we observed a notable portion of elements extracted only by TE (i.e., limitedly or not available), as well as a general low abundance with the hydroxylamine hydrochloride 0.5 M extraction (step 2 of SE2) and with hydrogen peroxide 8.8 M one (step 3 of SE1 and SE2, Fig. 2).

There is, however, a significant variance among the different samples (Fig. 1), suggesting that plastic-trace element interaction is a complex environmental process. Different environmental factors of the surrounding environment affect the elemental speciation on plastic, similar to other particulate matter in waters [21]. For example, redox processes are known to affect the elemental speciation through the water column, affecting the
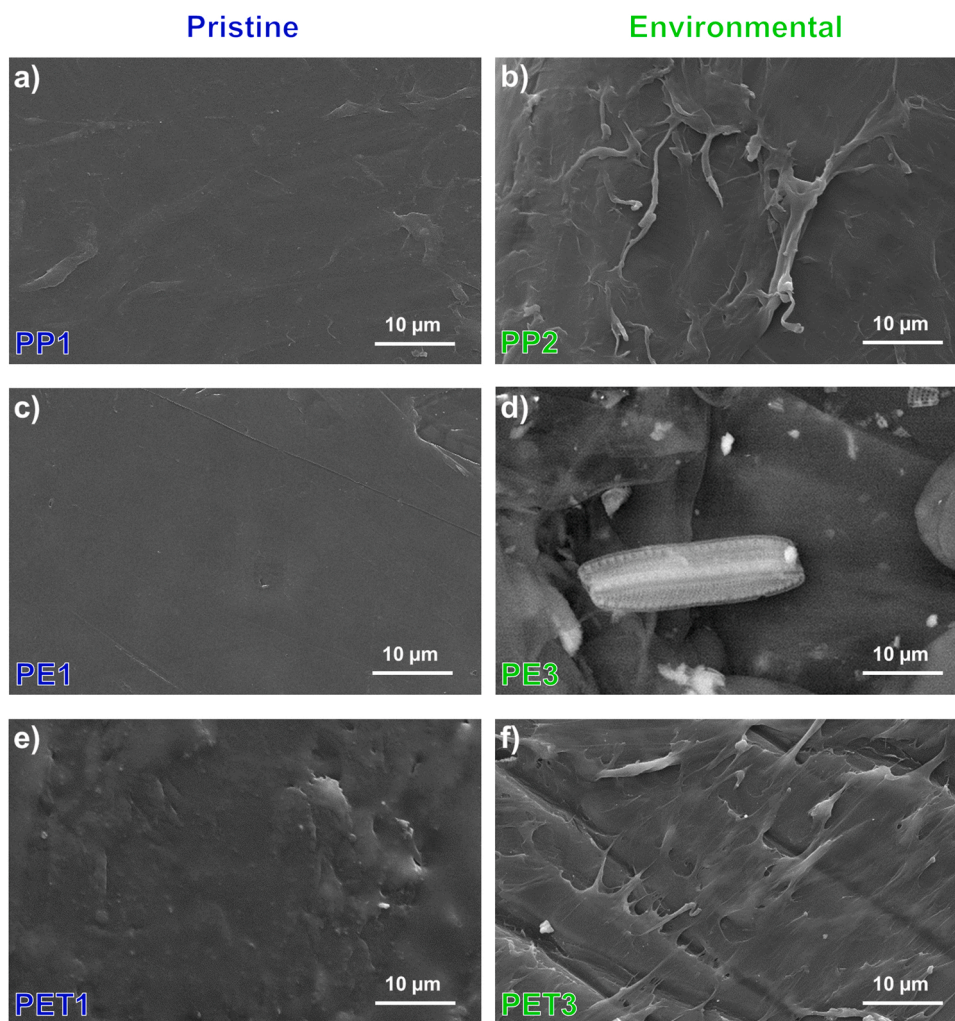
Pristine   **Environmental**

Fig. 5. SEM micrographs of pristine (a, c, e) and environmental samples (b, d, f). In sample PE3 (panel d), the residue of a diatom frustule is well visible. Other pictures are listed in Fig. S8.

species dissolved or present in particulate matter and colloids [2]. Therefore, future strategies will include similar analyses on macroscopic plastic litter in different water environments (e.g., along a depth gradient) to verify specific trends in elemental speciation derived by surrounding water chemical conditions.

