Methods for sampling and detection of microplastics in water and sediment: A critical review

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Abstract:

Microplastics are widespread contaminants, virtually present in all environmental compartments. However, knowledge on sources, fate and environmental concentration over time and space still is limited due to the laborious and varied analytical procedures currently used. In this work we critically review the methods currently used for sampling and detection of microplastics, identifying flaws in study design and suggesting promising alternatives. This work provides insights on bulk sample collection, separation, digestion, identification and quantification, and mitigation of cross-contamination. The sampling of microplastics will improve in representativeness and reproducibility through the determination of bulk sample volume, filter's pore size, density separation and digestion solutions, but also through use of novel methods, such as the enhancement of visual identification by staining dyes, and the generalized use of chemical characterization.

Keywords: Microplastic, Cross-contamination, ecosystems, Environmental Concentration, Fenton's Reagent, Determinant Factor.

Introduction

Microplastics, small plastic pieces <5 mm intentionally produced to be used in consumer products (e.g. as exfoliants in cosmetics) and in activities as abrasives (e.g. air blasting) (primary microplastics) or resulting fromthe fragmentation of larger objects (secondary microplastics), are highly persistent contaminants potentially harmful to organisms or ecosystems [14]. From the Artic [20] to the Antarctic seawater [13], to sediments [8,34], rivers [69,71,83], soil [110] and even the air we breathe [29], microplastics are currently present in all environmental matrixes. Concerns over these particles have led to a growing literature attempting to quantify microplastics in the environment and their effects on organisms [4]. However, the lack of universal and validated methods led to a wide range of analytical approaches, compromising a large-scale interpretation of current results.

Currently, research groups may struggle with selecting methods for sampling microplastics due to the large range of available options. In this review, we attempt to summarize the most relevant methods of sampling microplastics from sediment and water, providing criticism and perspectives of future developments. A search using the terms "sampling/extraction/determination/ methods microplastics" was performed on Web of Science in May 2018, selecting 49 works only directly related to the development of sampling procedures for microplastics. Google Scholar was also used to gather data on the methods used in sampling studies in water (N = 20) Information has been organized into sampling steps: bulk sample collection, separation, digestion, and identification. At last, we suggest five measures to reduce cross-contamination due to its importance in the validation of results.

Collection of water and sediment samples

Collection of water and sediment samples is the first step of microplastics sampling methodologies (Table 1). The choice between sampled medium is dependent on available equipment but also the objective of the work. For instance, sampling the water column may be the most adequate medium if the objective is to determine the exposure of pelagic organisms. However, microplastic distribution is largely influenced by meteorological, temporal and geographical factors that may compromise reproducibility of the results. On the other hand, methodology and quantity of sampled material may influence representativeness of results. Results are usually expressed as total microplastics per unit of sample (e.g. L^{-1}, in water), sometimes providing detailed classifications of size classes, color and shape (e.g. fiber, particle, fragment). We suggest authors provide all the data available, expressing their results in both total microplastics, but also in classes, which should be standardized. A uniform classification of what is considered a microplastics should also be proposed, clarifying the inclusion (or not) of pieces such as fibers and polymeric rubbers. For these reasons, most current research on environmental concentrations of microplastics represent a snapshot in time and space, limiting interpretation between studies.
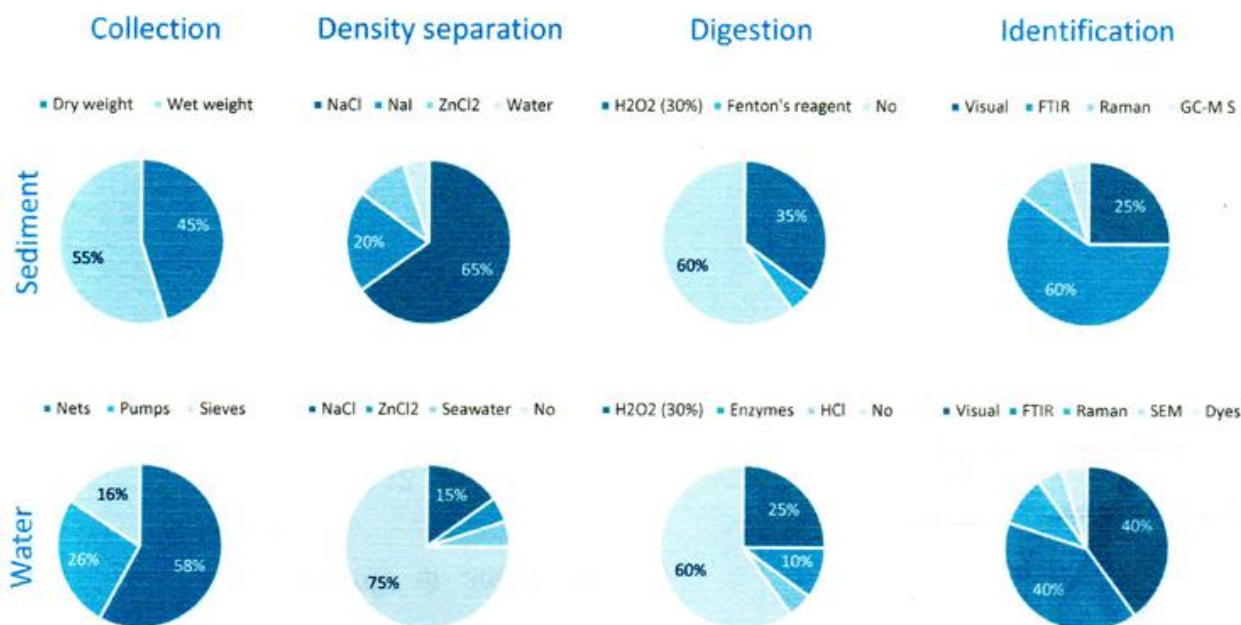
Fig. 1. Details from sampling methods reviewed from the literature for microplastics in sediment (top row, N = 20) and water (bottom row, N ¼ 20) regarding collection, density separation, digestion, and identification.

