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Preservation, storage, and sample preparation methods for freshwater microplastics – a comprehensive review†

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Due to their long-lasting negative effects on the environment and detrimental impact on the health of living organisms, microplastics (MPs), found in both water and sediment matrices, have attracted researchers' attention recently. Although various research and reviews have been conducted about MP occurrence and abundance in aqueous environments, less attention has been paid to freshwater matrices through which MPs enter oceans and seas. Freshwater is a vital source of drinking water supply, irrigation systems, and animal feeding systems, and it directly or indirectly affects human health. Thereby, it becomes important to study the occurrence of MPs in freshwater reserves, such as lakes and set up standardized methods for sample collection, storage, and preparation for analysis. Several recent studies have established best-practice in MP characterization and analysis. However, only a few studies have depicted the importance of the pre-analysis phase, including sampling methods, storage, preservation, and preparation strategies. Therefore, this review delineates different sampling methods from freshwater compartments – the surface and column of water and sediments, followed by storage and preservation of obtained samples. Finally, preparation (pre-treatment and extraction) methods have been elaborated, which are necessary to purify MP samples before further investigations. This review aims to provide a clear understanding of the analytical steps and tools, leading to accurate investigations regarding MP's occurrence in future studies.

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Environmental significance

Due to their long-lasting negative effects on the environment and detrimental impact on the health of living organisms, microplastics (MPs), found in both water and sediment matrices, have attracted researchers' attention recently. Although various research and reviews have been conducted about MP's occurrence and abundance in aqueous environments, less attention has been paid to freshwater matrices through which MPs enter oceans and seas. Freshwater is a vital source of drinking water supply, irrigation systems, and animal feeding systems, and it directly or indirectly affects human health. Thereby, it becomes important to study the occurrence of microplastics in freshwater reserves, such as lakes and set up standardized methods for sample collection, storage, and preparation for analysis. To perform research on microplastic detection and analysis, it is crucial to have high-quality sample preservation, storage, and pre-treatment. To the best of the authors' knowledge, this manuscript is the first review that shows the significance of storage and preservation processes has been overlooked. The review aims at summarizing different pre-analysis and pre-detection methods for microplastics in freshwater matrices, followed by highlighting the significance of storage and preservation, besides sampling and preparation methods.

1. Introduction

The production of plastics has been continuously increasing and is estimated to be doubled in the upcoming 20 years[1,2] leading to a continual flow of plastic trash into the environment. Some of these plastics, which are smaller than 5 mm and larger than 1 μm, are called microplastics (MPs). MPs can enter the environment through different external sources, including wastewater treatment effluent,[3] agricultural fields,[4] urbanization,[5] and even fishing nets.[6] Based on their manufacturing and release into the environment, MPs are classified into two major categories: primary and secondary. Primary MPs have been intentionally produced in very fine sizes to be utilized in different applications, including, and not limited to, sand-blasting[7] and cosmetic products.[8] Recent research shows that about 35% of primary MPs in water matrices stem from cloth washing.[9] On the other hand, secondary MPs are generated through the fragmentation of plastic debris in the environment because of natural phenomena including weathering, solar UV radiation, tidal waves, *etc.*[10]

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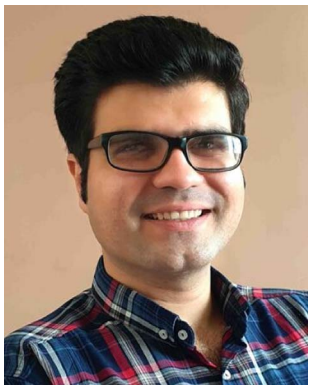
Different anthropogenic activities could also affect MP abundance in the environment. In a study, a comparison between particle presence in fish guts in urbanized and less-urbanized locations revealed a significant difference, showing the importance of urbanization in MP pollution.[11] Sediment records also revealed the high impact of urbanization on MP presence in the sediment. It has been reported that some indicators like an abundance of PET and smaller particle size at the surface level show the significance of urbanization. Agricultural activities, on the other hand, proved to be an important factor in less-urbanized areas.[12]

Wastewater treatment plants' outflow is another factor causing a meaningful difference in MP abundance. It is shown that despite the relatively high rate of MP removal in sewage and wastewater treatment (up to 99%), a sheer number of MPs enter the environment.[13] It should be noted that the entrapped particle in the wastewater treatment plant sludge is mainly used as fertilizer in agricultural lands, ending up in the environment through draining and stormwaters.[14] Wear and tear of tires has also been reported as an anthropogenic source. One study revealed that the average flow of tire particles in the environment is 0.81 kg per year.[15]

Additionally, fishing nets and cages are another anthropogenic sources of MP presence in areas that rely on fish as their



Behnam Nayebi, a PhD student at York University's Civil Engineering department, specializes in detecting and analyzing microplastics in Lake Ontario. His research during his PhD has focused on developing and optimizing methods for detecting microplastics in water and sediment samples.

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Rama Pulicharla holds a bachelor's degree in pharmacy and a master's degree in pharmaceutical sciences. Her PhD, obtained from Institut National de la Recherche Scientifique (Eau, Terre et Environnement, INRS-ETE, Université du Québec, Canada), concentrated on water science. During her doctoral studies, her main areas of focus involved the development of analytical methods and the investigation of the degradation of emerging contaminants in water sources. Rama Pulicharla has authored numerous peer-reviewed articles related to her research. Currently, she serves as a post-doctoral researcher at York University in Canada, where she continues her exploration of emerging contaminants. Her research primarily centers around environmental chemistry, with an emphasis on monitoring and implementing environmentally friendly treatment technologies for emerging contaminants.

Dr Shooka Karimpour is an Assistant Professor of Civil Engineering at York University since 2019 where she leads the Environmental HydroDynamics (EHD) lab. She is also recipient of several international and national awards including Kefeer medal by Canadian Society of Civil Engineering. Turbulent mixing and entrainment play a significant role in freshwater and marine ecosystems, for instance, on sediment and nutrient transport and availability. Shooka's research is to investigate how these processes are induced and how they affect mass and contaminant transport. Currently, her team is working on entrainment of multiphase flow, focusing on aerated flow and microplastic contaminants.

Dr Satinder K. Brar is Professor at Civil Engineering Department and the first James and Joanne Love Chair in Environmental Engineering at York University since January 2019. Her research interests lie in the development of finished products (formulations) of value-added bioproducts based on wastewater and wastewater sludge, such as enzymes, organic acids, platform chemicals, biocontrol agents, biopesticides, butanol and biohydrogen. She is also interested in the fate of endocrine disruptive compounds, pharmaceuticals, nanoparticles, and other toxic organic compounds during value-addition of wastewater and wastewater sludge in order to find suitable biological detoxification technologies.

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Fig. 1 Anthropogenic parameters affecting microplastics (MP) abundance in freshwater environment.

food resource, including the Chi River, Thailand.[6] In the study of MP abundance in three Gogian dams, the abundance of fibers was assumed to be related to fishing ropes and nets.[16]

Domestic garbage landfills were revealed to have the most effect on MP abundance in lake environments.[17] A study revealed that there are MPs in the landfill leachate and the treatment of leachate can decrease the MP presence by up to 50%.[18]

These findings reveal the significance of anthropogenic activities in the MP abundance in freshwater compartments, which can be harmful to different biotas. Different anthropogenic parameters affecting the abundance of MPs in the freshwater environment are shown in Fig. 1.

Most of the MPs detected in freshwater compartments are made of polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyethylene terephthalate (PET). These materials are highly recalcitrant and tend to accumulate in different environments,[19] resulting in prolonged exposure and thereby harming living organisms, including humans.[20] Further, their ability to adsorb on different types of toxic materials, for instance, heavy metals, perfluoro carboxylic acids, and other emerging contaminants[21–23] allow them to act as vectors for contaminants, leading to severe damages to organisms. Other than the parent polymers, plastic additives also affect hormonal modulation and apoptosis, causing damage to organisms' cells and carcinogenesis.[24,25]

Although MPs' presence has been reported in many marine environments, only a few studies[26] have investigated the presence of these emerging pollutants in freshwater compartments such as lakes and rivers. Freshwater compartments are vital matrices since they host a wide range of plants and animals, such as fish, one of the primary human food resources. Besides, these matrices are used as a source of drinking water and for recreational purposes.[27] Moreover, it has been mentioned that MPs are roughly 80% more abundant in terrestrial compartments, including lakes and rivers than in marine environments.[26] Also, it is reported that approximately 2 million tons of plastics enter the oceans through tributaries and rivers annually.[13,28] Accordingly, without analyzing MP presence in freshwater environments, understanding their abundance in marine environments seems not sufficient.