Considering these multiple and complex factors of variability in metal speciation, the SE approaches described here represent a valid option for a broadly comprehensive assessment of the surface-bound element pool. Relying on a single DE or a direct TE approach provide less information about the readily exchangeable pool of elements (see for example the abundant values of TEs in Fig. S4 compared to DEs in Fig. 3), possibly leading to an overestimation of the ecological risk [9].

Streamlining of SEs (for example through a procedure as illustrated in this paper, Fig. S1) can reduce the extra effort and costs required for a sequential multi-step process, compared to DE approaches. This setup also permits the use of a low amount of sample to perform the extraction (60 mg of sample), permitting the possibility to perform a sequential speciation scheme while avoiding sample loss in the filtration and washing phases (other reports show a range generally between 100 and 500 mg of sample; [13,14,59]). This sample size is still high for a real microplastic environmental sample and requires further improvements for the application in monitoring programs, but it is worth considering that sequential extractions are quasi-non-destructive methods enabling the sample characterization after the extraction steps (e.g., through FT-IR or Raman spectroscopy).

The analysis of elemental concentrations from TE (through concentrated acid digestion) can yield useful information on the extractable fraction from SE or DE approaches, to draw insights on the distribution and sources of elements found in environmental plastics (especially in the comparison of elements possibly adsorbed from the environment vs those present in the plastic matrix). In summary, to establish a reliable and informative extraction approach that assesses the interaction of elements between plastic and the environment, one should consider: a) a sequential extraction protocol focused on the more labile forms of bonding (as SE1 tested in this study) followed by total digestion (TE); or b) a single step direct extraction of labile and/or acid dissolvable elements (DE1 or DE2 tested in this study) followed by complete acid digestion if the application of the system proposed in this study (see Section 2.3.3) is not feasible. The second approach will clearly reduce the available information on elemental speciation but avoids problems in sample handling and consequent sample contamination (or loss).

Finally, the low concentration of elements on environmental plastic may still present a challenge, as confirmed by the number of samples showing concentrations below the MDL. This calls for solid quality assurance procedures to ensure robust and accurate analyses. However, the application of sequential extractions can shed light on the interaction with plastics of more abundant elements anyway (e.g., micronutrients).

4. Conclusions

This study explores the potential of single and sequential extractions to analyze specific fractions of trace elements bonded to plastic particles,

addressing the influencing factors (i.e., polymer type and environmental ageing). Several reagents were tested both as single extracts and in sequential extraction schemes, and an experimental setup for extraction used for sediments is firstly reported to facilitate sample handling and avoid sample loss. The findings of this study helped to further evidence the likelihood of enrichment in trace elements on plastic after its dispersion in the environment and highlight their most likely sources. Our results also show that extraction schemes can be a feasible and cost-effective tool for understanding plastic-trace element interactions in an environmental context. The application of a sequential extraction scheme focused on weakly bonded elements (i.e., SE1) followed by an acid digestion (TE) is the suggested way forward to shed light on the mechanisms underlying this environmental process. Still, the limited sample amount imposed by typical microplastic samples and the high heterogeneity of this matrix present notable challenges for their wider application. Next steps in the fine tuning of extraction techniques towards a harmonized protocol include the analysis of environmental plastics sampled from different lake waters or along a depth gradient to better investigate the role of the surrounding environment on elemental speciation and a direct comparison with other instrumental methods [49] for a stronger validation of these approaches.

Environmental implication

Plastic pollution is a known environmental threat. Most of its environmental consequences are yet still to be understood, such as the interaction of plastic with trace elements. A very limited set of analytical approaches has been tested so far to evaluate this interaction, hampering an affordable ecological risk assessment. Therefore, this study aims to shed light on how different extraction methods provides specific information about the origin of elements extractable from environmental plastics through the comparison of different extraction protocols. This comparison is a first, important step toward a specific plastic-trace element extraction protocol.