Table 1 Methods of sample collection in water and sediment.

Sample	Type	Advantages	Disadvantage
Water	Neuston and Manta nets	Easy to use; Sample large volumes of water; Largely used (good to compare between locations); Produces large numbers of microplastics for further testing.	Expensive equipment; Requires boat; Time-consuming; Potential contamination by vessel and tow ropes; Lower limit of detection is 333 μm.
	Plankton net	Easy to use; Lowest limit of detection 100 mm; Quick to use; Samples medium volumes of water.	Expensive equipment; Requires boat; Static sampling requires water flow; May become clogged or break; Sampling of lower volumes of water than Manta trawl.
	Sieving	Does not require specialized equipment nor boat; Easy to collect samples.	Laborious and time consuming; Samples medium volumes; Manual transfer of water with buckets
	Pumps	Samples large volumes of water; Effortless; Allows choice of mesh size.	Requires equipment; Requires energy to work; Potential contamination by the apparatus; May be difficult to carry between sampling locations.
	Filtration or Sieving ex situ	Easy to collect samples; Known volume of water; Allows choice of mesh size.	Sampling of low volumes; Transportation of water samples to the lab; Potential contamination by the apparatus;

			Time consuming depending on mesh size.
Sediment	Beach sediment collection	Easy to implement; Rapid sampling; Allows collection of large volumes of sample or replicates.	Variation with sampled area and depth.
	Seabed collection (Grab sampler, box corer, gravity core)	Easy to use; May allow replicates.	Expensive equipment; Requires boat; Variation with sampled area and depth; Sampling may disturb sediment surface.

Water sampling

Microplastics are distributed in the water column dependent on their properties, such as density, shape, size, adsorption of chemicals and biofouling, and on environmental conditions such as water density, wind, currents and waves. Thus, quantity and quality of microplastics recovered are highly dependent on sampling location and depth. Sampling and processing methods are similar for both fresh and saltwater samples, enabling a future standardization of methods. However, differences can be found in the distribution of microplastics in each system, influenced by environmental characteristics, such as hydrodynamic profiles, as well as density. The differences in density of fresh and saltwater, respectively 1.00 g cm^{-3} and 1.03 g cm^{-3}, may lead to distinct distribution of microplastics in the water column in each system (i.e. generally, microplastics will be deeper in the water column in freshwater systems). Thus, depth and location may need to be adjusted depending on the sampling location and salinity. Representativeness may be dependent on the sampling of large volumes ofwater, often achieved by reduction in situ through the use of nets, sieves or pumps. Towing of neuston nets or manta nets (333 nm) allows the sampling of near-surface or surface water, respectively. Bongo nets are paired nets used to gather replicate samples from the water column. Plankton nets are usually hauled or towed at low velocities since the small mesh size (~100 mm) can quickly become clogged. Besides horizontal towing or hauling, these nets allow vertical or obliquely sampling. A flow meter should be attached to all these nets to allow estimation of water volume sampled and the expression of results by m^3. An alternative to nets includes water pumps that can be comprised by intakes from the vessel, deck pumps or even used at coastal areas.

Mesh size may largely influence concentrations reported. For instance, a nylon net (100 mm) revealed concentrations almost a hundred times higher than a manta net (333 mm), 0.1 and 0.00135 MP L^{-1} respectively (Vermaire et al., 2016) [99]. Nonetheless, manta nets allow the sampling of large volumes of water and are widely used allowing some standardization of methods. Plankton nets also have smaller mesh sizes (~100 mm), allowing sampling under a minute and recovering concentrations 30 times higher than manta nets [28]. However, plankton nets must be deployed for a short period of time due to clogging with organic and mineral material in suspension, limiting the volume of water sampled. Regarding fibers, an 80 mmmesh is able to filter 250 times more fibers than a 330 mm net [30]. An alternative is to use a pump to collect water on a vessel [24] or on shore [49]. For instance Lenz and Labrenz [59] have developed a large portable filtration device based on pumps. On shore filtration or sieving using buckets is possible, but time consuming and demanding. Water samples can also be collected in glass bottles and processed in the lab. Dubaish and Liebezeit [25] collected 100mL of surfacewater, reporting high variability of the method. Thus, collection of higher volumes of water may be required to achieve representativeness. Reduction of sample size can also be achieved in the lab by a pre-treatment using steel meshes of decreasing sizes [114]. Regarding contamination, nylon nets and pumping systems may be a source of microplastic contamination whereas metal sieves and glass bottles avoid the use of plastic materials. However, these plastic free materials are usually associated with processing limited volumes of water. Thus, it may lead to a difficult choice between representativeness and avoiding potential contamination. Defining a minimum volume of sample to achieve representativeness could facilitate this decision and alleviate sampling efforts.

