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Abstract:

Microplastics and nanoplastics are a new type of emerging pollutant that appears in the environment. The difference between those two is based on the particle size. The studies about microplastics and nanoplastics have recently increased because of the growing social awareness and because of the biological effects they provoke. To clarify these effects, proper sampling and identification methods are required. The lack of standard methods up to now makes the studies and the comparisons hard.

This chapter provides an overview of the main existing methods of sampling, sample preparation, and the identification of debris comprise of microplastics (MPs) and nanoplastics (NPs) in nature. In conclusion, the methods employed for these purposes depend on the type of material, the size, and the matrix in which the polymer is involved.

Keywords: sampling; microplastics; nanoplastics; sample preparation; detection; identification

1. Introduction

Since utilisation of natural latex for producing tires (John Boyd Dunlop, 1888), the world of plastic polymers underwent amazing development, with the production of a great variety of materials resistant to UV, high temperature, and mechanical stress. As a consequence, plastic polymers became resistant to biodegradation and polymer debris accumulated in the environment, resulting in environmental pollution. These plastic debris comprise MPs and NPs. As well explained by Gigault et al. (2021), the distinction between microparticles and nanoparticles takes into consideration both size- and size-dependent properties. Also, as defined by the US National Nanotechnology Initiative, the dimension of nanomaterials ranges between 1 and 100 nm, and their specific physicochemical and biological properties are absent in microsize materials. This will be approached later in this chapter.

Since about 2015, the interest in small-size MPs and NPs raised because their presence in water, food, air and soil represents some risks which still remain unclear (Becker et al. 2020). In the human body, these small particles can pass the major physiological barriers that are the skin, lung, and gut, and accumulate in several organs such as the liver and brain. In the blood and organs, they might interact with macromolecules such as proteins. In the human body (Gillois et al., 2018, Li et al., 2020) they could affect the intestinal microbial consortium and mucus, with possible consequences on the immune system. They are responsible for immunity disorders in mice (Hirt et al., 2020).

Being small, MPs and NPs display a high specific surface area and can therefore interact with a wide range of organics and minerals in the environment. Their physicochemical properties drive their transport pathways and fate in the environment, with several consequences.

Ecotoxicological concerns related to MPs and NPs:

- Transport, infiltration in sediments and residence time of ecological importance: exposure time of organisms (Waldschläger and Schüttrumpf. 2020).
- Adsorption, transport, and release of metals and organic pollutants in the environment.

- Release of potentially toxic plastic additives in the environment.
- MPs and NPs transport by wind and water,
- Possible chronic health effects linked to air contamination by MPs and NPs (Saha et al. 2020), transport mechanisms of plastics poorly understood.
- Photodegradation with release of harmful COVs (Lomonaco et al. 2020)

Qualitative and quantitative monitoring of MPs and NPs is necessary to identify the polymer variety in environmental samples, to assess environmental pollution and contamination of living organisms by MPs and NPs, and to understand the environmental fate of MPs and NPs (i.e. their degradation pathways). Moreover, standardized analytical methods for identifying and quantifying the most abundant types of plastics found in the environment are necessary for data comparison (Ribeiro et al. 2020). They are urgently needed by academics, industry, environmental and nongovernmental organizations, regulation bodies, and politics (Becker et al. 2020; Elert et al. 2017); La Nasa et al. 2020).

MPs analysis involves 3 steps: sampling, sample preparation and detection.

The heterogeneous distribution of MPs and NPs in environmental samples requires representative sampling (Klein et al. 2018). The amount of samples depends on the concentration of particles and their size class. Sample preparation depends on the complexity of environmental sample and on the detection method used. Sampling and sample preparation methods are important in terms of representativeness and method harmonization (Becker et al. 2020; Elert et al .2017).

This review will focus on the detection of MPs and NPs in samples taken from the environment.

2. Sample collection for the detection of microplastics (MPs) and nanoplastics (NPs)

The strategy to obtain samples for the detection of MPs and NPs changes depending on the source: high-tide line, beaches and different depths of the water column, among others. This is because of the difference in the concentration and type of MPs in all of them. In general, as detailed by Hidalgo-Ruz et al. (2012), three types of sampling are distinguished: selective, bulk, and by volume-reduced.