CRediT authorship contribution statement

Gilberto Binda: Funding acquisition, Investigation, Writing – original draft. **Stefano Carnati**: Investigation, Formal analysis. **Davide Spanu**: Formal analysis, Data curation. **Arianna Bellasi**: Investigation, Writing – review & editing. **Rachel Hurley**: Conceptualization, Writing – review & editing. **Roberta Bettinetti**: Supervision, Data curation. **Damiano Monticelli**: Conceptualization, Formal analysis. **Andrea Pozzi**: Data curation, Writing – review & editing. **Luca Nizzetto**: Funding acquisition, Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data are available as supplementary material attached to the paper.

Acknowledgments

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.131330.

References

[1] Ahamed, T., Brown, S.P., Salehi, M., 2020. Investigate the role of biofilm and water chemistry on lead deposition onto and release from polyethylene: an implication for potable water pipes. J Hazard Mater 400, 123253. https://doi.org/10.1016/j.jhazmat.2020.123253.

[2] Baalousha, M., Stoll, S., Motelica-Heino, M., Guigues, N., Braibant, G., Huneau, F., et al., 2019. Suspended particulate matter determines physical speciation of Fe, Mn, and trace metals in surface waters of Loire watershed. Environ Sci Pollut Res 26, 5251–5266. https://doi.org/10.1007/s11356-018-1416-5.

[3] Bai, X., Li, F., Ma, L., Li, C., 2022. Weathering of geotextiles under ultraviolet exposure: a neglected source of microfibers from coastal reclamation. Sci Total Environ 804, 150168. https://doi.org/10.1016/j.scitotenv.2021.150168.

[4] Bellasi, A., Binda, G., Boldrocchi, G., Pozzi, A., Bettinetti, R., 2022. What are lake beaches made of? An assessment of plastic beach litter on the shores of Como Bay (Italy). Appl Sci 12, 5388. https://doi.org/10.3390/app12115388.

[5] Bellasi, A., Binda, G., Pozzi, A., Boldrocchi, G., Bettinetti, R., 2021. The extraction of microplastics from sediments: an overview of existing methods and the proposal of a new and green alternative. Chemosphere 278, 130357. https://doi.org/10.1016/j.chemosphere.2021.130357.

[6] Bellasi, A., Binda, G., Pozzi, A., Galafassi, S., Volta, P., Bettinetti, R., 2020. Microplastic contamination in freshwater environments: a review, focusing on interactions with sediments and benthic organisms. Environments 7, 30. https://doi.org/10.3390/environments7040030.

[7] Binda, G., Bellasi, A., Spanu, D., Pozzi, A., Cavallo, D., Bettinetti, R., 2021. Evaluating the environmental impacts of personal protective equipment use by the general population during the COVID-19 pandemic: a case study of Lombardy (Northern Italy). Environments 8, 33. https://doi.org/10.3390/environments8040033.

[8] Binda, G., Pozzi, A., Livio, F., Piasini, P., Zhang, C., 2018. Anomalously high concentration of Ni as sulphide phase in sediment and in water of a mountain catchment with serpentinite bedrock. J Geochem Explor 190, 58–68. https://doi.org/10.1016/j.gexplo.2018.02.014.

[9] Binda, G., Spanu, D., Monticelli, D., Pozzi, A., Bellasi, A., Bettinetti, R., et al., 2021. Unfolding the interaction between microplastics and (trace) elements in water: a critical review. Water Res 204, 117637. https://doi.org/10.1016/j.watres.2021.117637.

[10] Binda, G., Zanetti, G., Bellasi, A., Spanu, D., Boldrocchi, G., Bettinetti, R., et al., 2023. Physicochemical and biological ageing processes of (micro)plastics in the environment: a multi-tiered study on polyethylene. Environ Sci Pollut Res 30, 6298–6312. https://doi.org/10.1007/s11356-022-22599-4.

[11] Bradney, L., Wijesekara, H., Palansooriya, K.N., Obadamudalige, N., Bolan, N.S., Ok, Y.S., et al., 2019. Particulate plastics as a vector for toxic trace-element uptake by aquatic and terrestrial organisms and human health risk. Environ Int 131, 104937. https://doi.org/10.1016/j.envint.2019.104937.

