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Notes

Microplastics in water and sediments: Sampling, detection, characterization methods & quality control- A review

Revisión de métodos de muestreo, detección, caracterización de microplásticos y control de calidad en columna de agua y sedimentos

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Abstract

Microplastics (MPs) are already considered emerging pollutants because they are found in every analyzed waterbody and because they can have adverse effects on human health. Estimating the amount and type of MPs in surface waters is relevant to understand the problem. However, there is still no established methodology for the quantitative and qualitative determination of these pollutants in aquatic environments, and because of this lack of standardization, the comparison between articles becomes complicated. A standard methodology could allow future research to focus on solving the problem. This review presents the advantages and limitations of the different methods for sampling, detection, and characterization of PMs in water and sediments, so that future experimental investigations of microplastics can use the methods presented in the literature in an informed manner.

Keywords: Microplastics, methodology, comparison, emerging pollutants.

Resumen

Los microplásticos (MPs) ya se consideran como contaminantes emergentes debido a que se encuentran en todos los cuerpos de agua que se han analizado y a que pueden tener efectos adversos en la salud humana. Estimar la cantidad y el tipo de MPs en aguas superficiales es relevante para poder entender la problemática. Sin embargo, aún no se tiene una metodología establecida para la determinación cuantitativa y cualitativa de estos contaminantes en medios acuáticos y por esta falta de estandarización la comparación entre artículos se vuelve complicada.

Una metodología estándar podría hacer que futuras investigaciones se enfoquen a la resolución del problema. La presente revisión expone ventajas y limitantes de los diferentes métodos de muestro, detección y caracterización de MPs en agua y sedimentos, con el fin de que futuras investigaciones experimentales de microplásticos puedan utilizar los métodos expuestos en la literatura de manera informada.

Palabras clave: microplásticos, metodología, comparación, contaminantes emergentes.

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Introduction

The introduction of plastics in the 1950s was a turning point in human history. As a lightweight, versatile, oxygen- and moisture-resistant, and low-cost material, plastic is very practical as a replacement for other packaging materials (Andrady, 2011). Currently, more than 5 300 types of polymers are synthesized, of which approximately 300 million tons of waste are generated per year, and only 14 % are recycled (ONU, 2020). Of the remaining plastics (86 %), it is estimated that between one and three million tons enter the oceans annually due to poor waste management practices, illegal dumping, tourism, runoff, and other sources (Zhang *et al*., 2020b). According to the World Wildlife Fund (World Wildlife Fund WWF, 2019) by 2030, this number will increase to nine million tons.

Since plastics are not biodegradable products, they become microplastics (MPs; 1-5 000 μm in their longest dimension) through time and exposure to the environment. The MPs generated through this process are called secondary MPs and are the most abundant in water bodies. Some MPs are intentionally manufactured for specific applications such as microbeads used in exfoliants or detergents and are called primary MPs (Lambert, 2018; Wessel, Lockridge, Battiste, & Cebrian, 2016). MPs are starting to be considered emergent pollutants as they have been found in every studied waterbody (Ambrosini *et al*., 2019). They are of great interest because they have health effects on fauna and flora, present a potential for trophic transfer, and are considered a potential risk to human health (Zhang *et al*., 2020b).

Currently, it is sought to know how they are transported in rivers or other media, to estimate how much and how MPs reach water bodies of economic, social, and ecological relevance, such as lakes, rivers, and oceans, and to be able to propose remediation and/or mitigation measures (Ambrosini *et al*., 2019; Lambert, 2018; Liu, Wang, Wei, Song, & Li, 2019).

Researchers seeking to perform such modeling have faced that MP detection and characterization research lacks measurement standards. This causes the comparison between articles or the use of the information to become complicated and sometimes impossible (Correia-Prata, Da-Costa, Duarte, & Rocha-Santos, 2019). There are several articles internationally that show results of the detection and characterization of MPs, but few are representative and reproducible. From now on, to carry out robust research that works as a basis for MP modeling and remediation of water bodies, the methodology to be followed must be considered.

This review aims to present and compare different techniques for sampling, separation, detection, and/or characterization of MPs in water bodies and sediments, and to point out key quality control procedures at each step.

Sample collection

Sampling is the first part of the experimental section of a study, so it must be carried out in such a way that the sample is representative of the water body of interest. The sampling of MPs has been a topic of interest since polymers have different sizes, shapes, and densities, and therefore the same MPs are not sampled with one equipment as with another, just as the same MPs are not found at different depths in a water column. Next, the methods found in the literature for sampling MPs in water and sediments will be discussed.

Sediments

The following types of sampling are generally performed in sediments.

Deliberate sampling (critical or judgmental)

These are methods that are applied in most studies (Zhang *et al*., 2020a) They are performed by directly picking MPs from environmental samples. This type of method is suitable for collecting MPs from one to six mm (Karlsson *et al*., 2017). The tools commonly used are box samplers, steel shovels, or "Van Veen" dredges (Rocha-Santos & Duarte, 2015).

This way of sampling has the advantages that it is simple, fast, and that the tools can be used in many environmental conditions (rain, currents, etc.). But its disadvantages are the subjectivity of human visual recognition, the limited sampling range, the high probability of losing part of the sample, and that it is a disturbed sample due to the movement generated by the tool (Zhang *et al*., 2020a).