Several review papers have investigated microplastic sampling, preparation, and analysis methods for obtaining microplastic samples from both marine and freshwater environments. However, there is a significant knowledge gap in finding protocols for the preservation of obtained samples till further investigations. MPs are materials made up of polymers and have been proven to be affected by different temperatures

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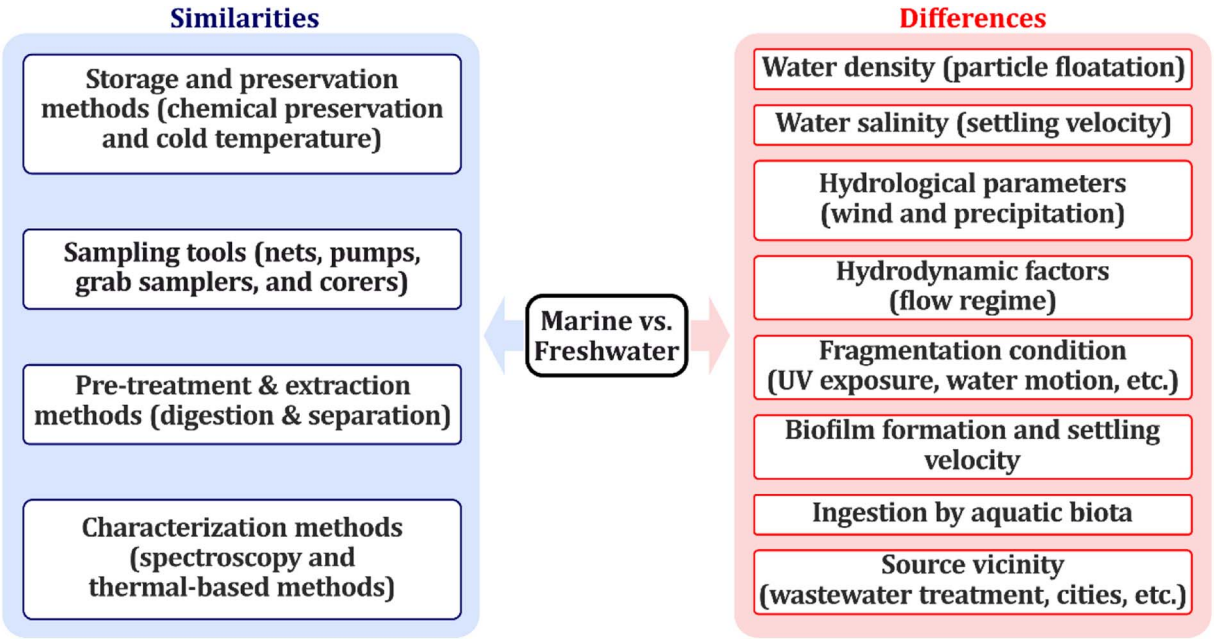

Fig. 2 Similarities and differences between MP research in marine and freshwater environments.

and chemicals. It should be noted that marine and freshwater environments have some similarities and differences, which should be considered in MP research, some of which have been identified in Fig. 2. Methods for sample storage, preservation, digestion/extraction, and characterization are similar for both compartments. However, the ambient properties and their vicinities to the pollution sources are different. Marine and freshwater are different in terms of density and salinity. Tursi *et al.*, have shown that these two factors can significantly affect the MPs distribution. Many plastic polymers have densities marginally smaller or larger than freshwater. Due to the increase in marine water density, compared to freshwater, it has been discussed that in marine environments floating MPs may outnumber those in freshwater environments.[29] However, it should be considered that buoyancy is affected by other factors apart from the density, for instance, flow currents, particle size, and biofilm formation.[30] Another example is areas with higher expected fibers, as explained below: only sampling methods should be adopted that aren't selective and mesh-based methods should be avoided. In addition, it was shown in another study that an increase in salinity leads to a decrease in the settling velocity of particles.[31] Settling velocity is an important factor since it can affect the abundance of MPs in water and sediment of both freshwater and marine environments. From a hydrodynamic perspective, rivers are highly dynamic systems, with smaller depths and higher speeds, compared to oceans which cause the resuspension and settling behaviors of particles as well as biofilm formation.[32,33] Environmental factors like UV, wind, and rain can be different in freshwater and oceans. UV exposure combined with water movement speed up the fragmentation process, and particles are more exposed to the sun and tidal waves in oceans compared to lakes. Therefore, the fragmentation of particles may increase the number of fragments in the marine environment compared to the higher abundance of fibers in rivers and lakes.[34] Another important factor affecting the shape distribution is the vicinity to the MP sources like wastewater treatments. In general, freshwater environments are closer to cities and wastewater treatments, which affects the abundance of microfibers in these environments in comparison with marine compartments.[35] In a comparative study in China, it was observed that microfibers are the most dominant in rivers and estuaries. However, fragments were predominant in the marine environment which shows the impact of the source vicinity as well as environmental parameters.[36] Biofilm formation is also different in freshwater compared to marine environments. It has been proven that PP fragments have a better biofilm condition for freshwater algae, impacting the distribution of specific MPs in freshwater and marine environments.[37] Biofilm formation is also affected by the shape of particles. Microfibers show a faster sinking velocity when they expose to biofilm formation. This is important since the distribution of MPs is different in marine and freshwater environments, thus the abundance in water and sediment of freshwater is affected by their settling velocities.[30] Biotas can mistake MPs for their food resources, being contaminated by these toxic materials. Issac *et al.*, reported that species inhabiting freshwater environments may experience increased levels of exposure, especially in close proximity to industrial and densely populated regions, where concentrations of hydrophobic toxins and microplastics are potentially elevated.[38]

In order for microplastic research to be precise, it is crucial to consider the potential effect of various parameters, including temperature, chemicals, *etc.*, on microplastics. Since procedures for extracting, quantifying, and analyzing MPs in labs are time-consuming, finding adequate preservative and storage materials is crucial to prevent sample deterioration over time

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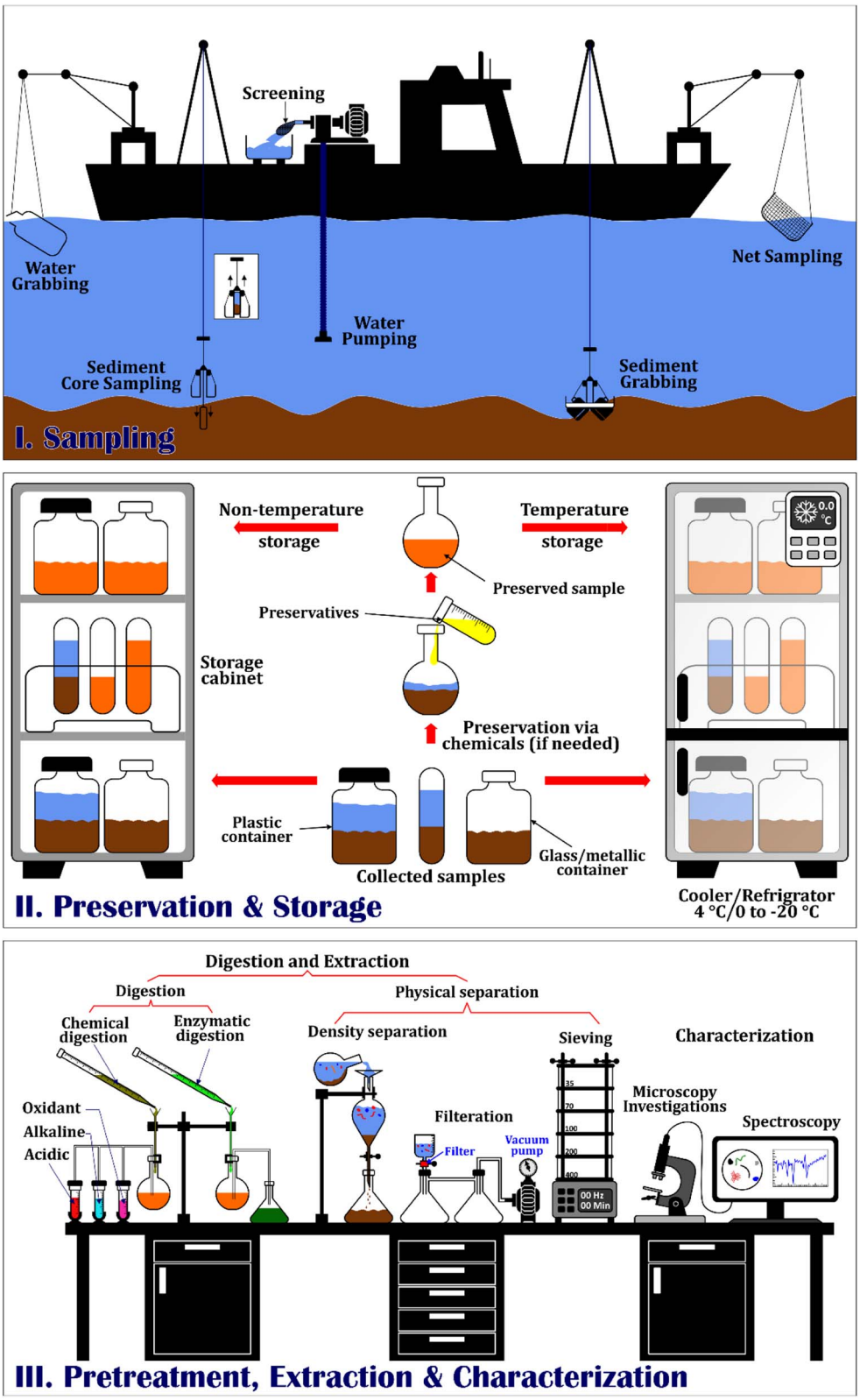