Selective sampling is used for samples taken from beaches. This method consists of the identification and collection of MPs directly by the person conducting the study (Hidalgo-Ruz et al., 2012). To make this real, plastics must have a size perceptible to the subject (Hidalgo-Ruz et al., 2012). Therefore, the main limitation of this type of sampling is the analysis of MPs with sizes below this perceptible range and the human factor for their identification (Crawford and Quinn, 2017).

Bulk sampling refers to taking a volume in which MPs are believed to be found in the medium to be studied (Hidalgo-Ruz et al., 2012). Selecting this method is due to three factors: i) that the samples can be coated with other materials; ii) that there are many MPs of a small size in a volume that is not very large in comparison; iii) that MPs cannot be recognized with the naked eye (Hidalgo-Ruz et al., 2012).

The main advantage lies in the fact that many MPs can be extracted in a single intake, although in contrast, the concentration of MPs based on the total size of the sample can be small, as the volume of the sample is not reduced. Furthermore, depending on where these samples are taken, they may or may not be representative of the environment as a whole since the distribution of MPs over the entire surface is uneven.

The last method discussed is sampling by volume reduction. In the same way as in the previous case, samples would be taken from the place where the study is to be carried out. However, the water sample is subjected to a filtration prior to the analysis (Hidalgo-Ruz et al., 2012).

Contrary to bulk sampling, in this method a very large study area is covered since most of the sample is eliminated, leaving only the solid part that is larger than that of the filter. The main limitation lies

in the size of the filter available, since the smaller MPs will be eliminated along with the rest of the sample, which causes interesting data to be lost depending on the study (Crawford and Quinn, 2017).

Once the sampling methods that can be used and the cases in which they are used are described, the type of samples that can be obtained will be developed.

2.1.Aqueous phase sampling

The water samples are taken directly from the area in which the study is to be carried out. The mass of water is taken and treated to obtain the data to be interpreted. Although the sample is extracted from the same area, the concentration of MPs in it varies depending on the characteristics of the material, such as size and shape, as well as the adhered substances, in addition to the environmental conditions of each area, depth, and location.

Nets are often used to collect MPs from water samples. The chosen network will depend on the depth and the area in which the study is carried out. (Wang and Wang 2018). If the samples belong to a more superficial part, the neuston (Rivers et al. 2019) or manta (Schönlau et al. 2020), (Kazour et al., 2019) will be normally used. The pore diameters of this type of nets are approximately 333 µm (Wang and Wang 2018; Prata et al. 2019; Schönlau et al. 2020; Rivers et al. 2019), smaller than the size of MPs that are usually found in those zones. Although the most common size of this type of net is the one mentioned above, it can vary depending on the study, as is the case of the one carried out by Suaria et al. 2020, which used a 200 µm neuston-type. For samples collected in the water column, the type of net used is the bongo type (Wang et al. 2018; Prata et al. 2019). This is characterized by being a double net that allows obtaining simultaneous equal samples at two points. For studies that require greater precision due to the fact that the particle size of MPs is even smaller than those mentioned above, the plankton type is used (Prata et al. 2019), whose pore diameter is 100 µm.

Although less frequent, another type of equipment is used to obtain samples (Prata et al., 2019), which are pumps (Schönlau et al., 2020). The system to obtain water by pumps is capable of collecting several cubic meters of water per hour (Tamminga et al., 2019). The water is taken and filtered to

collect MPs larger than those used for the filters. Filters can range from 500 µm (Schönlau et al., 2020), 300 µm (Tamminga et al., 2019), (Schönlau et al., 2020), to 50 µm (Schönlau et al., 2020). This technology is also used for a specific depth where extraction is guaranteed. This system is used for the superficial study of the water, that is, approximately 5 cm under the top of water.

2.2. Sediment Sample

MPs that remain longer in the environment are those collected in sediment samples (Van Cauwenberghe et al., 2015). Due to the density or accumulation of other substances on the surface of plastics, they remain sunken and subjected to different actions of the environment, leading to the degradation of the original plastics into smaller and more disparate pieces distributed at different depths. As reported by Wang et al. (2018), bulk sampling is usually used to obtain sediment samples, although they are also obtained selectively on beaches and by volume reduction.