Bulk sample

These are less common methods for sampling sediments as the entire sample is preserved. Adequate when the recognition of MPs with the naked eye is complicated (Dümichen, Braun, Bannick, Jekel, & Senz, 2015; Gong & Xie, 2020; Zhang *et al*., 2020a; Zhang *et al*., 2020b). For this type of sampling, more sophisticated tools such as drills or corers are used.

The advantages of this type of sampling are that it allows relating the concentration of MPs to events in time, such as the implementation of regulations or others (Stock, Kochleus, Bänsch-Baltruschat, Brennholt, & Reifferscheid, 2019), that it is an undisturbed sample and that there is no loss of sediment when sampling, which allows the sampled volume to be established in the record for future reproducibility of the method. The main disadvantages of these samples are that the tools required are more expensive than those used for purposive sampling and that they typically contain unwanted materials, increasing the workload (Wang & Wang, 2018).

Some authors have proposed novel tools that allow global sampling but are expensive and impractical for regular monitoring; Mani, Primpke, and Lorenz (2019) used a diving bell to minimize sample disturbance and Frei *et al*. (2019) collected sediment by freeze coring.

Quality control in sediment sampling

Factors that are important to consider in the sampling strategy are that the distribution of MPs is irregular and is influenced by: the properties of the polymer and its additives, the properties of the water body, and seasonally fluctuating environmental conditions (waves, wind, deposition of river materials). The concentration of MPs can also vary by zone (industrial activities along the river or water body) and by sampling depth as it has been observed that there are higher concentrations (transects) that can generate an over-or underestimation of polymers (Adomat & Grischek, 2021; Correia-Prata *et al*., 2019; Hanvey *et al*., 2017).

Standards specifying the number of replicates to be performed, depth, specific sampling points, and sample volume to be analyzed have not been established and therefore few studies provide this information. According to Correia-Prata *et al*. (2019), it is necessary to define the sampling depth in sediments since in the first 5 cm the concentration of MPs is higher than in deeper layers. The number of replicates should be defined since the collection of a point sample is not representative of an entire water body because sediment matrices are influenced by the factors mentioned above (Adomat & Grischek, 2020). Besley, Vijver, Behrens, and Bosker (2016) recommend taking 11 samples per 100 meters on the beach (should be standardized for other water bodies) and according to MSFD Technical Subgroup on Marine Litter (2013), sampling should be done upstream to facilitate data interpretation. The sampling volume also depends on the objective of the investigation and the minimum required for further analysis in the laboratory (Adomat & Grischek, 2020; Koelmans *et al*., 2019).

The units in which the concentration of MPs in sediments is reported to vary between each study but could be comparable if data such as sampling depth, density, and sediment dry weight are reported in each analysis until a standard metric is defined (Adomat & Grischek, 2020; Van Cauwenberghe, Devriese, Robbens, & Janssen, 2015).

Water column

In water, the following types of sampling are generally performed.

Sample concentration method

These are methods in which samples are filtered and/or sieved at the site of interest, retaining the MPs for later analysis (Gong & Xie, 2020). This type of sampling is the most used in water column sampling (Correia-Prata *et al*., 2019).

The tools to perform this type of sampling are commonly boat trawls, composed of a rigid frame that holds a rectangular or circular net opening at the surface and at the end a collector where the sample is concentrated. The U.S. National Oceanic and Atmospheric Administration (NOAA) supports this methodology and guides its methods manual (Masura, Baker, Foster, Arthur, & Herring, 2015). The net types are distinguished from each other by the depth at which it is sampled and their size. They can all be fitted with the desired net aperture size, but suppliers generally handle standard sizes. The above nets are divided into the following subtypes and their characteristics.

Trawls

- Plankton trawl: Basic trawl that handles a pore opening size of 100 to 500 μm and can be used for surface waters at different depths (Boyle & Ömerci, 2020).
- Manta trawl: Smaller and more manageable net for sampling MPs in the first few centimeters of the water column, thanks to the floats between which the net frame is located. It also handles a pore opening size of 100 to 500 μm (Gong & Xie, 2020).
- Neutson trawl: A net that can be used during storms and for near-surface waters, commonly used in marine environments. It allows the sampling of different depths (Correia-Prata *et al*., 2019).
- Bongo trawl: Ideal net for mid-level waters. It consists of a structure with a double hoop that allows obtaining two samples in each sampling. In this

way, samples are obtained in duplicate or can be sampled with two plankton nets of different sizes (Gong & Xie, 2020).

All the described nets have several advantages: they can sample large volumes of water making the sample representative; the sampling depths can be adjusted to specify in the methodology this data that has been omitted in many studies, and the sample is concentrated reducing the cost of transporting it in large containers.

They also have disadvantages in common: they require an aquatic means of transport (boat); when smaller mesh opening sizes are used (<300 μm) the nets become clogged and when larger ones are used, the quantification of MPs may be underestimated. Currently, the most common net aperture size has a diameter of 300 μm (Correia-Prata *et al*., 2019; Gong & Xie, 2020; Li, Liu, & Chen, 2017).

Pumps for in-situ sample reduction coupled with filters or sieves

Centrifugal, Teflon, or eccentric screw pumps have been used to sample MPs at certain water depths to feed cascade steel sieves (Stock *et al*., 2019).