Based on the 20 studies on water sampling, we found that the most used are nets (11), followed by pumps (5) and sieves (3) (Fig. 1). Only one study used bottles and buckets to collect water samples. Only 8 of these studies stated the approximate volume of sampling (10–2000 L). This information should always be present since it may determine the representativeness of the results. NOAA recommends the use of manta nets followed by sieving (0.3 mm) and filtration (0.3 mm) [70].

Sediment sampling

The distribution of microplastics on sediments is uneven, largely influenced by their properties and environmental factors, such as winds and currents. Results will be largely dependent on the sampling area (e.g. high tide line, intertidal areas, transects) and depth since some areas may contain higher concentrations of microplastics. For instance, collection

of sediments on the tide-line, the high accumulation area for microplastics, may result in overestimation [38]. Collection of microplastics on beaches include direct sampling with forceps, sieving and collection of sediment samples. Collection of samples from the seabed requires a vessel and the use of specialized equipment that is lowered to the seabed to collect the samples (e.g. grab sampler, box corer). An accurate estimation of microplastic concentration in sediment samples may require definition of sampling depth, since the top 1–5 cmpresents higher concentrations than the top 10 cm, and number of replicates, since 11 samples are recommended per each 100 m of beach to estimate microplastic concentration at a 90% confidence level [6]. Sample weight (25–3000 g) or volume (0.05–1.2 L) largely varies between studies, potentially affecting representativeness. Even though only 9 of 20 studies reported sediment concentrations in g, kg or L of dry weight (Fig. 1), this method is recommended to eliminate variations related to humidity. NOAA recommends the use of 400 g (w.w.) per replicate, followed by drying and weighing to adjust the results[70], while the MSFD technical subgroup [73] recommends using at least five replicates of the top 5 cm of sediment.

Separation of microplastics from samples

Microplastics must be separated from water and sediment samples in order to be quantified and characterized. Samples may be subjected to two separation steps: (1) a reduction step that allows to reduce sample volume, for instance, through the use of nets during collection or bulk collection followed by sieving; (2) a separation step usually through filtration and/or density separation. Density separation by the use of NaCl is recommended by both MSFD technical subgroup (2013) [73] and NOAA [70].

Filtration or sieving

Filtering or sieving is the most frequent method in separation of microplastics from water samples and for the supernatant containing plastics from density separation of sediment samples. Filter's pore size or sieve's mesh can vary greatly. Pore or mesh size determine the lower size of microplastics detected. However, small pore or mesh sizes may also result in quick obstruction by organic and mineral matter. In sediments, samples may be subjected to a pre-treatment such as larger sieves (e.g. 4 works used sieving as a pre-treatment to reduce sample volume), followed by density separation and filtration of the supernatant through filters (16) or sieves (4). In the reviewed works, pore or mesh size varies between 0.3 and 200 mm, with 3works lacking this information. In water, the reduction step usually is related to sample collection (e.g. manta nets) followed by filtration (16) or sieving (4) with pore or mesh sizes varying from 0.45 to 55500 mm, with 3 works not expressing this information (Fig. 1). Thus, a standardized pore or mesh size should be defined to allow comparison between works, even though this is sometimes dependent on protocol constrains (which could be overcome by creating a universal sampling protocol), and this information should always be clearly expressed.

Density separation: flotation and elutriation

Differences in density can be used to separate plastics (0.8–1.6 g cm^{-3}) (Table 2) from sediment (2.7 g cm^{-3}), usually by carefully mixing the sediment with salt saturated solutions and collecting the supernatant containing microplastics for further filtration [82]. It is accepted that solutions >1.4 g cm^{-3} are required to separate microplastics from sediments, since their density is dependent on polymer type, additive concentration, and even adsorbed substances and organisms [80]. A simple density separation using water is able to recover some types of plastics, such as PE (polyethylene) and PP (polypropylene), from soil samples [110] or fibers from sediments, due to their shape and large surface [80]. NaCl is one of themost used salts for density separation, as it is highly available, cheap and eco-friendly [78]. Reagent grade NaCl is recommend since it achieves slightly higher densities and thus has a higher extraction efficiency for slightly heavier polymers, such as HDPE (high density polyethylene) [84]. However, Quinn et al. [80] found that NaCl (1.2 g cm^{-3}) had low recovery rates (<90%) and larger error bars, as well as NaBr (1.4 g cm^{-3}), while both NaI (1.6 g cm^{-3}) and ZnBr2 (1.7 g cm^{-3}) were able to separate heavier polymers with good recovery rates (99%) and tight error bars. Furthermore, separation using NaI and ZnBr$_2$ requires a single washing of the sediment, while NaCl requires three. However, NaI reacts with cellulose filters, turning them black and complicating visual identification, while ZnBr is hazardous to the environment and expensive, which can be overcome by continuous reuse through careful filtration and density adjustment. NaI is also able to recover oleophobic fibers (93.3%), better than CaCl$_2$ (69%) [21], and with MeOH is able to recover most microplastics from marine snow (90–98%) [111]. Following Kedzierski et al. [52] protocol using an elutriation column, NaI can be recycled up to 10 cycles through rising and evaporation steps, having similar costs to NaCl. Thus, the use of NaI is the recommended since it is environmentally safe and can be recycled for several cycles, as long as it is not used with a cellulose filter. The Sediment-Microplastic Isolation, an apparatus comprised of two tubes connected by a valve that allows separation of the supernatant and sediment, has been used with ZnCl$_2$ (1.5 g cm^{-3}) with an efficiency of 95.8% [18], and could be used with other salt saturated solutions such as NaI. In the reviewed works, all sediments were subjected to density separation, using NaCl (13), ZnCl$_2$ (4), NaI (2) and deionized water (1), while only 5 works on water samples used density separation using NaCl (3), ZnCl$_2$ (1), and seawater (1) (Fig. 1).