[12] Cao, Y., Zhao, M., Ma, X., Song, Y., Zuo, S., Li, H., et al., 2021. A critical review on the interactions of microplastics with heavy metals: mechanism and their combined effect on organisms and humans. Sci Total Environ 788, 147620. https://doi.org/10.1016/j.scitotenv.2021.147620.

[13] Catrouillet, C., Davranche, M., Khatib, I., Fauny, C., Wahl, A., Gigault, J., 2021. Metals in microplastics: determining which are additive, adsorbed, and bioavailable. Environ Sci Process Impacts 23, 553–558. https://doi.org/10.1039/D1EM00017A.

[14] Chen, G., Fu, Q., Tan, X., Yang, H., Luo, Y., Shen, M., et al., 2022. Speciation and release risk of heavy metals bonded on simulated naturally-aged microplastics prepared from artificially broken macroplastics. Environ Pollut 295, 118695. https://doi.org/10.1016/j.envpol.2021.118695.

[15] Chen, Q., Wang, Q., Zhang, C., Zhang, J., Dong, Z., Xu, Q., 2021. Aging simulation of thin-film plastics in different environments to examine the formation of microplastic. Water Res 202, 117462. https://doi.org/10.1016/j.watres.2021.117462.

[16] Ciceri, E., Giussani, B., Pozzi, A., Dossi, C., Recchia, S., 2008. Problems in the application of the three-step BCR sequential extraction to low amounts of sediments: an alternative validated route. Talanta 76, 621–626. https://doi.org/10.1016/j.talanta.2008.04.006.

[17] Ding, T., Wei, L., Hou, Z., Li, J., Zhang, C., Lin, D., 2022. Microplastics altered contaminant behavior and toxicity in natural waters. J Hazard Mater 425, 127908. https://doi.org/10.1016/j.jhazmat.2021.127908.

[18] Djaoudi, K., Onrubia, J., Angel, T., Boukra, A., Guesnay, L., Portas, A., et al., 2022. Seawater copper content controls biofilm bioaccumulation and microbial community on microplastics. Sci Total Environ 814, 152278. https://doi.org/10.1016/j.scitotenv.2021.152278.

[19] Dohnalkova, A.C., Marshall, M.J., Arey, B.W., Williams, K.H., Buck, E.C., Fredrickson, J.K., 2011. Imaging hydrated microbial extracellular polymers: comparative analysis by electron microscopy. Appl Environ Microbiol 77, 1254–1262. https://doi.org/10.1128/AEM.02001-10.

[20] European Commission. Guidance on monitoring of marine litter in European seas. MSFD Technical Subgroup on Marine Litter. Publications Office, LU; 2013.

[21] Feng, C., Guo, X., Yin, S., Tian, C., Li, Y., Shen, Z., 2017. Heavy metal partitioning of suspended particulate matter–water and sediment–water in the Yangtze Estuary. Chemosphere 185, 717–725. https://doi.org/10.1016/j.chemosphere.2017.07.075.

[22] Feng, W., Huang, C., Tan, X., Tang, N., Zhang, L., Li, H., et al., 2022. Release of the additive metals from 3 commonly used plastics during the degradation under the treatment of UV irradiation. Ecotoxicology 31, 75–84. https://doi.org/10.1007/s10646-021-02467-6.

[23] Gao, F., Li, J., Sun, C., Zhang, L., Jiang, F., Cao, W., et al., 2019. Study on the capability and characteristics of heavy metals enriched on microplastics in marine environment. Mar Pollut Bull 144, 61–67. https://doi.org/10.1016/j.marpolbul.2019.04.039.

[24] Gryschko, R., Kuhnle, R., Terytze, K., Breuer, J., Stahr, K., 2005. Soil extraction of readily soluble heavy metals and as with 1 M NH4NO3-solution. Evaluation of DIN 19730. J Soils Sediment 5, 101–106. https://doi.org/10.1065/jss2004.10.119.

[25] Guan, J., Qi, K., Wang, J., Wang, W., Wang, Z., Lu, N., et al., 2020. Microplastics as an emerging anthropogenic vector of trace metals in freshwater: significance of biofilms and comparison with natural substrates. Water Res 184, 116205. https://doi.org/10.1016/j.watres.2020.116205.