They have the advantage that they can be constructed of different materials and allow sampling from the surface to depths of six to one hundred meters. The number of MPs obtained with this method can be determined from the choice of the aperture size of the sieves and/or coupled filters (Correia-Prata *et al*., 2019).

Some authors such as Coreira et al. (2019) do not recommend its use as they can contaminate the sample. Sedlak, Sutton, Box, Sun and Lin (2017) recommend it for sampling MPs smaller than 300 μm as this is the detection limit in trawls. For example, Enders, Lenz, Stedmon and Nielsen (2015) detected marine MPs down to 10 µm by pumping groundwater from a depth of 3 m with a pump that did not contaminate the sample as it was made of titanium and stainless steel. There is also no standardization of sampling times with pumps yet, as they can work for hours at the same sampling point or minutes at different stations (Correia-Prata *et al*., 2019).

Bulk sampling

With this type of sampling, all sizes of MPs can be collected, but the sample volume is small, so the representativeness of the sample may be affected (Wang & Wang, 2018). The tool used to perform the global water samples is discussed below.

Storage containers

Glass containers (to avoid sample contamination) are normally used and transported to the laboratory for further analysis. Its advantages are that it can achieve representativeness (depending on factors such as volume, size of MPs, etc.) and is more economical due to the simple equipment used (Wang & Wang, 2018).

The main disadvantage is that glass containers cannot process large volumes that ensure sample quality, so variations in this method have been found. Such a disadvantage can be overcome by defining a minimum sample volume (Correia-Prata *et al*., 2019) obtained from the analysis of how much the sample changes at different volumes and establishing the ideal one. This sampling is recommended for MPs < 100 m (Koelmans *et al*., 2019; Wang & Wang, 2018).

Quality control in water column sampling

For concentrated samples, it is important to include a flow meter that estimates the volume of water filtered, in the case of trawls the amount of flow can be calculated with the size of the net opening and the length of the transect, which in turn can be calculated as the distance between the start and endpoints (Eriksen *et al*., 2018). There is still no standardization as to the volume of water to be sampled, but all research should report it (Cutroneo *et al*., 2020). Koelmans *et al*. (2019) propose a minimum volume of 500 liters for surface water sampled with nets (MPs > 300 μm). They also propose that for MPs <100-300 μm much smaller sample volumes are required (Cabernard, Roscher, Lorenz, Gerdts, & Pimpke, 2018).

According to Sedlak *et al*. (2017), it is convenient to combine methods or to use one over the other, depending on the size of the MPs to be studied. The authors for example performed drags with manta nets for sampling MPs > 355 μm. For MPs from 20 μm to 5 mm, they used a specially designed and built pump, which includes a filter to capture particles from a volume of 10 liters of surface water. For nano-plastics (NPs; < 1 μm) they filled a 1-liter glass bottle with surface water at each site. This procedure can be modified if the analytical laboratory determines that a larger sample is required to identify MPs. This division allowed them to be more accurate in their estimates.

During sampling, potential sources of contamination can be clothing, equipment that is in contact with the sample (container, container paint), and devices used to move the sample from collectors to storage containers. For this reason, it is recommended to use 100 % cotton clothing, nitrile or latex gloves, and that laboratory instruments are not made of plastic (preferably glass or metal). It is also important to pre-wash laboratory utensils and equipment with distilled or pre-filtered water or a 70 % ethanol solution (Cutroneo *et al*., 2020). To minimize errors, it is recommended to have a procedural blank (pure water) and an enriched blank (pure water with a known number of MPs) (Mai, Bao, Shi, Wong, & Zeng, 2017).

For the sampling method to be reproducible, it is essential to report everything carried out, such as the depth at which it is sampled, the speed and duration of the trawl, and all the chosen parameters (Fok, Ling-Lam, Li, & Xu, 2019).

MPs separation from the sample

Samples collected in the field for MP identification contain natural organic and inorganic particles that must be removed for further analysis (Peller, Nelson, Babu, Iceman, & Kostelnik, 2020; Yang, Zhang, Kang, Wang, & Wu, 2021).

Before separation or identification in sediments, it is desirable to perform drying to facilitate subsequent techniques and report results in dry weight, as moisture varies among different sediments. Various drying methods have been used, but oven drying is the most common, simplest, and fastest (Adomat & Grischek, 2020). The main separation techniques are divided into:

Filtration or sieving

In sieving and filtration, sediment samples are passed through sieves or filters of different sizes. The sieve material is usually stainless steel or copper, and the filter material is fiberglass, cellulose nitrate, polycarbonate, or nylon (some authors do not recommend the latter because of possible contamination). The pore diameter of the filter is much smaller than that used in the sieve (0.22-20 m) (Bretas, Mendoza-Roca, & Bes-Piá, 2020; Cutroneo *et al*., 2020; Fok *et al*., 2019).

Sieving is usually performed before other separation methods and filtration is performed afterward. Performing filtration without having carried out some density separation or digestion can generate filter saturation (Kang, Ji, Zhao, & Wei, 2020). Both methods can go hand in hand (Gong & Xie, 2020).