Table 2 Separation of polymer types by solutions used in density separation.

Polymer	Density (g cm⁻3)	Water 1 g cm⁻3	NaCl 1.2 g cm⁻3	NaI 1.6 g cm⁻3	ZnBr₂ 1.7 g cm⁻3
PP	0.9 – 0.91	+	+	+	+
PE	0.92 – 0.97	+	+	+	+
PA	1.02 – 1.05	–	+	+	+
PS	1.04 – 1.1	–	+	+	+
Acrylic	1.09 – 1.20	–	+	+	+
PMA	1.17 – 1.20	–	+	+	+
PU	1.2	–	+	+	+
PVC	1.16 – 1.58	–	±	+	+
PVA	1.19 – 1.31	–	±	+	+
Alkyd	1.24 – 2.10	–	–	+	+
Polyester	1.24 – 2.3	–	–	+	+
PET	1.37 – 1.45	–	–	+	+
POM	1.41 – 1.61	–	–	±	+

Label: þ: separation, ±: possible separation, e: not separated. Polymers: PE: polyethylene, PP: polypropylene, PS: polystyrene; PA: polyamide (nylon), POM: polyoxymethylene, PVA: polyvinyl alcohol, PVC: polyvinylchloride, PMA: poly methyl acrylate, PET: polyethylene terephthalate, PU: polyurethane (polymer density adapted from Ref. [44]).

In elutriation, usually a liquid such as water is injected at the bottom of a column, allowing the separation of buoyant microplastics from the settling organic matter and sediment [53]. Microplastics are collected in a mesh in the column and then separated using dense solutions (e.g. NaI) [11,54]. The advantage of elutriation is the cheap and efficient separation of microplastics from large volumes of sediments, allowing higher environmental representativeness, and reduction of sample volume undergoing density separation [54]. However, this method takes at least 1 h per sample (comprised of 3 subsamples) and requires previous sieving by size range [54]. The Munich Plastic Sediment Separator (MPSS) uses a similar system, where a dense solution of ZnCl (1.6 – 1.7 kg L⁻1) is injected at the bottom of the column, allowing microplastics to ascend and be collected in the supernatant or by direct vacuum filtration, but at greater time expense (e.g. settling phase may take 1a – 2 h) [45]. Nuelle et al. [78] also used a pre-step of fluidization using NaCl and air-induced overflowto reduce sample size up to 80%.

Oil has also been tested as a separation method due to the hydrophobic properties of plastics. Imhof et al., [45] used pine oil along with froth conditioner to improve wetting, reduce surface tension and mediate the detachment of plastics from sediment in deionized water, with low recovery rates (55.0%) particularly of high density plastics. Canola oil has also been used, with recovery rates of 96.1%, shorter sampling time (~2 h) compared to salt saturated solutions and little retention of organicmatter [21]. A drop of olive oil has also been added to salt saturated solutions to help gather plastic particles in the supernatant, improving recovery rates from 64% to 82% [51]. Even though oil seems to have limitations in separating plastics and requires a cleaning step with a detergent, it can be combined with saturated solutions to improve recovery rates.

NaCl is a highly available, cheap and innocuous separation solution, advantages that led to its recommendation by both the MSFD technical subgroup (2013) [73] and NOAA [70]. However, NaCl limitations, namely the low recovery of higher density polymers Table 2, classify this solution as unsuitable for microplastic separation, since it leads to underestimation of environmental concentrations. Thus, recovery of microplastics, especially of heavier polymers, can be improved through the use of higher density solutions, in which we propose NaI due to its high density, safety and possibility of reuse, and possibly in combination with separation columns or the use of oil to improve recovery rates.

Unusual methods for separation and quantification of microplastics
Other unusual methods have been used in the separation and quantification of microplastics. For instance, Felsing et al. [33] separated microplastics (63e5000 mm) using a Korona-Walzen- Scheider electrostatic bell separator, reducing sample volume by 99%. Shimizu et al., [89] propose using the frequency of impact of microplastics in solution with an electrode, caused by Brownian motion, to infer concentration. Flow cytometry with visual stochastic network embedding (viSNE) also allows quantification of small microplastics (1–20 mm) in water samples[85]. However, limitations of these methods include availability of specialized equipment, time spent in each sample, incapability of characterization of polymer types, potential saturation of measuring equipment and changes in surface charge of plastics due to weathering and biofouling.