Fig. 3 Different pre-analysis phases in MPs studies (starting from steps 1 to 3).

and ensure higher sample quality for analysis. Therefore, this review paper aims to provide a detailed methodology for pre-analysis methods, including sampling, sample storage and preservation, and sample preparation (extraction and pre-treatment) to have high-quality data reflecting MPs abundance in the freshwater water and sediment. First, since these

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freshwater sources are immense, it is vital to carefully select the sampling sites, giving an accurate image of the whole environment. Second, storage and preservation of collected samples are also necessary since, without proper preservation and storage methods, the quality of samples and MPs can change due to biological activities. Finally, extraction and pre-treatment of samples should be conducted since obtained samples contain impurities (organic tissues, silts, sands, *etc.*) which can attach to MPs and cause deviations in results for both qualitative and quantitative estimations about MPs. As shown in Fig. 3, this review briefly explains different applied sampling methods for freshwater water and sediment sampling, including using nets, pumps, and bulk sampling for water and utilizing corer and grabbers for sediment sampling. Then, the most dominant part of this research relates to the importance of sample preservation and storage using different physical, chemical, and biological methods. Afterward, obtained samples would be purified before moving to the analysis step with various digestion and separation methods. Considering the importance of pre-analysis measures and to the best of our knowledge, no comprehensive studies have focused on this area, and this critical step of MP studies is often overlooked in all review papers. In this sense, the primary objectives of this paper are:

(1) Review the current state of sampling and preparation (extraction and pre-treatment) methods for freshwater water and sediment samples and other intermediate steps before the analysis stage.

(2) Provide data relating to different chemicals, storage materials, and preservation temperatures that should be used to improve the representativeness of obtained samples.

(3) Make recommendations for using different preservatives and storage materials based on the studied variables.

2. Sampling

Sampling is the first and one of the most crucial steps in MP detection studies and involves careful selection of the site for sampling and the sampling procedure to be employed. There are three different sampling procedures in any given matrix: selective, bulk, and volume-reduced methods.[39] Selective sampling detects plastic debris using the naked eye and is common when desired MPs are larger – about 1 to 5 mm, for example, large MPs in sediment samples, preferably on the shorelines.[40] Bulk sampling describes techniques that take a sample from the environment in its entirety without diluting it. This method is used when the entire sample volume needs to be explored and a smaller sample size could result in inaccurate results.[41] The other type of sampling is volume-reduced methods and refers to procedures by those larger areas of investigation can be mapped and analyzed. This technique could be used both for water and sediment sampling but is preferable for water sampling, and the main applied instruments are nets and sieves.[40,42,43] In several cases, these methods were combined and performed together to increase sampling accuracy. For instance, pumping large areas followed by sieving is the combination of bulk and volume-reduced methods used in MP studies.[44] Different sampling methods have been used considering the goals of studies in freshwater compartments, elaborated on in the following sections.

2.1 Water sampling

Based on the study goals and site accessibility, various sampling methods are proposed, among which volume-reduced methods have been widely used in water sampling. Bulk sampling is another method which is less efficient for water sampling since it requires a high amount of water to be representative, decreasing its feasibility. This method is mostly used when the main objective of the study is to evaluate fibers that can pass through volume-reduced sampling instruments.[45,46] Each sampling method has merits and demerits that should be considered before choosing the method. A comparison between different sampling methods for freshwater water sampling is summarized in Table 1.

When a more extensive sampling area is preferred, nets hauled by boats or individuals, or stationary places, such as bridges, are more favorable. However, to minimize early clogging and avoid missing smaller particles, this approach necessitates the careful selection of the nets. Thereby, for smaller MPs ($\leq$330 μm), bulk sampling and collecting samples through pumps, followed by filters and grab samplers can be used.

2.2 Sediment sampling

Apart from the water matrix, MPs have been abundantly found in freshwater sediments. Most plastic compounds in aquatic environments have different states of buoyancy based on their density, as shown in Table S1.†[47] It is estimated that roughly 70% to 90% of MPs are present in the sediment.[48] However, the percentages might change based on ambient characteristics such as hydrodynamic regimes and biofouling. Different factors can affect the presence of MPs in water and sediment as shown in Fig. 4. In general, low density particles are afloat and high density particles are sunk in the sediment. However, this behavior may be different in real conditions. For instance, in a turbulent flow, even positively buoyant particles can be transferred through the vortical structures to deeper water and, *via* other processes, such as density currents, be trapped in the sediment. Furthermore, MPs are known to possess hard and easy-to-colonize surfaces, facilitating the microorganisms' colonization. Also, the surface area of plastic particles on the micro and nanoscale grows, increasing open pores for microorganisms' colonization, causing even light MPs to settle in sediments.[47,49–52] The combined effect of biofouling and turbulent-induced mixing can lead to the presence of positively buoyant MPs in sediments.[30]

Like water sampling methods, the procedures for MPs collection in sediments are selective, bulk, and volume-reduced sampling. The leading equipment for selective sampling of sediments is tweezers, and this procedure is efficient for detecting larger MPs.[53] Further, from riverbanks/lakeshores, samples can be collected using stainless steel shovels, spoons, and spatulas,[54] while sampling from the bottom of lakes can be done using different grabbers, corers, and samplers.[39,55] The primary sampling equipment is Ekman, Van Veen grab, and the

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Table 1 Advantages and disadvantages of sampling using nets, grab samplers, and pumps

Method	Advantage	Disadvantage
Nets	Able to analyze larger study areas and catch higher concentrations of MPs	Underestimating small particles, particularly fiber, which could easily escape High risk of secondary MP contamination through exposure to air and net materials (nylon)
	Preferable when the MP detection is done through the naked eye	Less accurate reported sampling volumes due to inaccuracy of volume calculating through flow meters or mathematical operations More number of preparation steps, increasing airborne contamination of samples
	Low price and easy accessibility of nets with large mesh size (>330 μm)	Low repeatability to assure volume accuracy High cost of nets with a small mesh size (<330 μm)
Grabs	Could investigate the broader size of MPs by selecting smaller filters and sieves Able to be used in an environment where net sampling is tough Able to be applied for sampling from deeper columns of water with Niskin bottles	Small volumes of grabs cause high variability between samples
	Decrease the risk of secondary pollution due to shorter contact time with the sampling compartment and using non-plastic containers Can be conducted by citizen science method, increasing the accuracy of sampling Appropriate reports of MP abundance in the precise volumes	Lower particle concentration compared to nets, culminating in more probable false zero reports
	Non-plastic grab samplers could be heated up to 500 °C before sampling to eliminate any potential residues Require only one filtration, decreasing the risk of airborne contamination High repeatability to assure volume accuracy	Difficulty in the transportation of large volumes of bulk samples to the laboratory
Pumps	Could investigate the broader size of MPs by selecting smaller filters and sieves	Risk of secondary plastic contamination through the materials of pumps, ropes, and filters
	Able to be used in an environment where net sampling is not applicable High repeatability to assure volume accuracy	Lower particle concentration compared to nets, culminating in more probable false zero reports High risk of the clogging of limited filters area when a large amount of water is investigated Fragmentation of MPs to nanoplastics due to shear stresses caused by pumps blades

Box Corer. For sediment sampling from depth, a columnar sampler (inner diameter = 7 cm, height = 100 cm) is required.[51] Corer-based methods are also preferable when the bottom of the water bodies is hard, leading to the malfunction of grab-based methods, which cannot close efficiently.[56] However, corer-based methods are ineffective when the risk of core freezing is high. The number of sample replicates is another factor affecting the accuracy of sediment sampling which is reported to be varying from 2 to 5 times.[57] The sampling depth is also important, and it is noted that most MPs occur in the top 5 cm of the sediment layers, however, some recent studies included the collection of deeper sediments for vertical analysis of MP appearance in the samples.[1,58,59] Further, a recent study proved that MPs abundance decreases with an increase in sediment depth.[60] Another study showed that bigger MPs (4–5 mm) are more abundant in shallower layers, but the number of MPs raised by an increase in depth for smaller MPs (<2 mm). The number was 40 items per kg of wet sediment (items per kg WW) in 0–10 cm and 110 items per kg WW in 40–50 cm.[61]

3. Sample storage, preservation, and contamination control

After obtaining samples, they are often transported to the laboratories and stored before analysis to prevent physico-chemical and microbial reactions.[56] This process may take time to transfer samples from sampling sites to the labs, altering the quality of obtained samples. As a result, optimizing the storage conditions, storage containers, temperature, and preservation chemicals becomes imperative.