[26] Hahladakis, J.N., Velis, C.A., Weber, R., Iacovidou, E., Purnell, P., 2018. An overview of chemical additives present in plastics: migration, release, fate and environmental impact during their use, disposal and recycling. J Hazard Mater 344, 179–199. https://doi.org/10.1016/j.jhazmat.2017.10.014.

[27] Hildebrandt, L., von der Au, M., Zimmermann, T., Reese, A., Ludwig, J., Pröfrock, D., 2020. A metrologically traceable protocol for the quantification of trace metals in different types of microplastic. PLoS ONE 15, 1–18. https://doi.org/10.1371/journal.pone.0236120.

[28] Hites, R.A., 2019. Correcting for censored environmental measurements. Environ Sci Technol 53, 11059–11060. https://doi.org/10.1021/acs.est.9b05042.

[29] Hodson, M.E., Duffus-Hodson, C.A., Clark, A., Prendergast-Miller, M.T., Thorpe, K. L., 2017. Plastic bag derived-microplastics as a vector for metal exposure in terrestrial invertebrates. Environ Sci Technol 51, 4714–4721. https://doi.org/10.1021/acs.est.7b00635.

[30] Holmes, L.A., Thompson, R.C., Turner, A., 2020. In vitro avian bioaccessibility of metals adsorbed to microplastic pellets. Environ Pollut 261, 114107. https://doi.org/10.1016/j.envpol.2020.114107.

[31] Horváth M, Halász G, Kucanová E, Kuciková B, Fekete I, Remeteiová D, et al. Sequential extraction studies on aquatic sediment and biofilm samples for the assessment of heavy metal mobility. Microchem J, XIV Hungarian – Italian symposium on spectrochemistry: analytical techniques and preservation of natural resources, Sumeg (Hungary), 5–7 October 2011, 107; 2013. p. 121–5. ⟨https://doi.org/10.1016/j.microc.2012.05.024⟩.

[32] Jansson, A., Möller, K., Gevert, T., 2003. Degradation of post-consumer polypropylene materials exposed to simulated recycling—mechanical properties. Polym Degrad Stab 82, 37–46. https://doi.org/10.1016/S0141-3910(03)00160-5.

[33] Klöckner, P., Reemtsma, T., Wagner, S., 2021. The diverse metal composition of plastic items and its implications. Sci Total Environ 764, 142870. https://doi.org/10.1016/j.scitotenv.2020.142870.

[34] Koelmans, A.A., Mohamed Nor, N.H., Hermsen, E., Kooi, M., Mintenig, S.M., De France, J., 2019. Microplastics in freshwaters and drinking water: critical review and assessment of data quality. Water Res 155, 410–422. https://doi.org/10.1016/j.watres.2019.02.054.

[35] Lang, M., Yu, X., Liu, J., Xia, T., Wang, T., Jia, H., et al., 2020. Fenton aging significantly affects the heavy metal adsorption capacity of polystyrene microplastics. Sci Total Environ 722, 137762. https://doi.org/10.1016/j.scitotenv.2020.137762.

[36] Leiser, R., Wu, G.M., Neu, T.R., Wendt-Potthoff, K., 2020. Biofouling, metal sorption and aggregation are related to sinking of microplastics in a stratified reservoir. Water Res 176, 115748. https://doi.org/10.1016/j.watres.2020.115748.

[37] Liao, Y., liang Yang, J. yan, 2020. Microplastic serves as a potential vector for Cr in an in-vitro human digestive model. Sci Total Environ 703, 134805. https://doi.org/10.1016/j.scitotenv.2019.134805.

[38] Liu, S., Shi, J., Wang, J., Dai, Y., Li, H., Li, J., et al., 2021. Interactions between microplastics and heavy metals in aquatic environments: a review. Front Microbiol 12. https://doi.org/10.3389/fmicb.2021.652520.

[39] Luo, H., Liu, C., He, D., Xu, J., Sun, J., Li, J., et al., 2022. Environmental behaviors of microplastics in aquatic systems: a systematic review on degradation, adsorption, toxicity and biofilm under aging conditions. J Hazard Mater 423, 126915. https://doi.org/10.1016/j.jhazmat.2021.126915.