Sample processing: removal of organic matter

Environmental samples contain biologic material. For instance, Crichton et al. [21] reported that sediment from beaches contained 0.5–7.0% of biologic material. Biological material is often confused with plastics (e.g. darker algae fragments), leading to overestimation of environmental concentrations and increasing the number of particles subjected to further analysis. Thus, there is a need to create a simple method of digestion capable of reducing organic matter without affecting the structural or chemical integrity of polymers [33,72]. Nonetheless, the need for digestion varies depending on the quantity of organic matter in each sample. For instance, not all the reviewed studies performed a digestion step: in sediment seven used H_2O_2 (30%) and one Fenton's reagent (H_2O_2 with ferrous iron catalyst) and in water five used H_2O_2 (30%), two enzymatic digestion and one HCl (5 –10%) (Fig. 1). NOOA recommends the use of H_2O_2 (30%) with Fe(II) solution (0.05 M)(sulfate (Fenton's reagent) heated at 75ºC to a glass beaker containing the microplastics fraction for both water and sediment samples. The use of a digestion step is highly recommended when identification is mainly based on visual inspection. However, most works did not remove organic matter from their samples, possibly because authors deemed them low in organic matter. The NOOA oxidizingmethod has yet to be widely used, with a single study mentioning its use– an example of the difficulties in standardization. Digestion protocols, and their uniformization, are even more important when processing biota samples (e.g. fish). Besides oxidizing methods, digestion can also be acidic, alkaline or enzymatic (Table 3).

Acid digestion

Acid digestion may be used to degrade organic matter. However, some polymers (e.g. nylon, PET e polyethylene terephthalate) have low resistance to acids and may also be degraded, especially in high concentration and high temperatures [79]. Yet, there must be an optimum of concentration and temperature used to efficiently remove biologicmaterial in a reasonable period of time. For instance, Naidoo et al. [75] found that heating nitric acid (HNO_3, 55%) to 80ºC allows to digest fish tissues 26 times faster. Nonetheless, caution is advised when heating digestion solutions above 60ºC, since these temperatures may destroy microplastics[74]. Hydrochloric acid (HCl) seems to be the least effective treatment in treating large quantities of biologic material [15,66,111]. Nonetheless, Karami et al. [50] reported that HCl (37%) at 25ºC had a digestion efficiency >95% but with melting of PET. This difference may reflect different protocols, with variations in concentration and temperature that affect digestion efficiency. Nitric acid (HNO3) is widely used in acid digestion. Yet, nitric acid may leave oily residues or tissue debris, cause loss of nylon and melting of PS (polystyrene), LDPE (low density polyethylene), PET and HDPE, or yellowing of polymers, including Claessens et al. 2013[11] PP (polypropylene), PVC (polyvinyl chloride) and PET [10,11,22,50,66]. Naidoo et al. [75] reports PE, HDPE, PS, polyester and PVC survived HNO_3 (55%) at room temperature for a month, only with degradation of nylon and whitening of PVC. Once again, resistance of polymers to digestion is dependent on various factors, such as the presence of organic matter in the sample (leading to lower degradation of polymers) [11] and temperature of the solution. In this case, it is more realistic to think that HNO_3 will have some effects on the integrity of plastics, since heating is needed to achieve digestion in a timely manner. Thus, acid digestion may be used with caution since it may lead to underestimation of microplastics in environmental samples.

Alkali digestion

Alkali digestion is an alternative to acid digestion with great potential. However, alkali digestion may also damage or discolour plastics [79], leave oily residues and bone fragments[22,66] or redeposit tissue residues on plastic surfaces, complicating characterization by vibrational spectroscopy [101]. KOH has good digestion of organic matter and recovery of plastics [10,74]. Protocols using KOH (10%) at 60ºC overnight [66] or 60ºC for 24 h [22] showed to be one of the most effective digestive treatments, as well as NaOH [15]. Nonetheless, KOH may cause discoloration of nylon, PE and uPVC (unplasticized PVC), degradation of nylon, polyester, PE, PC (polycarbonate), PET, PVC, LDPE, CA (cellulose acetate) [15,50,56,66,74]. NaOH also may cause CA, PA, PET degradation and color change in PVC and PET [22]. Regarding digestion efficiency, Kühn et al. [56] tested several samples of organic matter often found in beaches (i.e. seaweed, squid beaks, Polychaeta beaks, sheep wool, seal whiskers, fish otoliths, bird feathers, manila rope, metal fish hook, paraffin and palm fat) revealing that fish otoliths, squid beaks, paraffin and palm fat survived the digestion process with KOH (1 M) for 2 days at room temperature. Thus, hard parts and fats seem to not be fully digested by alkali. Acid and alkali digestion may also be used sequentially (e.g. NaOH and HNO_3) with good digestion of biologic material and recovery rates [81].