The concentration in which MPs are found depends mainly on the depth and the area where they are extracted. It is common to take these samples on beaches because they are dragged by the high tide line, where the accumulation is large (Wang et al., 2018; Prata et al., 2019; Hidalgo-Ruz et al., 2012), and from the seabed (Wang et al., 2018; Zhang et al., 2020), although they can also be extracted at different depths of the water column (Prata et al., 2019).

As already mentioned, the most used method to obtain these samples is coarse sampling. Selective sampling can be done manually (Crawford and Quinn, 2017) or by using spoons, tweezers, and other gadgets that allow the person to pick up the MPs desired by the user directly and at the same moment. Another option to extract MPs from beaches is to submerge a metal mesh in the sand (Kunz et al., 2016) or a stainless-steel shovel (Song et al., 2015), with a surface area of 50x50 cm. This allows one to obtain sediment samples of the superficial layer, up to 5 cm deep, or others deeper, up to 10 cm. After collecting what remains in the mesh or in the spoon, the MPs are mixed with the sand from the beach, so the entire sample is taken, which could be called a bulk sampling, and is passed through a sieve to perform separation (Crawford & Quinn, 2017). On the seabed, although the investigations

146 are minor due to the great depths reached, there are some authors such as Zhang et al. (2020) who
147 place several hollow stainless-steel boxes distributed over the entire surface that covers the study
148 where MPs are deposited, dragged by the current and sunk, as already mentioned, to be later collected
149 and studied.

150 **2.3. Biota Sample**

151 As MPs are found immersed in marine ecosystems, it is easy to think that the species that live in these
152 environments can be directly influenced by their presence and even intervene in their vital functions.
153 The ingestion of this material can be both direct because it is immersed in large bodies of water and
154 indirect, following the trophic chain of the ecosystem, as shown in Figure 1.

155

156 Insert figure 1

157

158 To obtain this type of sample, it is necessary to perform work to collect marine species that may have
159 been affected by living with this type of material in their habitat, either by taking them directly from
160 the environment or for a study based on species that enter our diet, they can be purchased directly in
161 food stores (Griet et al., 2015).

162

163 **3. Sample Preparation**

164 Once the samples have been obtained, it is necessary to extract only the part that belongs to the MPs
165 and discard the rest. When samples are taken mainly from beaches and bodies of water, the part
166 removed will be water, sand, and organic or inorganic substances adhered to the surface of the MPs.
167 Therefore, the main steps will be common, namely reduction in sample volume, as long as it has not
168 been done previously, and thus purification (Prata et al., 2019).

169 **3.1. Separation of MPs and NPs from the sample**

The most used reduction and purification techniques are density separation, sieving, filtration, and digestion. These techniques can be used individually or in combination for best results, e.g., digestion followed by sieving or density separation and filtration. The selection of one technique will depend on the specific case of each type of sample.

In the next section, we will go on to explain in detail what each of the mentioned techniques consists of and in which cases its application is useful.

3.1.1. Filtration and Sieving

To perform the separation of MPs from sand or water, the sample was passed through a filter. MPs and other substances larger than the filter pore will remain in it, thus separating them from the smaller ones.

The filters used are generally cellulose, whose typical pore sizes range between 20 and 43 µm (Wang et al., 2017 ; Thiele et al., 2019). Although smaller sizes have also been achieved by changing the type of material, there are cellulose nitrate filters capable of reaching 5 µm in diameter and up to 1.2 µm made of fiberglass (Thiele et al., 2019).

One of the alternatives to increase the effectiveness of the technique, as proposed by Funck et al. (2020), would be to pass the sample through the filter, applying some pressure on it. As can be detected, the filters mentioned above are made of very fragile materials, so if this technique is applied to them, they will break. Because of this, it is necessary for the filters to be made of a more resistant material to avoid breakage. Funck et al. (2020) used several 10 µm, 50 µm and 100 µm stainless steel filters. This method is interesting when a rapid extraction of MPs is required due to the high volume of samples.

The sieving differs from filtration by the use of a sieve to separate the MPs from the rest of the sample. Usually, the sieves that are used have a mesh size between 0.038 - 4.750 mm (Fu et al., 2020) and are

made of a resistant material. For example, steel (Löder et al., 2015) allows the passage of water or other substances correctly, without breaking or deforming easily.

The process is as simple as passing the sample through sieves of the desired size. To separate MPs of different sizes, the process can be repeated several times with sieves of different sizes, from the largest to the smallest (Moore et al., 2002). This method is especially effective for water samples and sediment samples that have already separated the attached matter from the MP (Löder et al., 2015).