Sieve mesh sizes are chosen according to the size range of the MPs to be collected and are generally in the range of 38-4750 µm (Bretas *et al*., 2020; Wang & Wang, 2018). Classifying MPs by size is relevant as this parameter is related to their migration behavior in the environment and determines the ease of entry of MPs into organisms (Li, Busquets, & Campos, 2019). There is still no standard in sieve sizes, but some studies have used a 500 µm sieve to obtain size fractions larger and smaller than 500 µm, which is reasonable since MPs > 500 μm can be visually identified (Hidalgo-Ruz, Guttow, Thompson, & Thiel, 2012; Mai *et al*., 2017).

The dry sieving technique for MPs in sediments is limited by the thickness of the sediment; the finer the sediment the more agglomeration it will present. Therefore, dry sieving is recommended for particle sizes of 40-125 mm, and for smaller sizes, wet sieving is suggested (Adomat & Grischek, 2021; Retsch, 2015).

Chemical/biochemical technique: Digestion methods

The need for digestion is related to the amount of organic matter present in the sample, so it is mostly used for marine sediments or biological samples, but some authors recommend it for any sample. The main objective of digestion is to remove the organic material while the MPs are the least affected (Kang *et al*., 2020). It is highly recommended to remove organic matter when a visual analysis will be done (it can be confused with MPs) (Gong & Xie, 2020; Masura *et al*., 2015). The most common digestion methods are oxidative digestion by adding acidic, alkaline, and/or enzymatic compounds.

Oxidative digestion has been studied by many authors who have varied the concentration and exposure time of oxidants. The oxidant that has proved to be the most efficient is H_2O_2 (Bretas *et al*., 2020). Nuelle, Dekiff, Remy and Fries (2014) reported in their research that 30-35 % H2O2 solutions presented higher organic matter removal in sediment samples than NaOH (20, 30, 30, 40, and 50 %) and HCl (20 %) solutions, but had effects on the color and size of MPs. A modification that has been explored to decrease reaction times and perform the experiment at room temperature is to carry out, as recommended by NOAA, a Fenton reaction, which consists of mixing H_2O_2 and a catalyst (Fe (II)) (Adomat & Grischek, 2021; Babuponnusami & Muthukumar, 2013; Masura *et al*., 2015).

Acid digestion has different effects depending on the type of MP. Some studies that have analyzed the use of HNO_3 found that it is more efficient in removing organic material than other acids but dissolves polymers such as Polystyrene (PS) and Polyethylene (PE) (Claessens, Van Cauwenberghe, Vandegehuchte, & Janssen, 2013; Stock *et al*., 2019). The least recommended acid is HCl as it does not remove all organic matter (Stock *et al*., 2019; Strungaru, Jijie, Nicoara, Plavan, & Faggio, 2019).

Alkaline digestion affects other types of MPs, for example, Polycarbonate (PC), Polyethylene terephthalate (PET), and Polyvinyl chloride (PVC) were disintegrated using 10 M NaOH (Stock *et al*., 2019). The use of KOH has shown promise as Kühn *et al*. (2017) found that it only affects cellulose acetate (CA) and other "biodegradable" plastics.

Enzymatic degradation is efficient for degrading organic matter without affecting polymers, but it is expensive and alone has been used only on sediment or biota samples. Some enzymes used are cellulase, lipase, protease, and chitinase but the one that presented efficiencies up to 97 % is proteinase-k (Bretas *et al*., 2020; Cutroneo *et al*., 2020; Pico, Alfarhan, & Barcelo, 2019). Its main disadvantages are that it takes a long time to digest the samples and that it requires specific conditions to maintain the enzymes (Adomat & Grischek, 2021).

Physical technique: Density separation

The specific density of polymers varies considerably (0.8-1.6 g/cm^3), without considering additives or adsorbed contaminants. Because sediments have densities of approximately 2.65 g/cm^3, the difference can be used for light MPs to separate from the sediment with the help of a denser compound (Bretas *et al*., 2020; Stock *et al*., 2019; Wang & Wang, 2018). Regardless of the salt used, the dried sample must be mixed with the solution, allowed to settle and finally, the remaining floating MPs are separated by filtration, either with vacuum or normal pressure (Cutroneo, *et al*., 2020; Mai *et al*., 2017; Zhang *et al*., 2020a). The density separation method is commonly applied after some digestion methods to remove organic matter (Pico *et al*., 2019).

A sub-method developed from the density separation principle is elutriation which is based on the use of an upward flow of gas or liquid within a column (Kang *et al*., 2020). Some authors suggest placing sieves on top of the column to separate the MPs according to their size (Kedzierski *et al*., 2018; Ruggero, Gori, & Lubello, 2020). Elutriation proves to be economically attractive and efficient, reaching recovery percentages of 93 to 98 %. Its only disadvantage is that it takes a long time to perform (Claessens *et al*., 2013). The Munich plastic-sediment separator (MPSS) uses a similar system and exhibits recoveries of 100 and 95.5 % for MPs of sizes 1-5 mm and < 1 mm, respectively (Correia-

Prata *et al*., 2019; Imhof, Schmid, Niessner, Ivleva, & Laforsch 2012; Stock *et al*., 2019).

There are various salts used for density separation. NOAA, recommend using NaCl (Masura *et al*., 2015; MSFD Technical Subgroup on Marine Litter, 2013) because it is economical and environmentally friendly. But its low density (1.2 g/cm^3) may generate an underestimation of MPs as it does not separate high-density polymers (PVC or PET) or polymers containing additives (Gong & Xie, 2020; Stock *et al*., 2019).