[40] Luo, H., Zhao, Y., Li, Y., Xiang, Y., He, D., Pan, X., 2020. Aging of microplastics affects their surface properties, thermal decomposition, additives leaching and interactions in simulated fluids. Sci Total Environ 714, 136862. https://doi.org/10.1016/j.scitotenv.2020.136862.

[41] Martínez, K.I., González-Mota, R., Soto-Bernal, J.J., Rosales-Candelas, I., 2021. Evaluation by IR spectroscopy of the degradation of different types of commercial polyethylene exposed to UV radiation and domestic compost in ambient conditions. J Appl Polym Sci 138, 50158. https://doi.org/10.1002/app.50158.

[42] May, T.W., Wiedmeyer, R.H., 1998. A table of polyatomic interferences in ICP-MS. Spectroscopy 19, 150–155.

[43] Meng, J., Xu, B., Liu, F., Li, W., Sy, N., Zhou, X., et al., 2021. Effects of chemical and natural ageing on the release of potentially toxic metal additives in commercial PVC microplastics. Chemosphere 283, 131274. https://doi.org/10.1016/j.chemosphere.2021.131274.

[44] Min, K., Cuiffi, J.D., Mathers, R.T., 2020. Ranking environmental degradation trends of plastic marine debris based on physical properties and molecular structure. Nat Commun 11, 727. https://doi.org/10.1038/s41467-020-14538-z.

[45] Monticelli, D., Castelletti, A., Civati, D., Recchia, S., Dossi, C., 2019. How to efficiently produce ultrapure acids. Int J Anal Chem 2019, 1–5. https://doi.org/10.1155/2019/5180610.

[46] Nava, V., Leoni, B., 2021. A critical review of interactions between microplastics, microalgae and aquatic ecosystem function. Water Res 188, 116476. https://doi.org/10.1016/j.watres.2020.116476.

[47] Pal, A., Paul, A.K., 2008. Microbial extracellular polymeric substances: central elements in heavy metal bioremediation. Indian J Microbiol 48, 49. https://doi.org/10.1007/s12088-008-0006-5.

[48] Paluselli, A., Fauvelle, V., Galgani, F., Sempéré, R., 2019. Phthalate release from plastic fragments and degradation in seawater. Environ Sci Technol 53, 166–175. https://doi.org/10.1021/acs.est.8b05083.

[49] Pořízka, P., Brunnbauer, L., Porkert, M., Rozman, U., Marolt, G., Holub, D., et al., 2023. Laser-based tools for the identification and characterization of aged microplastics with developed biofilm. Chemosphere 313, 137373. https://doi.org/10.1016/j.chemosphere.2022.137373.

[50] Pueyo, M., López-Sánchez, J.F., Rauret, G., 2004. Assessment of CaCl2, NaNO3 and NH4NO 3 extraction procedures for the study of Cd, Cu, Pb and Zn extractability in contaminated soils. Anal Chim Acta 504, 217–226. https://doi.org/10.1016/j.aca.2003.10.047.

[51] Rahman, M.M., Al-Sulaimi, S., Farooque, A.M., 2018. Characterization of new and fouled SWRO membranes by ATR/FTIR spectroscopy. Appl Water Sci 8, 183. https://doi.org/10.1007/s13201-018-0806-7.

[52] Rauret, G., López-Sánchez, J.F., Sahuquillo, A., Rubio, R., Davidson, C., Ure, A., et al., 1999. Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. J Environ Monit 1, 57–61. https://doi.org/10.1039/A807854H.

[53] Ren, S.Y., Sun, Q., Ni, H.G., Wang, J., 2020. A minimalist approach to quantify emission factor of microplastic by mechanical abrasion. Chemosphere 245, 125630. https://doi.org/10.1016/j.chemosphere.2019.125630.

[54] Richard, H., Carpenter, E.J., Komada, T., Palmer, P.T., Rochman, C.M., 2019. Biofilm facilitates metal accumulation onto microplastics in estuarine waters. Sci Total Environ 683, 600–608. https://doi.org/10.1016/j.scitotenv.2019.04.331.