3.1.2. Flotation and elutriation

The method of separation by flotation uses the physical property of the density between the material and other substances. This is possible because of the Bouyant force of the liquid with a density higher than that of the object to be separated. Immersing the material, it displaces its same volume in the liquid where it is introduced, resulting in a thrust force equal to the displaced volume. If the material weighs less than that of the displaced volume, part of it is suspended above the liquid until the weight of the material and the thrust are the same value (Hwang, 2001). On the other hand, if the material weighs more than the volume of the displaced liquid, it will sink.

The density of polymers varies as a function of the physicochemical properties that differentiate each other, such as molecular weight, degree of polymerization, or cross-linking of the chains. Within the same type, there may also be variations, depending on the degree of crystallinity. Those with the highest crystallinity are also those with the highest density, due to the increasing degree of compaction between the polymer chains of the macromolecules that make up the material (Callister, 2016). Taking the example of low-density polyethylene (LDPE) and its antagonist, high-density polyethylene (HDPE), their mean densities are 0.9245 g / cm^3 and 0.9585 g / cm^3. Although these values are quite similar, there are some commercial polymers that increase or decrease this value. Below, Table 1 shows the density ranges of some of the most commonly used polymers and, therefore, most commonly found in MPs research:

Insert table 1

On the other hand, it is also necessary to take into account the density of sediments and sand. Wang and Wang (2018), Hidalgo-Ruz et al. (2012), and Prata et al. (2019) note these values between 2.65-2.70 g / cm^3.

The separation method is based on the choice of one of the salts, mixed with distilled water (Quinn et al., 2016) until the densities shown in Table 2. As a result, the sample to be separated. On the surface, MPs will be separated from unwanted materials such as silica or other substances.

Insert table 2

Depending on the material desired to be separated, the type of liquid used will be chosen. Table 3 shows which solutions would be able to extract any of the MPs or sediments discussed above.

Insert table 3

After comparing, it could be considered that the best option is the direct selection of the solution with the highest density in order to guarantee the separation of any type of material. However, salts mixing that provide the highest densities and the best results also has the worst environmental impact. This is due to the type of chemical compounds. Furthermore, they are the highest costs (Wang et al., 2018). On the contrary, solutions with lower density such as those of water and those saturated with sodium chloride are very cheap, easily accessible to any laboratory, and their environmental impact is much lower than the previous ones. Hence, these advantages result in the high utilisation of low-density solutions in research.

There are many investigations that use different salts to make the separations. Quinn et al. (2016) compared different solutions to perform the separation: fresh water, NaCl, NaBr, NaI, and $ZnBr_2$. In this article, it is revealed that solutions based on NaI and $ZnBr_2$ obtain the highest polymer recovery rate, up to 99%, due to their high density. In the case of fresh water, the one with the lowest density will therefore have the worst recovery rates. Lastly, NaCl obtains recovery rates lower than those of

other salts but better than those of fresh water. However, it is one of the most widely used for the separation of MPs; since it is cheap, respectful to the environment, and for polymers with a density similar to those of HDPE, very commonly found in water, it allows obtaining a recovery of up to 90%.

Once this mixing process has been carried out, a filtration could be performed to finally separate the solution from the desired material, and the process can even be repeated several times to increase the recovery of the material (Woodall et al., 2014).

3.2. Purification: Removal of organic material

Purification is a methodology widely used for sediment samples. MPs submerged for very long periods of time, presenting adhesion of organic species such as zooplankton, phytoplankton, biofilms, or other marine microorganisms (Wang and Wang, 2018). These interfere in the characterization of the samples. For example, the biological material ranges between 0.5 - 7.0%, are those that are taken from beaches. Because of digestion, the organic matter adhering to the MPs is reduced without affecting the structural and chemical integrity of the polymers. This method is interesting for biological samples (Roch and Brinker, 2017), especially when it comes to examining collections from digestive tracts of these species (Kühn et al., 2017).

There are different methods of digestion depending on the chemical agent used: acid, alkali, oxidizing, and enzymatic. Currently, the most widely used methods are acid, alkali and oxidizing; however, the enzyme is gaining more and more prominence due to the fact that it presents a less aggressive character with treated MPs (Courtene-Jones et al., 2016), in addition to giving very good results purification.