Other authors have preferred to use NaI or $ZnCl_2$ in their research as its density is higher (1.6 g/cm^3) and it can separate polymers containing additives (Bretas *et al*., 2020; Gong & Xie, 2020; Rocha-Santos & Duarte, 2015). The use of $ZnCl_2$ has the following advantages: it presents recovery percentages of up to 99 %, it is economical (Fok *et al*., 2019; Stock *et al*., 2019) and the sample only needs one wash, while with NaCl it requires three (Correia-Prata *et al*., 2019). Its main disadvantage is that it is harmful to the environment and corrosive (Fok *et al*., 2019; Stock *et al*., 2019). The use of NaI has the main advantages that it has a recovery rate of 91 % (Fok *et al*., 2019), the need for only one wash, that it recovers oleophobic fibers, its low impact on the environment, and that it can be reused up to ten times. Its disadvantages are that it is very costly and reacts with cellulose filters, turning them black and making visual identification difficult. Authors such as Correia-Prata *et al*. (2019) and Fok *et al*. (2019) established that it is an ideal salt for density separation.

Finally, Frias, Pagter, Nash and O´Connor (2018) recommend sodium tungstate dihydrate (Na_2WO_4 $2H_2O$) in the "BASEMAN" sediment

MP monitoring project as it reaches a density of up to 1.4 g/cm^3, is inexpensive, and does not harm the environment.

On the other hand, some authors recommend a two-step separation technique, in which a low-density salt is used first, followed by a denser salt. This technique can decrease the amount of toxic salt used (Fok *et al*., 2019; Fu & Wang, 2019).

Quality control in separation

When examining samples taken in situ in the laboratory, some authors avoid contamination using a laminar flow hood or a fume hood (Adomat & Grischek, 2021; Stock *et al*., 2019; Wang, Yuan, Chen, & Wang, 2018). To correct the sample for contamination, analysis targets are recommended close to the area where the MP samples are analyzed and are studied in the same way (Koelmans *et al*., 2019). As all steps for MP identification are performed, likely, there will also be a loss of particles, so to establish the percentage recovery of MPs, Hermsen, Mintenig, Besseling and Koelmans (2018) recommend a positive control. Thorough cleaning of the materials and the laboratory is indispensable, as well as reporting the frequency in which it is performed, and the details of the solutions used for the cleaning (Adomat & Grischek, 2021).

For separation using filters or sieves there are factors to be taken into account; the shape of the MPs, for example, microfibers have a high length-to-width ratio, so they can be retained horizontally in the sieve or pass longitudinally (Bretas *et al*., 2020; Michielssen, Michielssen, Ji, & Duhaime, 2016); the presence of particulate solids that can block the filter reducing the efficiency of the process (Wang & Wang, 2018).

In the final stage of filtration, the filters are rinsed with ultrapure water to avoid the formation of salt crystals in the dried filters, the remaining solutions in the filter can be removed in an oven or dryer or at room temperature. The temperature should be chosen with prior analysis as some plastics melt at temperatures above 100 °C (Cutroneo *et al*., 2020).

Digestion separation should consider factors such as the resistance of the study polymers to the proposed digestions; the shape of the MPs, since particles with a large surface area tend to float with low-density salts and those with fibers or small MPs (200-400 μm) do not float easily (Kang *et al*., 2020); the amount of organic matter in the sample and the temperature of the reaction (Bretas *et al*., 2020; Correia-Prata *et al*., 2019). Treatment with different degradants and analysis of organic matter is also recommended since for example, acid and Fenton reactions degrade better organic contaminants that interfere with Infrared or Raman signals, alkaline reactions degrade better proteins, fats, or waxes and enzymatic degradation is more efficient in the degradation of biofilms and organic material (Mai *et al*., 2017; Masura *et al*., 2015; Lee & Chae, 2020). It is important to highlight that the effects of digestion compounds on organic chemicals that may be affiliated with MPs (additives or adsorbed material) have not been studied (Mai *et al*., 2017).

Detection and/or characterization

Visual methods

Through visual methods, MPs are optically identified and separated with tweezers or other tools (Rocha-Santos & Duarte, 2015). The method is simple and inexpensive, but it is subjective, and it has been proven in different investigations that the smaller the size of the MPs the more false positives are committed (Yang *et al*., 2021; Zhang *et al*., 2020a).

The visual method allows the classification of MPs by size, color, and shape, making it possible to infer their origin. Some authors propose that transparent MPs derive from single-use plastics or packaging and blue MPs are related to aquaculture or fishing activities (Fok *et al*., 2019). For this reason, it is recommended to improve the method for future research and to accompany it with chemical characterization (Bretas *et al*., 2020; Correia-Prata *et al*., 2019; Gong & Xie, 2020). The most commonly used equipment in visual methods are the following sections.

Light microscopes

These instruments are suitable for large-volume samples, especially when analytical equipment is not available (Wang & Wang, 2018). Different types of microscopes have been used for the analysis of MPs, but the most common around the world are Stereomicroscopes or dissecting microscopes (10-40 X magnification) (Yang *et al*., 2021). The smallest detected by this type of microscope is 100 μm (Hanvey *et al*., 2017).