[55] Rogers, K.L., Carreres-Calabuig, J.A., Gorokhova, E., Posth, N.R., 2020. Micro-by-micro interactions: how microorganisms influence the fate of marine microplastics. Limnol Oceanogr Lett 5, 18–36. https://doi.org/10.1002/lol2.10136.

[56] Rozman, U., Filker, S., Kalčíková, G., 2023. Monitoring of biofilm development and physico-chemical changes of floating microplastics at the air-water interface. Environ Pollut 322, 121157. https://doi.org/10.1016/j.envpol.2023.121157.

[57] Rummel, C.D., Jahnke, A., Gorokhova, E., Kühnel, D., Schmitt-Jansen, M., 2017. Impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment. Environ Sci Technol Lett 4, 258–267. https://doi.org/10.1021/acs.estlett.7b00164.

[58] Seeley, M.E., Song, B., Passie, R., Hale, R.C., 2020. Microplastics affect sedimentary microbial communities and nitrogen cycling. Nat Commun 11, 2372. https://doi.org/10.1038/s41467-020-16235-3.

[59] Smith, E.C., Turner, A., 2020. Mobilisation kinetics of Br, Cd, Cr, Hg, Pb and Sb in microplastics exposed to simulated, dietary-adapted digestive conditions of seabirds. Sci Total Environ 733, 138802. https://doi.org/10.1016/j.scitotenv.2020.138802.

[60] Smith, I.L., Stanton, T., Law, A., 2021. Plastic habitats: algal biofilms on photic and aphotic plastics. J Hazard Mater Lett 2, 100038. https://doi.org/10.1016/j.hazl.2021.100038.

[61] Sturm, M.T., Schuhen, K., Horn, H., 2022. Method for rapid biofilm cultivation on microplastics and investigation of its effect on the agglomeration and removal of microplastics using organosilanes. Sci Total Environ 806, 151388. https://doi.org/10.1016/j.scitotenv.2021.151388.

[62] Turner, A., Filella, M., 2021. Hazardous metal additives in plastics and their environmental impacts. Environ Int 156, 106622. https://doi.org/10.1016/j.envint.2021.106622.

[63] Turner, A., Filella, M., 2020. The influence of additives on the fate of plastics in the marine environment, exemplified with barium sulphate. Mar Pollut Bull 158, 111352. https://doi.org/10.1016/j.marpolbul.2020.111352.

[64] United States Environmental Protection Agency. Definition and procedure for the determination of the method detection limit, revision 2; 2016.

[65] Wang, T., Ma, Y., Ji, R., 2021. Aging processes of polyethylene mulch films and preparation of microplastics with environmental characteristics. Bull Environ Contam Toxicol 107, 736–740. https://doi.org/10.1007/s00128-020-02975-x.

[66] Wang, Y., Wang, X., Li, Y., Li, J., Wang, F., Xia, S., et al., 2020. Biofilm alters tetracycline and copper adsorption behaviors onto polyethylene microplastics. Chem Eng J 392, 123808. https://doi.org/10.1016/j.cej.2019.123808.

[67] Wei, X., Liu, Y., Zhan, Q., Zhang, P., Zhao, D., Xu, B., et al., 2018. Effect of Si soil amendments on As, Cd, and Pb bioavailability in contaminated paddy soils. Paddy Water Environ 16, 173–181. https://doi.org/10.1007/s10333-017-0629-4.

[68] Wu, C., Tanaka, K., Tani, Y., Bi, X., Liu, J., Yu, Q., 2022. Effect of particle size on the colonization of biofilms and the potential of biofilm-covered microplastics as metal carriers. Sci Total Environ 821, 153265. https://doi.org/10.1016/j.scitotenv.2022.153265.

[69] Xie, M., Huang, J.-L., Lin, Z., Chen, R., Tan, Q.-G., 2021. Field to laboratory comparison of metal accumulation on aged microplastics in coastal waters. Sci Total Environ 797, 149108. https://doi.org/10.1016/j.scitotenv.2021.149108.

[70] Xie, Q., Li, Hengxiang, Li, Z., Zhang, H., Yuan, M., Wu, M., et al., 2022. Accumulation, chemical speciation and ecological risks of heavy metals on expanded polystyrene microplastics in seawater. Gondwana Res. https://doi.org/10.1016/j.gr.2022.01.017.