Purification consists of making a solution with one of the agents that appear in Table 4. At a certain concentration, time, and temperature, the sample is immersed in water to be treated to eliminate organic matter.

Insert table 4

There are parameters that can be varied to improve the efficiency of the process, such as temperature or concentration. Figure 2 is obtained from the study carried out by Karami et al. (2017). It can be observed that the efficiency of elimination of organic matter varies as follows:

Insert figure 2

As observed in Figure 2, it can be concluded:

- Alkali solutions give different results. The KOH solution is always maintained in high efficiency ranges, although the temperature varies. The maximum value was reached at 40 ° C. However, solutions based on NaOH depend on the temperature variation, increasing the efficiency as the temperature increases.
- Acid solutions based on HCl and HNO_3, at a concentration of 5% by volume, show similar behaviour with temperature ranges, even with a small decrease to 50 ° C. As soon as the concentration is increased to 37% HCl and 69% HNO_3, clearly the efficiency increases from the lowest temperatures, and although for HCl it increases slightly, in the case of HNO_3 it decreases as the temperature increases.
- Oxidizing solutions based on H_2O_2 present high extraction efficiency values from 40 ° C and progressively increase as the temperature increases.

The main drawback when the temperature and concentration are increasing is that not only the presence of organic matter but also the most sensitive polymers can be degraded, falsifying the subsequent results. This can be clearly seen because the color of the MPs varies with increasing temperature in alkali and oxidizing solutions (Karami et al., 2017), (Roch and Brinker, 2017). Regarding acid solutions, at the highest concentrations, some MPs such as LDPE, HDPE, PP and PET melt even at room temperature (Karami et al., 2017).

Although mainly the agents described separately are used, combinations of various of them were also made to proceed with the digestion. For example, Roch and Brinker (2017) perform digestion from the mixture of HNO_3 and NaOH. This method showed a MPs recovery rate of ≥95%, and all tested polymer types were recovered with only minor changes in weight, size, and color with the exception of polyamide.

4. Identification and quantification

Once the samples have been obtained and properly separated, as explained in the previous sections, a series of techniques and instruments for the study and characterization of the MPs that are obtained are necessary. The scientific community opts for the following for the identification of MPs:

i) Identification and optical microscopy
ii) Electron microscopy
iii) Fourier transform infrared spectroscopy
iv) Raman spectroscopy

4.1. Identification and optical microscopy

The optical identification of MPs is one of the most widely used techniques. It is carried out with the naked eye or by means of an optical microscope, depending on the size of the MPs in the samples obtained already separated from the possible substances not relevant for the study. Larger pieces can be directly identified and quantified; however, smaller ones require microscopy to ensure that the pieces are MP and will not be confused with sand, shell fragments, plants, minerals, or rocks of similar sizes (Kunz et al., 2016). Due to the possible confusion and mistakes produced by optical identification, Norén (2007) establishes a series of steps to follow to minimize these errors.

1. Guarantee the absence of cellular material or visible organic structures adhered to the MPs.

2. If the MP is fiber-shaped, it should have a similar width throughout its entire surface.
3. MPs must be homogeneous in shape, and they will be shown defined colors.
4. If the MP is discolored or transparent, a microscope with high magnification will be used. Fluorescence is recommended to exclude the organic origin.

The main aspects that are allowed to be obtained through these techniques are the type, source, state of degradation, and color (Hidalgo-Ruz et al., 2012). This technique is widely used because of its low cost compared to the other techniques that will be discussed.

4.2. Electron Microscopy

This type of microscopy differs from the previous one since it uses a high-intensity electron beam to generate high-resolution images. The one used for the identification of MPs is the so-called scanning electron microscope (SEM).

The electron beam falls on the surface of the sample, obtaining as a result image of it at high magnifications, up to one million, showing details less than 0.5 nm (Wang and Wang, 2018).

This technology makes it possible to differentiate the possible substances adhering to MPs from those that are not. In addition, it can be combined with other techniques to obtain more information. For example, to know the state of degradation and elemental composition of the samples, one can resort to X-ray energy dispersion spectroscopy (EDS) together with SEM (Fries et al., 2013).

The main problem with this technique is the high cost of the equipment and the difficulty in preparing the samples (Wang et al., 2018). They must be under vacuum conditions and treated to convert them into conductive materials (Fries et al., 2013) through which the electrons that generate the image circulate.