To optimize the visual method, dyes have been used to help differentiate polymers from other materials, Nile Red (NR) adsorbs on the polymer surface and is identified through fluorescence microscopy. This dye has proven to be the most efficient dye for staining MPs > 20 μm (Bretas *et al*., 2020), but has disadvantages such as staining of natural materials and poor staining of Polyvinyl Chloride (PVC) and Polyacetate (PA) (Shim, Song, Hong, & Jang, 2016). Melt (hot needle) tests have also been applied, in which MPs are confirmed by melting (Bretas *et al*., 2020; Fok *et al*., 2019), this method can cause damage to the polymer and is not recommended for MPs < 1 mm (Kang *et al*., 2020). These improvements are promising, economical, and are a good tool to standardize visual methods.

Scanning electron microscope (SEM)

The main difference between this type of microscopy and traditional microscopy is that SEM uses an electron beam instead of a light beam to magnify the image (Wang & Wang, 2018). SEM has a higher resolution than traditional microscopes, so its results are more accurate, and it can detect MPs down to 1 μm (Wang, Wagner, Ghosal, Bedi, & Wall, 2017). What most distinguishes the method is that it provides insight into particle morphology and weathering by observing cracks (Bretas *et al*., 2020; Wang & Wang, 2018; Zhung *et al*., 2016).

There are also modifications of the method (SEM), such as SEM coupled with energy-dispersive X-ray spectroscopy (SEM-EDS) that helps to differentiate natural material more easily from MPs through imaging and elemental analysis (Wang & Wang, 2018).

Despite being a successful method in identifying and characterizing the surface of MPs, sample preparation requires a lot of time and effort, so it is not feasible to use it on a large number of samples (Wang & Wang, 2018), in addition to the fact that like traditional microscopes, SEM cannot identify the type of polymer, except PVC (which contains chlorine) (Elkhatib & Oyanedel-Craver, 2020; Silva *et al*., 2018).

Vibrational spectroscopy methods

Two methods are mostly used for the detection and/or characterization of MPs. Käppler *et al*. (2016) recommend complementing the two techniques for the best results. Both have the advantages that they are non-destructive, accurate, and can be used as complementary to visual methods:

Fourier transform infrared transmission spectroscopy (FTIR)

It is based on the change of dipole moment phenomenon, from energy applied to the system (infrared light) that gives as a response a percentage of absorbance at different wavelengths (spectrum) that is characterized depending on the functional groups found. This method has two ways of identifying MPs; with the transmittance or reflectance configuration (Bretas *et al*., 2020).

The identification of MPs with FTIR is not affected by size, shape, etc. It has the advantage of being able to identify MPs without the result being affected by fluorescence. It not only provides information on MP type but also detects weathering of the material (important for modeling) (Gong & Xie, 2020; Käppler *et al*., 2016).

Its limitations are that it identifies MPs>500 μm, that it must be analyzed one particle at a time (time-consuming) and the results can be affected by the presence of CO_2 and H_2O (Zhang *et al*., 2020a).

Modifications have been made to the reflectance setup, such as attenuated total reflectance FTIR (ATR-FTIR), a more sensitive technique that improves information on irregular MPs and in contrast to FTIR can also be applied to thick or opaque samples (Shim *et al*., 2016), but in which the MPs run the risk of sticking to the glass and is time-consuming to perform. Its use is recommended only for MPs>300 μm (Bretas *et al*., 2020; Gong & Xie, 2020). Another modification is micro-FTIR, which generates a high-resolution map of the sample without the need for prescreening (MPs up to 20 μm), its disadvantage is that it is a more time-consuming and non-automatable technique; Imhof *et al*. (2016) were only able to analyze 1.6 % of a filter, using this method. This drawback can be overcome by using FTIR with a focal plane array (FPA) detector (Joachim-Löder, Kuczera, Mintenig, Lorenz, & Gerdts, 2015; Käppler *et al*., 2016).

Although not yet deeply studied, a promising future is expected for transmittance configuration in the analysis of MPs on the filter, the investigated particles should be thin (<100 μm) to avoid total absorption in the FTIR spectrum but larger than 5 microns to have sufficient absorbance (Joachim-Löder *et al*., 2015).

Raman Spectroscopy

The Raman Spectroscopy method is based on the phenomenon of the chemical bond polarizability of molecules in particles, caused by the application of external energy (monochromatic light). This yields as a response a light intensity percentage emitted at different frequencies and is known as a molecular spectrum (Correia-Prata *et al*., 2019). Such a spectrum allows knowing the chemical structure of the components in the sample (Araujo, Nolasco, Ribeiro, & Ribeiro-Claro, 2018).

This method allows obtaining information on non-polar functional groups adsorbed on the MP surface and local microscopic morphology (Zhang *et al*., 2020a). In addition, Raman techniques have less interference with water than FTIR methods (Araujo *et al*., 2018).

The main disadvantages of the Raman technique are that it takes a long time to process images (longer than FTIR), it is prone to fluorescence interference and can heat the sample due to the laser used as a light source, causing background emission and sometimes MP degradation (Araujo *et al*., 2018) and Raman equipment is up to 5 times more expensive than an FTIR one (Mai *et al*., 2017). Analysis times can be drastically reduced by using the software as used by Frère *et al*. (2016).