Oxidizing agents

Hydrogen peroxide (H_2O_2, 30–35%) is an oxidizing agent able to digest organic matter more efficiently than NaOH and HCl, with little to no degradation of polymers [78,79,111]. Nuelle et al. [78] reported resistance of PVC, PET, nylon, ABS (acrylonitrile butadiene styrene), PC, PUR (polyurethane), PP, LDPE, LLDPE (linear LDPE), HPDE to H_2O_2, with some discoloration, while Karami et al. [50] reported degradation of nylon and color change of PET following H_2O_2 (35%) treatment at 50ºC for 96 h. Digestion may also cause the production of foam that may lead to the reduction of microplastics retrieved [66]. Temperature of incubation seems to be a determinant factor on the efficiency of H_2O_2. For

instance Cole et al.[15] reported that incubation with H_2O_2 (35%) at room temperature for 7 days only degraded 25% of organic matter, whereas Avio et al. [2] reported to have used H_2O_2 (15%) at 50ºC overnight to efficiently remove organic matter. Zhao et al. [111] reports that 15% is preferred to 20% H_2O_2, and both these treatments have better results than HCl. Thus, H_2O_2 treatments may be able to efficiently remove organic matter with little effect on microplastic integrity.

Enzymatic digestion

Enzymes have been used as alternative digestion methods. Enzymatic digestion is less hazardous (e.g. can be used without a fume hood) and less likely to induce damage to microplastics [66]. However, enzyme efficiency will vary with type of organic material present in the sample [19]. Enzyme protocols include the predigestion of sediments with an industrial enzyme blend (2.5%) at 45ºC for 60 min followed by H_2O_2 (30%) removal of debris [21]. To digest fish tissues Karlsson et al. [51] used proteinase K (500 mgmL^{-1}) with $CaCl_2$ incubated at 50ºC for 2 h, followed by shaking (20 min) and further incubations (60ºC, 20min), then treated with H_2O_2 (30%) with recoveries of 97% butwith calciumdeposition over particles that may complicate further characterization. Protein K has also been used in seawater to digest biologic material retained in samples collected by plankton net, allowing a digestion efficiency up to 97% at 50ºC [15]. Courtene-Jones et al. [19] tested Tripsin, Collagenase and Papain with digestion efficiencies of 72–88% and no effect on polymers tested. L€oder et al. [64] proposed the use of a basic enzymatic purification protocol with 98.3% efficiency, based on the use of detergent (5% w/w sodium dodecyl sulfate), a sequential use of enzymes (protease, cellulase, chitinase) and two hydrogen peroxide treatments (one between enzyme treatments and one in the end), adding up to 13 days of sample processing. However, enzyme use is limited by its high price. Industrial Corolase 7089, sold in liquid form, has been presented as a less expensive enzyme that could be used in microplastic sampling with better results than chemical treatments [10]. Nonetheless, enzymes are still being used in a small scale and some protocols may require a following treatment with H_2O_2 to remove undigested debris.

Table 3 *Digestion methods for the removal of organic matter to improve the identification of microplastics, their efficiency and effects on synthetic polymers.*

Digestion	Treatment	Recovery rate	Polymer degradation	Organic matter degradation	Reference
Acid	HNO_3 (35%), 60ºC 1 h	n.a.	Fusion of PET and HDPE; destruction of PA	100%	[10]
	HNO_3 (65%), RT overnight, 60ºC 2 h, dilution 80ºC distilled water	n.a.	PA degradation; yellowing	n.a.	[22]
	HNO_3 (65%) and $HClO_4$ (65%) 4:1 overnight, boiled 10 min, dilution 80ºC distilled water	n.a.	PA degradation; yellowing	n.a.	[22]
	HNO3 (5e69%), RT 96 h	<95%	Melted LDPE and PP; color change in PP, PVC, PET; decrease Raman peaks	n.a.	[50]
	HNO3 (55%) RT 1 month	n.a.	Whitening of PVC, degradation of PA	n.a.	[75]
	HCl (5 – 37%), 25–60ºC 96 h	n.a.	Changes in PET and PVC	>95%	[50]
Alkali	NaOH, 60ºC 1 h	94%	No	100%	[10]
	NaOH (10 M), 60ºC 24 h	n.a.	CA degradation	n.a.	[22]
	$K_2S_2O_8$ (0.27 M) and NaOH (0.24 M), 65ºC 24 h	n.a.	CA degradation; Unpredictable weight increase	n.a.	[22]
	KOH (10%), RT 3 weeks	n.a.	No	n.a.	[22]
	KOH (10%), 60ºC 24 h	n.a.	CA degradation	n.a.	[22]
	KOH (10%), 50ºC 96 h	n.a.	Loss of PET and PVC	n.a.	[50]