3.1 Sample storage materials

The type of containers being used for microplastic sample storage is an important parameter to consider, as they can

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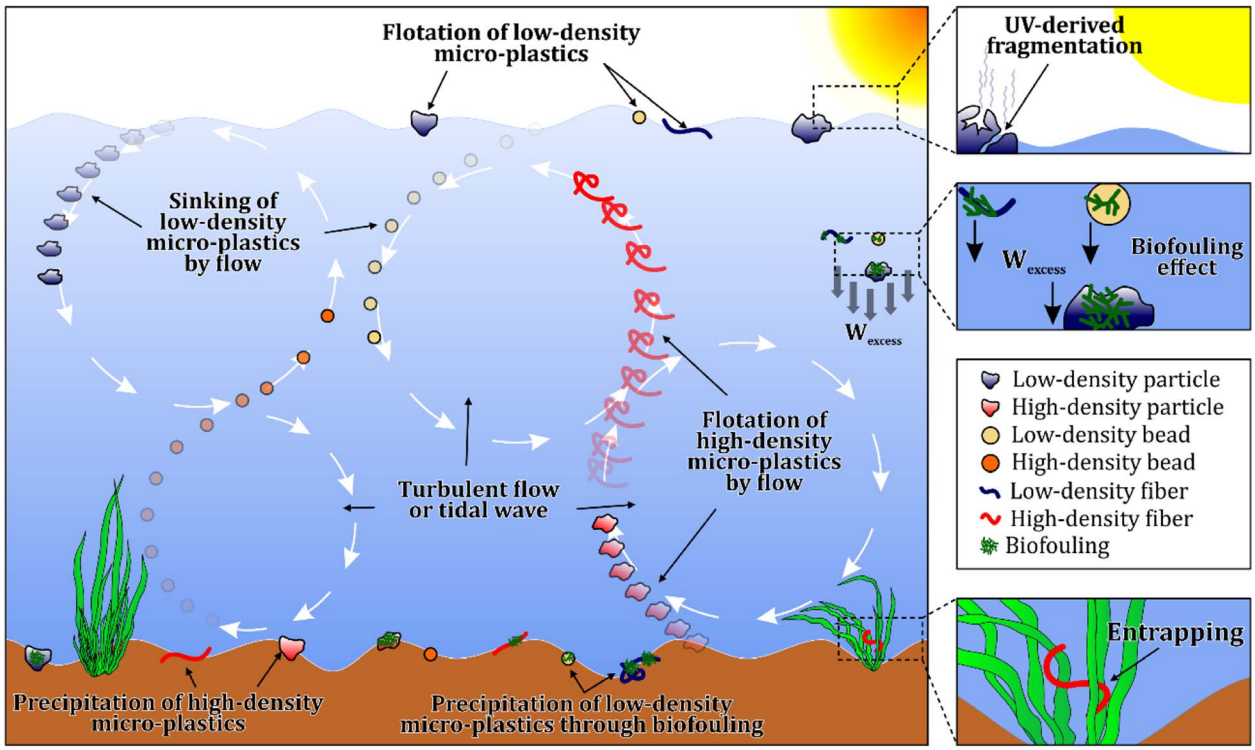

Fig. 4 Schematic of MP settling behavior affected by different parameters.

influence the stability and contamination of some analytes. The use of non-plastic containers, such as glass, steel or aluminum-made vessels, is preferable since these containers reduce unwanted plastic pollution.[42,62] However, some studies have also used polymer-based vessels, such as PE bags,[63] PET jars,[64] PP bags,[65,66] and Whirl-Pak or zip-lock bags.[67,68] Unlike glass containers, which can break due to falling, especially while sampling on boats, plastic-based vessels can be used in sampling aboard ships and boats. Nonetheless, due to the plastic abrasion, particularly by sediment grains, the above materials might cross-contaminate the samples with MPs, hence is not recommended to store sediment samples.[56] If it is not feasible to avoid storage of samples in polymeric containers, appropriate blanks should be considered in different stages of analysis, namely sampling, pre-analysis, and analysis phases. By subtracting the findings MPs from blank data, the over-estimation caused by the abrasion of plastic materials could be hindered. Field and laboratory blanks in the study of MP abundance in Lake Simcoe show 1 to 27 particles, which were subtracted from MP numbers before characterization analysis.[63] A study on different containers for storing food and water revealed that various factors such as physical stress and squeezing, scissoring, tearing, and cutting the bottles expand the abundance of MPs in the stored samples. These studies revealed that plastic containers could be a source of MP over-estimation/cross-contamination.[69,70] Besides, the aging process could increase plastic cross-contamination, another attribute of plastic containers.[71] In terms of recycling/non-recyclable plastic containers, it is proved that recyclable plastic-made containers release MPs into the samples 8 times higher than single-use plastic containers, showing the effect of plastic aging on the leachate of MPs into the samples.[70] Table 2 provides more information about the storage condition governed in different freshwater studies. It can be seen that only very few studies used polymeric storage containers to store the water and sediment materials, which is mainly related to avoiding adding unwanted plastic contamination due to the abrasion of polymeric containers. Researchers have used glass containers in most of the mentioned studies in Table 2. However, in conditions that sways related to the transportation of samples will happen, glass materials may fall and break. Therefore, metal-based containers, stainless steel or aluminum, should be considered to avoid the risk of glass breaking.

3.2 Sample storage temperature and preservation chemicals

Besides container type, maintaining the temperature of samples while transferring from sites to laboratories and storing them in laboratories to avoid sample deterioration, mainly caused by biological reactions, is essential.[56,72] Although microplastics are designed to be durable, recent reports have unveiled the presence of potential microbes that can degrade microplastics.[73–76] Sediment and soil microorganisms in marine and terrestrial ecosystems have been reported to biodegrade debris and MPs.[76–78] For instance, commercial synthetic organic polymers, such as polyesters polylactic acid (PLA), poly-caprolactone (PCL), polybutylene adipate terephthalate (PBAT) and polyhydroxybutyrate (PHB) contain polyester bonds, which can be degraded by enzymes (esterase), released by ubiquitous

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Table 2 Details of MP sampling and preservation in different studies