4.3. Fourier transform infrared spectroscopy

This technique, called Fourier transform infrared spectroscopy (FTIR), generates a spectrum of the material based on its chemical bonds and its component structure. These are specific to each one. An infrared beam is deposited on the sample, generating the vibration of part of the molecules that make

up it (Löder and Gerdts, 2015). The spectrum obtained is compared with those of already known materials and thus it is known with certainty which is the sample material (Wang and Wang, 2018), (Courtene-Jones et al., 2016). It also allows us to know, as in the previous case, the state of degradation of the polymer.

This technology is used not only for the identification of the type of MP (Kühn et al., 2017), but also for its quantification (Courtene-Jones et al., 2016) by differentiating them from materials that are not MPs.

Many studies opt for this technique that incorporates a variation, for example, FTIR with Attenuated Total Reflectance (ATR) (Kunz et al., 2016; Song et al., 2015; Kühn et al., 2017; Courtene-Jones et al., 2016). This variation with respect to the initial technique consists of the incorporation of an optically dense crystal that makes it possible to measure the changes produced in the infrared beam when striking the sample (ThermoFisher Scientific). FTIR-ATR is especially useful for the analysis of materials of sizes greater than 500 µm (Xu, Z. et al., 2020).

The main limitations of this method are the high cost of the equipment and the time (Wang et al., 2018) required to analyze and interpret the data obtained for the subsequent study.

4.4. Raman spectroscopy

The principle of operation of this technique is based on the use of a monochromatic laser beam that is irradiated on the sample. This causes a spectrum of light to be generated at different frequencies by the light absorbed and reflected from the beam, depending on the atomic composition and molecular structure of the material on which it falls (Löder and Gerdts, 2015). As in the generated previous case, the spectrum is specific for each material (Wang and Wang, 2018), so in this way the type of polymer or other substance belonging to each sample is identified quite precisely (Song et al., 2015; Löder and Gerdts, 2015) and allows quantifying the MPs present in each one (Song et al., 2015).

Raman spectroscopy can be combined with traditional light microscopy to detect MPs whose sizes are too small that they cannot be found otherwise. This technique, called micro-Raman, then makes it possible to work at different sizes, even counting MPs of a few micrometers (Löder and Gerdts, 2015). However, the use of this technique can generate results that generate errors when confused with other materials, due to the presence of additives or other chemicals, normally associated with MPs (Huppertsberg and Knepper, 2018).

After describing the main identification and quantification methods, it can be concluded:

1) According to the location of the research, a determined sampling type will be chosen to obtain a certain sample. This can be seen in Table 5.
2) Once the sample is obtained, its identification will also be based on the ease of manipulation and precision. Table 6 shows the main advantages and disadvantages of each one.

Insert table 5

Insert table 6

5. Advance Methods for the Detection of MPs and NPs

Pyrolysis-GCMS (Py-GC/MS) is one promising method for the direct quantification of MPs and NPs in environmental samples (Fabbri et al., 2000; La Nasa et al., 2020; Gigault et al., 2021). Pyrolysis-MS was first reported in 1948, and py-GC/MS was progressively developed with the advent of GC in 1952 and of chemically inert fused silica capillary columns in 1979 (Tsuge and Ohtani, 2002). Py-GC/MS is like a 'symbiotic technique' in which macromolecules (organic compounds) and polymers are degraded into small volatile fragments that are efficiently separated in a GC column. The obtained chromatogram, called the pyrogram, is polymer specific and individual peaks can be identified with mass spectrometry (Pico and Barcelo, 2020; Tsuge and Ohtani, 2002)(Figure 3 and 4).

Polymer degradation occurs through rapid sample heating (at >500°C for 10 s to 1 min), which is the first key parameter of a py/GC-MS system. This is obtained only with Curie point and tungsten filament pyrolyzers. The second key parameter is maintaining a high temperature throughout the whole analytical system, to avoid products condensation and adsorption in the system, except for the column head where the concentration of molecules occurs before chromatographic separation. The complete description of pyrolyser types and system configurations have been reviewed in Dehaut et al. (2020).