	KOH (10%), 40°C 96 h	n.a.	Loss of PET; yellowing of PA	n.a.	[50]
	KOH (1 M), RT 2 days	n.a.	Degradation of LDPE, CA, Cradonyl and PA.	Most, except otoliths, squid beaks, paraffin, palm fat	[56]
	NaOH (1 mol L^{-1}), 17.5 mL of 65% HNO$_3$ and 2.5 mL UP and drying	95%	Degradation of PA, PET, EPS, LDPE, PVC; color change in PVC and PET	n.a.	[81]
Oxidative	H$_2$O$_2$ (30%), 60°C for 1 h, 100°C for 7 h	n.a.	n.a.	n.a.	[32]
	H$_2$O$_2$ (35%), RT, 40°C 96 h	n.a.	Decrease in Raman peaks of PVC and PA	n.a.	[50]
	H$_2$O$_2$ (35%), RT, 50°C 96 h	n.a.	Degradation of PA; color change of PET; foam and oxidization	n.a.	[50]
	H2O2 (6%) 70°C for 24 h	78% (PE)	n.a.	n.a.	[93]
	H2O2 (30%), 60°C until evaporation	n.a.	n.a.	n.a.	[114]
Enzymatic	Corolase 7086, 60°C 1 h	93%	No	n.a.	[10]
	Tripsin, 38-42°C 30 min	n.a.	No	88%	[19]
	Collagenase, 38-42°C 30 min	n.a.	No	76%	[19]
	Papain, 38-42°C 30 min	n.a.	No	72%	[19]
	Pepsin (0.5%) and HCL (0.063 M), 35°C 2 h	n.a.	No	Incomplete	[22]
	15 mL Tris-HCl 60°C 60 min, proteinase K (500 mg/mL) and CaCl$_2$ 50°C 2 h, shaken 20 min, incubated 60°C 2 h, 30 mL H$_2$O$_2$ (30%) overnight	97%	Calcium layer	n.a.	[51]

n.a. – not available; RT – room temperature; Polymers: PA – polyamide (nylon), PE – polyethylene, PET – polyethylene terephthalate, LDPE – low density polyethylene, HDPE – high density polyethylene, PP – polypropylene, PS – polystyrene, PVC – polyvinyl chloride, CA – cellulose acetate, EPS – expanded polystyrene.

Less used digestion methods

Other methods include the use of microwaves, that seem to damage microplastics [51], ultrasonication, useful in combination with other methods (e.g. improved the digestion of sludge using NaOH from 43.5% to 50.7%, around 7.2%) [48] and the use of sodium hypochlorite (NaClO; 24.8 g L^{-1}) in distilled water (1:3 v/v) left overnight as an efficient method in the digestion of fish stomach contents, without affecting polymers and Raman spectra but potentially causing discoloration [16].

Identification, chemical characterization and quantification

Identification and quantification of microplastics is almost always done by visual inspection, even if followed by chemical characterization. From the reviewed studies on water and sediment (N ¼ 40), 50% used Fourier-transform infrared spectroscopy (FTIR) based methods, 32.5% visual inspection, 10% Raman spectroscopy, whereas electron microscopy, staining dyes and gas chromatography-mass spectroscopy were each used in 2.5% (Fig.1). However, electron microscopy was used in three more studies also subjected to FTIR.

Visual inspection

Visual inspection allows classification of particles as plastic based on physical characteristics, observed directly, or using a stereoscope or microscope. This is one of the most used and widely available methods of identification and quantification of plastic particles, even used as pre-selection when chemical characterization is performed. However, this method is subjective, may produce wide variations between observers and is highly time consuming. For instance, visual detection of microplastics directly in beach sediments by multiple observers had detections of 60e100%, varying with the individual, experience, fatigue and leading to overestimation (e.g. biologic material confused for black fragments) or underestimation (e.g. white fragments) of certain types and colors of microplastics[57]. Misclassification of other material as plastics, confirmed by following chemical analysis, is reported in 70% of presumed microplastics [44], for 32% of particles and 25% of fibers [58]. Researchers have tried to improve visual inspection by prodding particles with needles [87], testing plastics with a heated needle tip [9] and even melting plastics (130ºC, 3e5 s) in the sample to ease detection [110]. Visual inspection also allows the classification of plastic particles by size, color and shape, allowing to infer on their origin. The use of staining dyes is a low-cost method to ease visual identification. Unsatisfactory results have been reported for Oil red EGN, Eosin B, Hostasol Yellow 3G and Rose Bengal [66]. Problems arise from the affinity of the dye for plastics and the confounding effect of staining biogenic material in the sample, requiring a thorough digestion step. For instance, Rose-Bengal allows a 92% coloration of 25 mm microplastics, and following characterization by FTIR, but from all the stained particles only 22–99% were actually plastics [114]. Presently, Nile Red seems to be the most promising staining protocol for microplastics. Staining with Nile Red requires a short term of incubation (10–30 min), provides high recovery rates (96.6%) and allows vibrational spectroscopy with or without a short cleaning step with bleach [32,66]. Results are seen under an orange, red or green filter in the fluorescent microscope. Even though biogenic material, such as algae, seaweed, wood, feathers and mollusc shells are not stained [66] and weathering of plastics does not seem to affect staining [88], some types of plastics such as PC, PUR, PET and PVC have weak signals [32] and fibers are difficult to stain [94]. Since Nile Red is a solvatochromic dye, the fluorescence emission is dependent on the polarity of the solvent, possibly allowing classification of microplastics in large chemical groups based on fluorescent shift [66,88], especially through the analysis of images with suitable software. Thus, validation of a staining protocol may provide researchers with a simple and time effective tool to visually identifying plastics and improve the pre-selection method of particles to be submitted to chemical characterization.