Location	Sampling method	Storage, preservation, and pretreatment methods	Abundance	Reference
Lake water				
(1) Taihu Lake, China; (2) three lakes, Poland; (3) Lake Naivasha, Kenya; (4) Lakes Mead and Mohave, U.S. (5) Veeranam Lake, South India; (6) four lakes, Italy; (7) Dimon Lake, Carnic Alps; (8) Nine Lakes, Argentine; (9) Lake Guaíba, Brazil; (10) Red Hills Lake, India	Net sampling	Storage and preservation: (1, 10) 1 L glass container containing 5% methyl aldehyde; (2) 100 mL screw-capped vials; (3) poured in glass bottles and kept at 4 °C (4) glass jars containing isopropyl alcohol (5) a 250 mL amber bottle; (6) kept at 4 °C in glass vials containing 30% hydrogen peroxide; (7) frozen at −20 °C in plastic-free containers; (8) glass bottles at −20 °C; (9) 250 mL glass vial, stored at 0 °C Pretreatment: (1, 5, 8) 30% H_2O_2 digestion + drying; (2) 69% nitric acid + 30% H_2O_2 digestion; (3) 30% H_2O_2 and Fenton digestion + drying; (4) sieving + 30% H_2O_2 digestion and Fenton reagents + sieving + density separation (lithium metatungstate); (5, 7); (6) density separation (NaCl); (9) sieving + 35% H_2O_2 digestion and Fenton reagents + 35% H_2O_2 digestion + density separation (NaI); (10) sieving + drying + 30% H_2O_2 digestion and Fenton reagents + drying + density separation (NaCl)	(1) 3.4–25.8 items per L; (2) 4930 fibres per m^3; (3) 0.183 ± 0.017–0.633 ± 0.067 items per m^2; (4) 0.44–9.7 items per m^3; (5) 28 items per km^2; (6) 100 036 MPs per km^2; (7) absence of MPs; (8) 0.9 items per m^3; (9) 11.9 ± 0.6–61.2 ± 6.1 items per m^3; (10) 5.9 items per L	88, 91 and 100–106
(1) Lake; Simcoe, Ontario; (2) Rawal Lake, Pakistan; (3) La Salada lake, Argentina; (4) Remote; Mountain Lake, Switzerland	Grab sampling	Storage and preservation: (1) stored in pre-cleaned polyethylene or PET jars and preserved in 20% ethanol solution; (2) the glass bottles held at 4 °C; (3) 4% formalin Pretreatment: (1) sieving + rinsing; (2, 3) 30% H_2O_2 digestion + drying	(1) 0–0.7 items per L (grab); 0.4–1.3 items per m^3 (trawl); (2) 1.4 items per L (3) 140–180 items per m^3; (4) 2.6 microplastics and 4.4 fibres per litre	63 and 107–110
(1) Lake Donghu, China; (2) Ox-bow Lake, Nigeria	Pump sampling	Storage and preservation: (1) stored in 50 mL brown glass jars containing 5% methyl aldehyde and at 4 °C; (2) stored in a clean beaker and preserved at −3 °C Pretreatment: (1, 2) 30% H_2O_2 digestion + drying	(1) 7.4–29.6 items per L; (2) 1004–8329 items per m^3 (dry season); 201–8369 items per m^3 (wet season)	111 and 112
River water				
(1, 3) Gave de Pau River, France; (2) Milwaukee Rivers, USA; (4) Illinois, USA; (5) Vistula River, Poland; (6) North Saskatchewan River, Canada; (7) Three Manitoban Rivers; (8) Ganges River, India; (9) Citarum River, Indonesia; (10) Upper Guayllabamba River Basin, Ecuador; (11) Rogue River, USA; (12) Rhône and Têt Rivers, France; (13) Thames River, UK; (14) Magdalena River, Colombia; (15) Surabaya River,	Net sampling	Storage and preservation: (1, 2, 3, 9, 12, 14); (5) glass containers containing formaldehyde; (4, 6, 8, 10, 11; 16) glass jar, storing at 4 °C; (7; 15) stored in non-plastic containers and preserved with 70% ethanol; (13) 4% formalin; (17) zip-lock bag and preserved at 3 °C Pretreatment: (1) enzymatic digestion (biozyme F + biozyme SE) + 30% H_2O_2 digestion + drying + density separation ($ZnCl_2$); (2, 3, 7) 30% H_2O_2 digestion and Fenton reagents + sieving; (4, 8, 10) sieving + heating + 30% H_2O_2 digestion +	(1) 100.6 ± 99.9 fibers per m^3; (2) 0.42–2.71 particles per m^3; (3) 3.34 ± 0.20 particles per m^3; (4) 2.355 ± 0.375–5.733 ± 0.850 particles per m^3; (5) 1.6–2.55 particles per L; (6) 26.2 ± 18.4 particles per m^3; (7) 113 888–1241 085 particles per km^2; (8) 380–684 particles per 1000 m^3; (9) 0.0574 ± 0.025 particles per m^3; (10) 1584 particles per m^3; (11) 0.001–0.249 particles per m^3; (12) 11.6 ± 17.7 particles per m^3; (13) 14.2–24.8 particles	18, 58, 59, 67, 89, 90, 92 and 113–122

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Table 2 (*Contd.*)

Location	Sampling method	Storage, preservation, and pretreatment methods	Abundance	Reference
Indonesia; (16) Ofanto river, Italy; (17) Tamsui River, Taiwan		density separation (NaCl); (5, 17) sieving + 30% H_2O_2 digestion + drying; (6) sieving + heating + density separataion ($ZnCl_2$) + 30% H_2O_2 digestion and Fenton reagents + sieving; (9) sieving + 30% H_2O_2 digestion and Fenton reagents + heating + density separation ($ZnCl_2$); (11) sieving + digestion (KOH) + density separation (NaCl); (12) sieving + 40% H_2O_2 digestion + drying; (13) sieving + 40% KOH digestion + drying; (14) sieving + drying + density separation (NaCl); (15, 16) sieving + drying + 30% H_2O_2 digestion and Fenton reagents + density separation (NaCl)	per m^3; (14) 0.097–0.135 fibers per L; (15) 0.76–43.11 particles per m^3; (16) 0.9–13 particles per m^3	
(1) The Manas River, China; (2, 3) Ottawa River, Canada; (4) St. Lawrence River, Canada; (5) Netravathi River, India; (6) Fengshan River, Taiwan; (7) three rivers, UK; (8) Yellow River, China; (9) Maozhou River, China	Grab sampling	Storage and preservation: (1, 2; 6; 9) kept at 4 °C; (3) stored in Whirl-Pak bags; (4) Whirl-Pak bags and kept at −20 °C; (5, 7) stored in a glass container; (8) amber bottles with foil-lined caps, preserved in 4 °C Pretreatment: (1) Sieving + 30% H_2O_2 digestion and Fenton reagents; (2, 3, 7; 9) 30% H_2O_2 digestion; (4) oil separation + 30% H_2O_2 digestion + heating; (5, 6) sieving + drying + 30% H_2O_2 digestion and Fenton reagents + density separation ($ZnCl_2$); (8) centrifuging + density separation (NaCl) + 30% H_2O_2 digestion	(1) 32 particles per m^3: (2) 1.35 particles per m^3; (3) 0.11 particles per L; (4) 0.12 ± 0.01–0.16 ± 0.02 particles per L; (5) 288 particles per m^3; (6) 334–1058 particles per m^3; (7) 0.4 particles per L; (8) 497–930 particles per L; (9) 4–25.5 particles per m^3	68 and 123–130
(1) Ganges River, India; (2) Haihe River, China; (3) Yongjiang River, China; (4) Qin River, China	Pump sampling	Storage and preservation: (1) double-wrapped in foil and placed in polypropylene bags; (2) stored in glass jars covered with aluminum foils and preserved at 4 °C; (3) stored in a glass bottle and preserved in 5% methyl aldehyde; (4) stored in polypropylene containers in ice boxes Pretreatment: (1); (2) sieving + 30% H_2O_2 digestion + density separation (NaCl) + drying; (3) 30% H_2O_2 digestion and Fenton reagents; (4) 30% H_2O_2 digestion + density separation (NaCl)	(1) 0.038 particles per L; (2) 0.69–74.95 particles per m^3; (3) 500–7700 particles per m^3; (4) —	24, 65, 131 and 132
Lake sediment				
(1) Three Gorges Reservoir, China; (2) Yangtze River Basin, China; (3) Taihu Lake, China; (4) Lake Simcoe, Canada; (5) Lake Victoria, Africa; (6) Lakes Mead and Mohave, U.S; (7) Veeranam Lake, South India; (8) Dimon Lake, Italy; (9) Ox-bow Lake, Nigeria; (10) Red Hills Lake, India; (11) Vembanad Lake, India	Grab sampling	Storage and preservation: (1, 3) non-plastic container, preserved at 4 °C; (2) glass jar containing 5% formalin and then preserved at 4 °C; (4) amber glass bottles; (5, 8, 9) non-plastic bottle and frozen; (6) glass jars and preserved with isopropyl alcohol and stored at room temperature; (7, 10, 11) - Pretreatment: (1) 30% H_2O_2 digestion + drying + two steps density separation (NaCl + NaI); (2)	(1) 25–300 particles per kg ww; (2) 50–580 particles per kg; (3) 11.0–234.6 particles per kg dw; (4) 8.3–1070 particles per kg; (5) 0–1102 particles per kg dw; (6) 87.5–1010 particles per kg dw; (7) 309 particles per kg; (8) no MPs; (9) 347–4031 particles per kg; (10) 27 particles per kg; (11) 252.8 particles per m^2	63, 88, 100, 103, 105, 110, 111 and 133–136

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Table 2 (*Contd.*)

Location	Sampling method	Storage, preservation, and pretreatment methods	Abundance	Reference
		30% H_2O_2 digestion + drying + density separation (NaCl); (3) density separation (NaCl) + 30% H_2O_2 digestion; (4) sieved + drying + density separation ($CaCl_2$); (5) density separation (NaCl) + 30% H_2O_2 digestion and Fenton reagents + density separation (NaCl) + drying; (6) drying + density separation (lithium metatungstate) + sieving + 30% H_2O_2 digestion and Fenton reagents + sieving + density separation (lithium metatungstate); (7) sieving + drying +30% H_2O_2 digestion + density separation (Milli Q deionized water) + density separation ($ZnCl_2$); (8) density separation (NaCl) + drying; (10) sieving + drying + 30% H_2O_2 digestion and Fenton reagents + density separation (NaCl) + drying; (11) sieving + drying + 30% H_2O_2 digestion + density separation (NaCl) + drying		
(1) Yangtze Estuary, China; (2) Fuhe River Estuary, China; (3) Lake Ontario, Canada	Core sampling	Storage and preservation: (1) non-plastic containers, kept at 4 °C; (2); (3) PET bottles, stored at −25 °C Pretreatment: (1, 2) drying + density separation (NaCl) + 30% H_2O_2 digestion; (3) drying + density separation (sodium polytungstate) + density separation (deionized water)	(1) 10–60 particles per kg dw; (2) 1049 particles per kg; (3) 760 particles per kg dw	51, 64 and 137
River sediment				
(1) Yangtze River, China; (2) Karnaphuli River, Bangladesh	Grab sampler	Storage and preservation: (1, 2) stored in a non-plastic container and frozen Pretreatment: (1) density separation ($ZnCl_2$) + drying; (2) drying + density separation ($ZnCl_2$) + sieving + 30% H_2O_2 digestion and Fenton + density separation (NaCl) + sieving	(1) 34 particles per kg; (2) 22.3–59.5 items per kg dw	138 and 139