The traditional Raman method was used in early investigations of MPs detection. However, in more recent investigations the micro-Raman (m-Raman) technique has been more used due to its high performance to detect small MPs, Käppler *et al*. (2016) proved that compared to micro-FTIR the micro-Raman had a better detection of 5-20 μm MPs. Some authors state that it achieves the detection of polymers down to 1 μm (Li *et al*., 2017). This is valuable as it is possible that MPs<40 μm account for more than 60 % of ocean MPs (Enders *et al*., 2015).

Vibrational spectroscopy is limited by its high cost, availability of equipment, the time and effort required in sample analysis and processing, complex data treatment, the need for trained personnel, and limited detection, especially in contaminated MPs. Visual prescreening of particles is often used to reduce these practical problems but can induce bias (Correia-Prata *et al*., 2019; Rocha-Santos & Duarte, 2015).

Thermo-analytic methods

They are based on the relationship between physical properties and the temperature of MPs under controlled conditions. The thermogram obtained is used to identify the components of the MPs (Zhang *et al*., 2020a).

Pyrolysis-gas chromatography coupled to mass spectrometry (Py-GC/MS)

The objective of this method is to determine the chemical composition of MPs through their thermal decomposition (pyrolysis). The gas formed is separated on a chromatography column and the thermal spectrum obtained is compared with reference spectra (Zhang *et al*., 2020a

Its main advantages are that it can analyze the type of polymer and organic plastic additives in a single run (Nuelle *et al*., 2014), that it is suitable for quantitative analysis of a single type of MP (Li et al., 2019), the no need for sample pretreatment and its independence of the form of the MP or the presence of organic contaminants.

Its main disadvantages are that it is destructive, it does not provide information on size or shape, different polymers produce similar decomposition products and this can generate errors (Dümichen *et al*., 2015; Shim *et al*., 2016), it cannot identify synthetic fibers due to its low mass and low sensitivity of the indicator ions, the equipment can only analyze <5 mg per experiment and the size of the MPs it can identify is limited to 100-1500 μm (Peñalver, Arroyo-Manzanares, López-García, & Hernández-Córdoba, 2019). Other drawbacks encountered are that the equipment requires constant maintenance as debris from MP degradation can condense in the capillary between the pyrolysis chamber and the GC, contaminating the sample; analysis results are dependent on the size and purity of the MP and it does not detect inorganic additives or polymers with polar subunits (Lee & Chae, 2020; Li *et al*., 2019; Strungaru *et al*., 2019).

Modifications of this method have been performed such as Thermoextraction and desorption coupled with Gas Chromatography and Mass Spectroscopy (TED-GC-MS), in which a thermogravimetric analysis (TGA) is combined where the generated volatile products are concentrated by adsorbing on fibers subsequently analyzed by thermal desorption in GC-MS. The advantages of this method over Py-GC-MS are that the sample size can be larger (100 mg) (Boyle & Örmeci, 2020), it allows the identification and quantification of polymers in environmental samples without pre-selection, as it is independent of the purity of the MP and avoids clogging problems encountered in Py-GC-MS (Correia-Prata *et al*., 2019; Li *et al*., 2019; Peñalver *et al*., 2019).

Differential Scanning Calorimetry (DSC)

The principle of this method is that when changing from solid to liquid or the gas phase, the material absorbs heat and generates an endothermic peak at a specific temperature that allows chemical identification and particle mass determination of a specific polymer (Zhang *et al*., 2020a).This technique requires using reference polymers for the identification and matching of a sample with MP.

The main advantage of this method is that it is simple and fast, but it is also destructive, and the size of the MP has been proven to impact the qualitative and quantitative performance of DSC signals (Rodriguez-Chialanza, Sierra, Perez Prada, & Fornaro, 2018).

Majewsky, Bitter, Eiche and Horn (2016) performed a variation to DSC; thermogravimetric analysis coupled with differential scanning calorimetry (TGA-DSC). They found that the method has the advantages of both thermal techniques as it is simply operated, cost-effective, and can give information related to other impurities in the sample. The disadvantages are that it is dependent on the size and shape of the MP, it is destructive, there can be overlapping in the phase change and polymers such as PVC, PE, PA, and PET cannot be identified.

Some authors consider that vibrational and thermal methods can be complementary or can be chosen according to the objective of the investigation, as they provide valuable but different information. For modeling and mass balances and their monitoring thermal techniques are more suitable, while for the identification and detection of smaller MPs, vibrational methods are better (Primpke, Fischer, Lorenz, Gerdts, & Scholz-Böttcher, 2020).

The presented techniques are widely used but still have limitations; they can only be used for a few parameters; in the case of complex samples and/or with low content of MPs, the reliability of the obtained data is low. Therefore, the development of multi-parametric analytical methods with complex substrates and samples with a low concentration of MPs would have wider applications. In addition, faster detection methods are required. Because of this, the authors consider it necessary to improve and develop novel methods or equipment (Correia-Prata *et al*., 2019; Lambert, 2018; Zhang *et al*., 2020a).

Quality control of vibrational and thermal spectroscopy methods

The MSFD Technical Subgroup on Marine Litter (2013) recommends subjecting at least 10 % of the 100-500 μm MPs sampled and all suspected particles from 20-200 μm to this type of analysis. More than 10 % may be required for larger particles due to variability in visual identification (Correia-Prata *et al*., 2019). If separating with filtration, even if the measurement time is long, Koelmans *et al*. (2019) recommend analyzing from at least 25 % of the filter.