Chemical characterization

Chemical characterization of potential microplastics by Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy is highly recommended. The MSFD technical subgroup [73] recommends subjecting 10% of microplastics of sizes 100–5000 mm and all the suspected particles in the range of 20–100 mm to these methods, but more may be required for the larger sizes due varying accuracy in visual identification. Both vibrational spectroscopy methods are non-destructible, highly accurate, and complementary, producing a spectrum based on the interaction of light with molecules: FTIR produces an infrared spectrum resulting from the change in dipole moment, whereas Raman provides a molecular fingerprint spectrum based on the polarizability of chemical bonds [31,79]. Diverse FTIR techniques have been used in the characterization of microplastics, such as attenuated total reflection FTIR (ATR-FTIR) that improves the information on irregular microplastics, which in contrast to transmission FTIR is also applicable to thick or opaque samples [19,87] and micro-FTIR that produces a high-resolution map of the sample (down to 20 mm), without a preselection step [43,63,104]. Raman microscopy allows the characterization of microplastics <20 mm, but may be limited by weak signals, overcome by increasing measurement duration, and fluorescence interference, dependent on material characteristics such as color, biofouling and degradation [1]. Stimulated Raman scattering (SRS) has been used to identify microplastics on low Raman background filter membranes without pre-selection, scanning at six wavenumber settings and characterizing 1 cm^2 under 4.5 h, compared to the 24 h needed in Raman mapping [109]. However, the low Raman background filters used are highly expensive (about 6-7 times the price of the cellulose filters commonly used), limiting the wide application of this technique. Vibrational spectroscopy is limited by the high cost, availability of equipment, time and effort required in analyzing and processing samples, complex data treatment, need for skilled personal and limited detection, especially in weathered or contaminated microplastics[26,31,63,79,87,111]. Visual pre-selection of particles is often used to lessen these practical problems but may induce bias [79].

In pyrolysis-gas chromatography e mass spectrometry (Pyro- GC-MS), microplastics are thermally decomposed (pyrolyzed) under inert conditions and the gas formed is cryo trapped and separated on a chromatographic column, identified by mass spectrometry [27,87]. This method can provide the chemical characterization of a single microplastic or of a bulk sample, but it is destructive and provides no information regarding number, size or shape. Thermoextraction and desorption coupled with gas chromatography-mass spectroscopy (TED-GC-MS) combines a thermogravimetric analysis (TGA) for thermal degradation (100-600ºC) and solid phase extraction of plastic degradation products, further analyzed by thermal desorption in GC-MS (~3 h) [27,31]. The advantage of this method over Pyro-GC-MS is the use of relatively high sample masses and enabling measurement of complex heterogeneous matrices, allowing identification

and quantification of polymers in environmental samples without preselection [27]. Liquid chromatography uses an appropriate solvent for the polymer type and size exclusion chromatography in characterization, requiring large amounts of sample [31]. These methods present the advantage of analyzing relatively high masses, improving representativeness, but they are destructive, and the information provided is limited to chemical composition.

Turner [97] used a portable X-ray fluorescence (XRF) spectrometer to characterize the elemental composition of polymers through the diffraction and reflection of radiation, with potential use in the detection of some additives or adsorbed metals. Scanning electron microscope (SEM) with an energy-dispersive X-ray microanalyser has also been used to collect information of morphology and chemical composition of microplastics, requiring a previous pre-selection and mounting of the particles analyzed [36]. Fuller and Gautam [37] used a pressurized fluid extraction with solvents (methanol, hexane and dichloromethane), temperature and pressure conditions to separate the bulk microplastics fraction from soil samples.

Mitigation of cross-contamination

Due to the wide contamination of the environment with microplastics, including air [29], measures should be taken during sampling to reduce the contamination with these particles and fibers. The five rules to reduce cross-contamination of microplastic samples are: (1) using glass and metal equipment instead of plastics, which can introduce contamination; (2) avoiding the use of synthetic textiles during sampling or sample handling, preferring the use of 100% cotton lab coat; (3) cleaning the surfaces with 70% ethanol and paper towels, washing the equipment with acid followed by ultrapure water, using consumables directly from packaging and filtering all working solutions; (4) using open petri dishes, procedural blanks and replicates to control for airborne contamination; (5) keeping samples covered as much as possible and handling them in clean rooms with controlled air circulation, limited access (e.g. doors and windows closed) and limited circulation, preferentially in a fume hood or algae-culturing unit, or by covering the equipment during handling [15,26,95,105,107]. A fume hood can reduce 50% of the contamination [105] while covering samples during filtration, digestion and visual identification can reduce more than 90% of contamination [95].

Conclusion

All the Earth's ecosystems are currently contaminated with microplastics. However, due to the lack of uniformity and simplicity in sampling methods, little is known about their temporal and geographical distribution in the environments needed to assess organism exposure. Variations between research groups are found in all sampling steps. For instance, there is no standard net, pore or mesh size leading to different sizes of microplastics being sampled in each study. Representativeness and reproducibility of most results is uncertain, amplified by uncareful methodology descriptions lacking important details, such as volume of bulk sample. Thus, there is an urgent need for a validated, quick and simple methodology. This new sampling protocol should include: (a) measures to reduce cross-contamination; (b) how and where to collect bulk samples; (c) how to separate microplastics from bulk samples, possibly through direct filtration, setting a filter's pore size, or with

a previous exposure to a salt saturated solution, such as NaI; (d) a digestion protocol that is quick and has little effect on polymer integrity, possibly H_2O_2 or enzymes; (e) criteria for visual identification, with the aid of staining dyes, and recommended methods ofchemical characterization.

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