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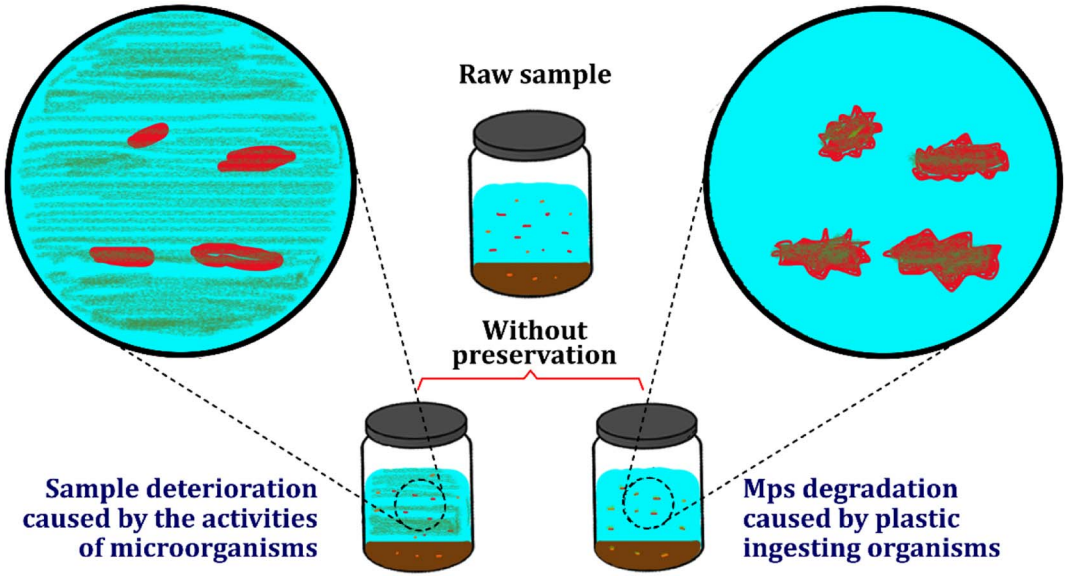

Fig. 5 Schematic of bacterial effect on unpreserved samples.

bacteria and fungi.[76] These potential plastic-eating microbes can attach to the surface of the polymer (formation of biofilms) and release extracellular enzymes, such as lipase, laccase, polyesterase, and lignin peroxidase, resulting in depolymerization (polymer to oligomers and dimers) and ultimately mineralization (carbon dioxide and water). Different synthetic MPs have different degrees of biodegradability and require suitable conditions-pH, temperature, nutrients, and humidity.

Although the process is very slow, to maintain the integrity of the MPs in collected samples, it becomes necessary to contain the microbial action and prevent biodegradation of the polymeric chains by providing unfavorable conditions, such as cold temperatures. It is a common practice to store environmental samples including water, soil, sediment, and sludge at 4 °C until further use and is often used for MPs as well. However, as 4 °C only slows down the bacterial activity, it is effective for only a short period (a few weeks, less than a month), as suggested for sludge and sediment samples.[79,80] For long-term storage, cryopreservation-freezing or ultra-freezing conditions (−20/−80 °C) are advised to suppress both biological and chemical reactions, which can otherwise affect the composition of the samples.[81] The schematic of sample and MPs deterioration caused by bacterial growth and activity is illustrated in Fig. 5. As seen in the figure, long-term storage of MPs without proper preservation and storage could, directly and indirectly, affect the quality of MPs contained samples. In other words, plastic-ingesting organisms may affect the MPs in the water and sediment, changing their characteristics and shapes and decreasing the accuracy of sample analysis (the right side of the figure).[82] Apart from plastic-ingesting organisms, algae, fungi, *etc.*, could grow inside the samples. As a result, the samples would need extensive pretreatment processes, particularly the digestion process, to eliminate the growth organisms, which increases the risk of the negative impact of digestion chemicals on the particles. Without extensive pretreatment methods, some particles, especially transparent MPs, could hide behind these impurities, adding error to the analysis process.

When storing the samples in ultra-freezing temperatures, it becomes necessary to study the effect of freeze–thaw cycles on the MPs. Recent reports have revealed that continuous freeze–thaw cycles can accelerate the aging of MPs and affect their physicochemical properties. Aged MPs are reported to have smaller sizes, increased specific surface area (SSA), and more oxygen-containing surface functional groups, leading to increased adsorption sites for co-existing contaminants, such as heavy metals and organic matter. A recent study by Sun *et al.* (2022) reported that the −25/+25 °C freeze–thaw cycle increased the SSA, pore size and pore volume of PVC. They have also reported that freeze–thaw cycle aging of PE results in increased pore size (from 29.8 nm to 123.4 nm) and pore volume due to local rupture of the microplastic surface due to water penetration during thawing.[83] Also, freeze–thaw cycling can destroy the amorphous region of the polymers, thereby increasing the crystallinity and making the polymer more brittle. A few reports also reveal the formation of stable aggregates in MPs suspension after exposure to 10 freeze–thaw cycles.[84,85] Barb and Mikucki (1959) reported that frozen suspensions of 50–100 nm polystyrene latex particles remained agglomerated even after thawing at room temperature.[84] Therefore, from these reports, it is evident that continuous freeze–thaw cycles can affect the characteristics of the MP sample and must be avoided to introduce any deviations in the results. One way to avoid continuous freeze–thaw cycles is to store the samples in aliquots. Another prospective course of action is lyophilization, as suggested recently by.[86] The collected samples can be subjected to centrifugal force to separate clear liquid and solid fractions containing the MPs. The liquid fraction can be filtered, and MPs collected on filter paper or discarded, and the solid fraction can be frozen overnight, followed by lyophilization or freeze-drying. The dry samples can then be passed

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through molecular sieves of desired sizes to obtain the MPs of interest. Freeze drying of sludge is a frequent practice and can be extrapolated to sediments containing MPs.[87] The lyophilization of samples will eliminate the storage of large amounts of samples/space requirements. Since the method does not use any solvent or elevated temperatures, the samples are supposed to retain their physicochemical and mechanical properties and can be stored at room temperature, or 4 °C, for long periods.

However, in some cases, freeze-drying may not kill all bacteria and it is safe to use a preservative, such as 5% methyl aldehyde, 20–70% alcohol, and formalin, to further inhibit microbial growth in free-dried samples. These are industrial disinfectants and have been commonly used for the preservation of various samples to retard both biological and natural chemical changes, which can affect the sample characteristics.[56,63,88–90] For instance, formalin 4% and formaldehyde were used to preserve lakes and rivers water samples with MPs ranging from 32 to 5000 μm.[90–92] Further, a recent investigation into the identification of fiber microplastic in Lake Simcoe used 20% ethanol to preserve water samples.[63] In other studies, samples collected by manta trawling were preserved in 70% ethanol solutions.[59,89] Table 2 expands further on the sampling and preservation of the collected samples from lakes, rivers, and sediments in recent studies. As can be observed in Table 2, a large number of lake and river water samples have been stored at low temperatures, 4 °C and 0 °C. However, in some of the studies, and based on the organic load of the samples, samples could be kept in freezers. Regarding the preservative chemicals, few studies have preserved their samples in chemical solutions, which could end up with inaccuracies in the results due to possible alteration of sample structures.