As mentioned above, many authors recommend combining vibratory and thermal methods with visual methods to reduce costs and increase the reliability of the study. Another aspect commented on by Ribeiro-Claro, Nolasco and Araujo (2017) is the urgency to include non-typical reference MPs (MPs from WWTPs or having attrition).

Some good practices have been tested to reduce fluorescence and weak signal problems in Raman spectroscopy. To minimize the former, proper digestion is important for the removal of organics that promote fluorescence. Sometimes even with digestion there remain traces of coloring agents, a proposed solution for these cases is to photobleach the sample, placing it under the laser until the fluorescent agent degrades, but it is not suitable for samples prone to photodegradation or pyrolysis and does not always work (Araujo *et al*., 2018). Another source of fluorescence can be the filter used with the equipment, for example, Ossman *et al*. (2017) analyzed some commercial filters and three filters designed by them and found that the aluminum-coated polycarbonate membrane filter (made by them) was the one that emitted the lowest fluorescence in the analysis of samples with MPs, it is important to highlight that any low background Raman filter is more expensive (seven times or more) than the traditional ones. To improve the signal, some authors have proposed to use improved detectors in the Raman spectrophotometer; for example, changing the commonly used charge-coupled device (CCD) for an Electron Multiplier Charge Coupled Device (EM-CCD), which allows the detector to overcome the readout noise limit, so this type of detectors will allow performing the samples in less time (Araujo *et al*., 2018; HORIBA, 2021).

Conclusions

It is concluded that despite the great progress that is being made in sampling, separation, and identification of MPs, there is still much to be covered. The lack of standardization of methods means that studies around the world cannot be compared and used for modeling. Modeling to estimate input-output and transformation flows is urgently needed to attack the root of the problem and implement adequate regulations and remediation measures.

It can also be concluded that it is important to delimit the investigations to a certain size range and types of MPs to perform more valuable and specific investigations. Therefore, a summary of the appropriate MP size for the types of sampling and detection and/or characterization is shown in Figure 1.

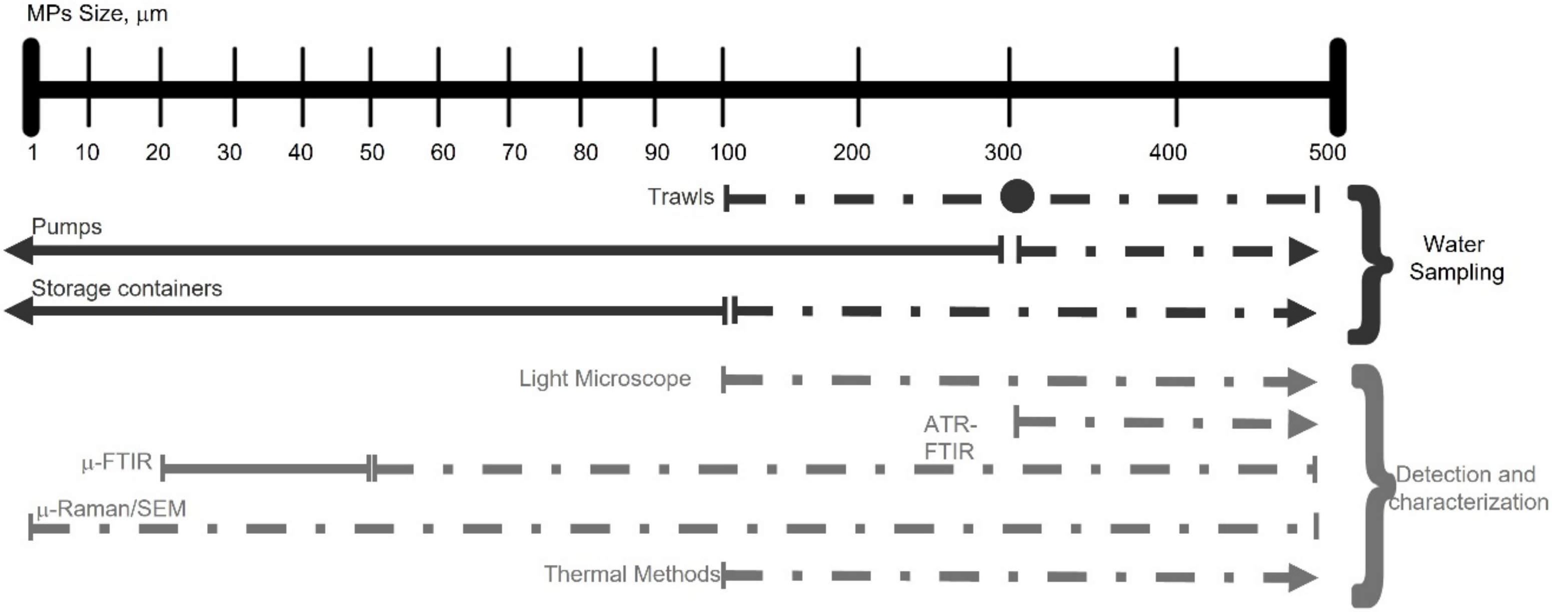

Figure 1. Sizes of related MPs that apply to certain types of sampling, detection, and characterization. The dotted lines represent the capacity, while the straight lines represent the recommendation for use as discussed in the review.

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