The selection of the preservative, however, depends on the chemical compatibility of the solvent and the chemical makeup of the MPs of interest. The most important factors that should be considered are the solubility condition of polymers and chemicals and the polarity condition of these materials. When polymers and chemicals have the highest difference in solubility and polarity states, the polymers will stay in a compatible condition. Otherwise, the situation is considered incompatible, causing changes in the polymer structure.[93] Since most of the MPs detected in freshwater compartments are attributed to PE, PP, PS, and PET, the chemical compatibility of these materials with solvents, such as alcohols, aldehydes, and formaldehyde, is summarized in Table 3. It should be noted that, as mentioned earlier, there is a giant gap in the analyzed research papers about the potential effects of different environmental and experimental conditions on the found microplastics. Therefore, the table is provided based on the chemical compatibility charts provided by polymer industries and polymer handbooks. It is evident that PP is highly resistant to solvents and chemicals and does not show any damage from exposure to them. To strengthen and cite, a recent study that evaluated PP samples under different solvers has shown that the mass, dimensions, or thermal properties of the samples remained unchanged.[94] Further, as per the results from the literature, PS, PP, and PET polymers have good compatibility in an ethanol solvent.[94,95] Solvolytic reactions, like alcoholysis (methanolysis), hydrolysis, transesterification, and glycolysis, generally attack the C–X bonds (where X is the heteroatom, such as O, N, Cl, in the polymeric chain), for example, polyesters (PET) or polyamides. Transesterification of PET using alcohols is a common technique for chemical recycling; however, it requires supercritical conditions, higher temperatures (180–220 °C), an inert atmosphere, and catalysts.[96–98] Therefore, preserving MP samples in alcohols, such as methanol, at 4 °C or under ambient conditions does not affect the chemistry of the polymer PET even after 30 days of constant exposure and can be used for the preservation of MP samples containing PET. Similarly, LDPE, HDPE, and PS show little to no damage, after 30 days of exposure, when preserved in ethanol, isopropanol or formaldehyde. Whereas PVC is susceptible to nucleophilic attack-both substitution, and elimination, followed by dichlorination, as suggested by.[99] The conjugate base of alcohols, or the alkoxides ($R–O^-$), are good nucleophiles and can attack the polymeric chain to alter its properties within 30 days of constant exposure (Table 3). The mentioned table is obtained based on the chemical compatibility of polymers at room temperature. More details regarding the storage, preservation, and precautions required for maintaining the quality of samples over longer times are given in Table 2. However, it is essential to notice that in almost all of the reviewed articles, the effect of preservation methods on MPs has yet to be analyzed, which may cause the misidentification of MPs since different conditions could cause changes in plastic physical and chemical characteristics. Therefore, it should be of the most important factors to be considered in the upcoming microplastic studies. Thermoplastic polyesters and Nylon-66 become brittle even in water after two months,[93] which shows the vital role of this missed part of microplastic research. Therefore, we strongly recommend devising spiking tests evaluating the effect of preservation/lack of preservation on the obtained results.

Table 3 Chemical compatibility of common polymers with preservation chemicals[a,b,c]

	MPs					
Solvent	LDPE	HDPE	PP	PS	PVC	PET
Ethanol	E	E	E	E	G	E
Methanol	E	E	E	G	G	G
Isopropyl alcohol	E	E	F	B	B	B
Formaldehyde 10%	E	E	E	E	E	—[d]

[a] E: 30 days of constant exposure causes no damage. Plastic may tolerate for years. [b] G: little or no damage after 30 days of constant exposure to the reagent. [c] B: some effect after 7 days to the reagent. The effect may be crazing, cracking, loss of strength or discoloration. [d] There is no conclusive evidence on the effect of formaldehyde on PET.

3.3 Quality assurance/quality control (Q_A/Q_C)

Besides storage and preservation methods, quality assurance is imperative for a more accurate MP analysis. Apart from sampling sites and laboratory storage, all the equipment should be cleaned before usage and be covered with aluminum or glass

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lids when applicable.[56,62] All surfaces should be rinsed with either Milli-Q water or alcohol and working under laminar airflow in laboratories and fume hoods would be preferable.[140] Although laminar flow hoods draw air through high-efficiency particulate air (HEPA) filters to make laminar airflow, preventing the entrance of uncontrolled air, all labs may not have access to this advanced equipment.[141] Also, bright colour sponges and other clothes can be used to ease the detection of potential secondary contaminations.[142] To avoid fiber contamination, researchers should wear cotton clothes and latex gloves, and the exposure of samples should be minimized by isolating them.[56] Considering specific spaces in labs that are not in laboratory traffic zones, pre-filtering all utilized solutions, and conducting blanks in different steps should also be considered.[62] Moreover, working solutions used in the laboratory processes, including preservation, preparation and extraction procedures, should be pre-filtered and kept in suitable containers.[62] Also, water sampler materials, *i.e.*, nets, pumps, and grabs should be selected meticulously to decrease the risk of unwanted secondary contamination originating from samplers. To do so, Karlsson *et al.* (2019) suggested the use of a net with an aluminum frame and nylon net. Apart from nets, non-plastic pumps should be used for water sampling *via* pumps, and they recommended using stainless steel pumps, filters, and ropes for submerging the pump from the boat.[143] Heating, cooling, or rinsing of the used lab instrument, including beakers, flasks, filtration systems should be done before sampling and analytical investigations.[62]

4. Sample preparation (extraction and pre-treatment)

After being transported to the laboratories, samples should be prepared for the next steps of MP analysis preparation. This is an essential and critical step in MP's quantitative and qualitative analysis.[144] Based on the environmental sampling parameters such as the load of organic/inorganic particles, and the size of impurities in samples, morphology of water/sediment surface, bottom gradient, vegetation, *etc.*, different steps, including but not limited to digestion and extraction are used for the preparation step of both water and sediments.[145,146]

4.1 Sample heating

To obtain an accurate picture of MP presence in water and sediment samples and eliminate the effects of humidity, various studies applied heating as the first step of the preparation procedure. The most frequent method for this purpose is drying samples at high temperatures (between 35 °C to 100 °C) until reaching a constant weight.[105,147,148] However, to avoid probable MP deformation, it is suggested to put samples in an oven under 60 °C.[39] It is imperative to acknowledge that permeation is a factor that can compromise the structural integrity of polymers. The rate of permeation of a polymer can be augmented by an increase in temperature, due to two primary reasons. Firstly, the solubility of the permeant in the polymer becomes more pronounced at elevated temperatures. Secondly, the polymer chains exhibit more extensive movements at higher temperatures, facilitating the diffusion of the permeant. Hence, it is vital to consider the heating temperature within a range that does not jeopardize the polymer structure.[93]

4.2 Sample digestion

Another pre-treatment step is to purify samples that indeed contain organic matrix. Two main water/sediment sample purification strategies are enzymatic and chemical digestion/oxidation of the organic matrix. Chemicals that are mainly added to digest organic matter can be divided into three main categories, acid, alkaline, and hydrogen peroxide-based oxidation.[140] It is proven that using acid and alkaline as digestion materials can result in the degradation and damage of MP structures.[149] Therefore, hydrogen peroxide-based methods have been widely used in studies as a digestive reagent in both water and sediment samples.[39,150] It is proven that the number and size of MPs are not affected by adding hydrogen peroxide, although they may be discolored.[148] Acidic digestion shows weight loss in PE, PET, PP, and PS structures since acids could dissolve these polymers and cause shifts in their functional group's spectroscopy. The alkaline process shows a significant surface change in PET films since this polymer consists of carbonate and ester linkages, which makes them susceptible to alkaline hydrolysis of these functional groups.[151] Enzymatic degradation could also be employed for sediments containing high amounts of planktons.[152] However, this method is mainly used when the study aims to evaluate MP presence in biota tissues. Besides, enzymatic methods are expensive, the preservation of enzymes is challenging, and different enzymes are required for degrading various organic compounds.[39] Another method for degrading organic compounds presenting in water and sediment samples is using Fenton reagents. This method is based on the reaction between hydrogen peroxide and Fe^{2+} ions to generate hydroxyl radicals, oxidizing the organic contaminants.[153] Using Fenton reagents combined with peroxidation is a method recommended by National Oceanic and Atmospheric Administration (NOAA).[153] In a study, 98% of organic load reduction and recovery rate with no alteration in polymer structures were achieved using the Fenton oxidation method.[154] 30% H_2O_2 was used for water samples' digestion in urban surface water in Wuhan, China, for 24 h and at room temperature, and the abundance of MPs was up to 8925 $\pm$ 1591 particles per m^3.[155] The combination of sediment samples drying, followed by digesting using 50% HCl and 30% H_2O_2 was utilized for water and sediment preparation, and 90.6 $\pm$ 2.9% to 93.5 $\pm$ 5.7% recovery rates for water samples and 96.8 $\pm$ 4.3% to 104.5 $\pm$ 3.7% for sediment were achieved for PET microplastics. As the authors mentioned, more than 100% of the recovery rate for sediment samples is related to errors that happened during the experiments.[156] Based on the quantity of organic matter present in samples, adding hydrogen peroxide to samples under temperatures and purity attracts the researcher's attention and is the method that has been widely used in studies related to freshwater sediment purification.[112,133,139,148,157] Based on the mentioned reasons, hydrogen peroxide-based methods

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Table 4 Advantages and disadvantages of different salt solutions used in density separation

Density separation solution	Density (g cm^{-3})	Advantage	Disadvantage
Deionized water (DI water)	1	Non-toxic, highly available, able to afloat lighter MPs	Unable to afloat high-density polymers
NaCl	1.2	Low cost, non-toxic, highly available, high recovery rate	Unable to afloat high-density polymers such as PET and PVC
$CaCl_2$	1.4	Low-cost, highly available	Unable to afloat high-density polymers, less efficiency in high organic load matrices
Sodium metatungstate (SPT)	1.4	Non-toxic, able to afloat high-density MPs	Expensive
NaI	1.5–1.8	Separation of very high-density polymers	Expensive, hard handling, time-consuming separation process
$ZnCl_2$	1.5–1.7	Toxic, separation of high-density polymers	Harmful, corrosive, blackening filter papers

are shown to be the best method for freshwater water and sediment samples due to their higher recovery rate and ease of application, which is following the provided literature review depicted in Table 2.

4.3 Density separation

The separation of MPs from inorganic compounds such as sands and silts is another important extraction method. This method is mainly used when the sample contains fine and coarse grains that may cause the entrapment of MPs in these ambiances.[39] The density of most MPs existing in freshwater bodies varies between 0.01–2.3 g cm^{-3} (Table 2). Some particles are buoyant with densities higher than ambient water, and others are negatively buoyant and will sink into a quiescent fluid.[30,47] It has been mentioned that only 46% of MPs float on the marine water's surface.[144] It should be noted that since the density of freshwater is lower than saline water, these values may be lower in freshwater environments. Therefore, density separation methods are used to separate entrapped MPs from water and sediment samples. However, it is mentioned that density separation is rarely used for extracting MPs from water samples.[57] Density separation methods are based on the floatation of lighter particles on the surface of high-density salt solutions (varies from 1.2 to 1.8 g cm^{-3}).[158] With all this considered, based on the density of the solution used for density separation, different types of MPs with various densities are extracted from the sediment samples.[42]

Different solutions and liquids are used in the density separation stage, the most frequently used of which is NaCl solution. These various materials and chemicals have merits and demerits listed in Table 4. In addition, some studies conducted two-step density separation to assure the highest separation of MPs. First, the sample bulks were separated using high-density NaCl to reduce bulk volume. Then, the residues were density-separated using denser solutions such as NaI to have broader spectra of size. The main reason for the mentioned method is to reduce the sample volume, leading to a decrease in the amount of required NaI, which is an expensive material.[57,133,157] In terms of the recovery rate of MPs using different salt solutions, saturated NaCl shows the lowest recovery rate (75.5%). To evaluate the effect of different density separation solutions on distinct polymers, NaCl, $CaCl_2$, $ZnCl_2$,

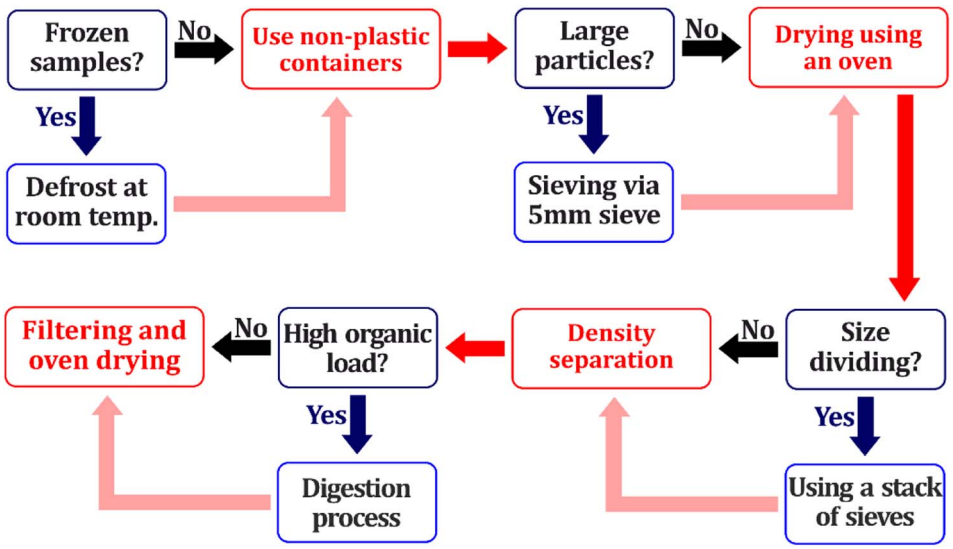

Fig. 6 A flowchart showing different pre-treatment and extraction steps.

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and NaI have been used to separate six types of commonly used polymers (PP, PE, HDPE, PS, PVC, and PET). The obtained results revealed that lighter MPs (PP, PE, HDPE, and PS) were highly recovered with all the density separation solutions (up to 97% of recovery rate) due to their high ability to float with any of the density separation solutions. On the other hand, PVC showed the lowest recovery rate with all the mentioned solutions, varying from 28% to 62%. This is also related to the harder floatation of heavier MPs on the surface of the solutions, decreasing the recovery rate.[159]

Despite the wide applicability of density separation methods using salts, these methods suffer from deficiencies. The two most significant disadvantages are the inability to separate smaller particles (<400 μm) of plastic particles and the generation of large amounts of waste.[160,161] This results are in accordance with another study's finding which showed the higher recovery rate of different types of MPs for larger particles.[162] Therefore, some researchers have proposed applying oil-based solutions.[163,164] These methods are based on the lipophilic characteristics of plastic compounds, which lead to the formation of a less hazardous alternative to salt-based methods.[39] The application of canola oil[163] and olive oil[164] for density separation has been suggested in various studies, which shows a promising recovery rate (92–97%) for light and heavy MPs. Combining some drops of oil with a salt solution has also been recommended for the density separation process.[165] The main advantages of using oils are their low cost and their high ability to separate light or heavy MPs. However, it has been proven that the hydrophilicity of polymeric particles may be affected through organic particle attachment to their surface, leading to the inaccuracy of separation using oils.[166] Considering the different preparation (pre-treatment and extraction) steps mentioned in the previous sections, the flowchart of sample preparation is illustrated in Fig. 6.

5. Conclusion and recommendations

In this review paper, besides sampling and preparation (pre-treatment and extraction) methods and for the first time, different preservation and storing methods were discussed. The pros and cons of various methods have been investigated. Based on the reviewed papers regarding MP studies in freshwater compartments, plastic-free materials, either glass or metal-based materials, are recommended to be utilized as container materials. In other words, using non-polymeric materials as containers decreases the risk of secondary contamination through the abrasion of plastic-made containers, leading to the overestimation and inaccuracy of MP studies. When using non-plastic materials is not accessible or feasible, using less abundant polymeric materials such as PVC to facilitate the detection of abrasive materials is recommended. In terms of sample preservation, using alcohol-based materials is suggested due to their lower toxicity and higher availability. When the long-term storage of the materials is desired, freezing and ultra-freezing temperatures are preferable since samples can be stored with minor changes in their structures. However, samples' thawing and freezing cycles, especially those with high ratios of organic loads such as tissues, should be considered to avoid the deterioration of samples. In the short-term storage of samples, on the other hand, keeping samples at refrigerator temperature (4 °C) is enough to maintain the quality of samples. Sampling, preparation and pretreatment, were other pre-analysis steps that have been mentioned in this review paper. Based on our study, different sampling and preparation methods could be applied based on the research goals, studied MPs size range, type of the matrix (either water or sediment), and sample organic and inorganic loads based on which different digestion and density separation methods should be applied.

It goes without mentioning that despite the importance of sample preservation and storage in MP studies, to the best of the authors' knowledge, none of the studies evaluate the importance of these parameters. Therefore, it is strongly advised that MPs researchers consider the impact of preservation and storage methods on their studies to make the data more reliable. The following hints are recommended to be in researchers' minds once conducting microplastic research:

- Devise parallel studies utilizing synthetic polymers to find the recovery rate of the studies while using different preservatives and containers.
- Conduct blank experiments in various stages of MP studies to reduce the impact of secondary contamination and determine the effect of different preservation and storage methods.
- Do the polymer characterization tests before and after using preservation methods to see any probable effect of preservation materials on microplastic structures. We strongly recommend devising experiments before and after applying any chemicals or heat to see if there are any changes in the polymer characteristics.
- For long-term storage, cryopreservation-freezing or ultra-freezing conditions (−20/−80 °C) are advised to suppress both biological and chemical reactions.

By considering these precautions in different pre-analysis phases of MP research – that is, sampling, preservation and storage, and preparation steps – the obtained results will be more reliable to give a more accurate picture of MP presence in freshwater environments.

Conflicts of interest

There are no conflicts to declare.

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