Classical applications of py-GC/MS are polymer characterization (specific composition and / or chemical structures, degradation mechanisms, and related kinetics) (Tsuge and Ohtani, 2002). The most recent applications of py-GC/MS are in fast polymer identification and quantification in environmental samples (Dehaut et al. 2020), through analysing their specific pyrolysis products. Their nature and proportion are immediately related to the applied pyrolysis temperature. The main products obtained for various polymers are reported by La Nasa et al. (2020)), and more products are described for other polymers such as polyurethane (PUR), polycaprolactam (PA-6), acrylonitrile butadiene styrene (ABS), nylon 66 (PA-66) and polytetrafluoroethylene (PTFE) (Dehaut et al. 2020).

In polymer mixtures or in field samples, accurate and specific polymer quantification can only be performed using specific markers. Such markers have been identified for only a few polymers. For polypropylene (PP), the major pyrolysis product present at temperatures between 560 and 700 °C is 2,4-dimethyl-1-heptene. It has been proposed as a marker of PP. For polyethylene (PE), no molecule was identified as a marker, but the target 97 and 83 m/z ions and also 55 m/z ions have been proposed as markers for α--alkenes, and for α, ω-alkadienes, respectively, which are pyrolysis products of PE. The proposed markers for polystyrene pyrolysis are the styrene dimer (2,4-diphenyl-1-butene) and the styrene trimer (2,4,6-triphenyl-1-hexene). The latter is detected below 600 ° C and absent at 700 ° C. Finaly, ε-caprolactam, and methyl methacrylate were proposed as markers for the pyrolysis of PA-6 and PMMA, respectively (Dehaut et al., 2020).

Considering these markers, the identification of MPs in environmental samples using py-GC/MS was often partial. Moreover, only a selected part of the sampled microparticles could usually be analysed. In addition, identification of fibers is challenging because of their low mass due to their shape. Therefore, identification of single particles with GCMS can be difficult and is dependent on the particle mass (Dehaut et al., 2020).

Insert figure 3

Insert figure 4

Moreover, pyrolysis of field samples generates complex pyrograms with nonspecific pyrolysis products, which are common to the pyrolysis of natural organic matter and polymers. Therefore, the analysis of field samples using py-GC/MS requires both sample purification and identification of specific polymer markers. In complex samples such as soil, sediments, or biological samples, interactions and aggregates between nanoplastics and matrice components need to be overcome to isolate nanoplastics. Quantitation should be the final step in nanoplastics analysis. In their excellent review, Cai et al. (2021) indicated that only 5 out of 33 screened papers published by the beginning of December 2020 presented real field data on detection and analysis of nanoplastics. The analyzed samples were seawater, snow, air, sand, and agricultural soil, and measurements were performed using thermochemical or spectroscopic methods.

Moreover, Py-GC/MS allows the identification, and determination of polymer mass per sample, but gives no information about particle number or morphology, which are required data for ecotoxicology studies (Cai et al. 2021, Bartkova et al. 2021)

Furthermore, lower limit of detection (LOD) are needed for nanoplastic determinations in field samples by py-GC/MS (LOD measured of 0.6 μg/L PMMA in water, and 52μg/g for PS in fish samples)(Cai et al., 2021).

6. Major challenges

The challenges about the sampling and sample preparation for the detection of MPs and NPs can vary as a function of the water matrices in which they are contened. The main problem that these issues are addressing are the unexistence of clear and standardized procedures for the sampling and detection that does not let a real and true comparison between different studies.

Despite this, there is no knowledge about how the MPs and NPs evolved in the aqueous solutions along time and so about the evolution of their ecotoxicity and persistence which make more difficult the sampling.

Beside these reasons, an increase in the awareness of the aqueous plastic pollution is needed.

So, as a future goal, the main challenge will consist of the development of standardized methodologies for sampling and for the detection and quantification of the micro- and nanoparticles in real water.

7. Conclusions and perspectives

MPs and NPs are present in the environment, including the biota. In this chapter, an overview of the main existing methods of sampling, sample preparation, and the identification of MPs and nanoplastics in all compartments is provided.

The main conclusions are that the methods employed for these purposes substantially depend on the type of material, the size of the polymer, and the matrix in which the polymer is involved.

The scarce information obtained about these procedures indicates the lack of standard methods and the need of an international agreement that allows for a routine application for comparison purposes. In addition, there exists a need to develop appropriate MPs and NPs risk assessment methods, including those regarding their interactions with other pollutants and with living organisms.

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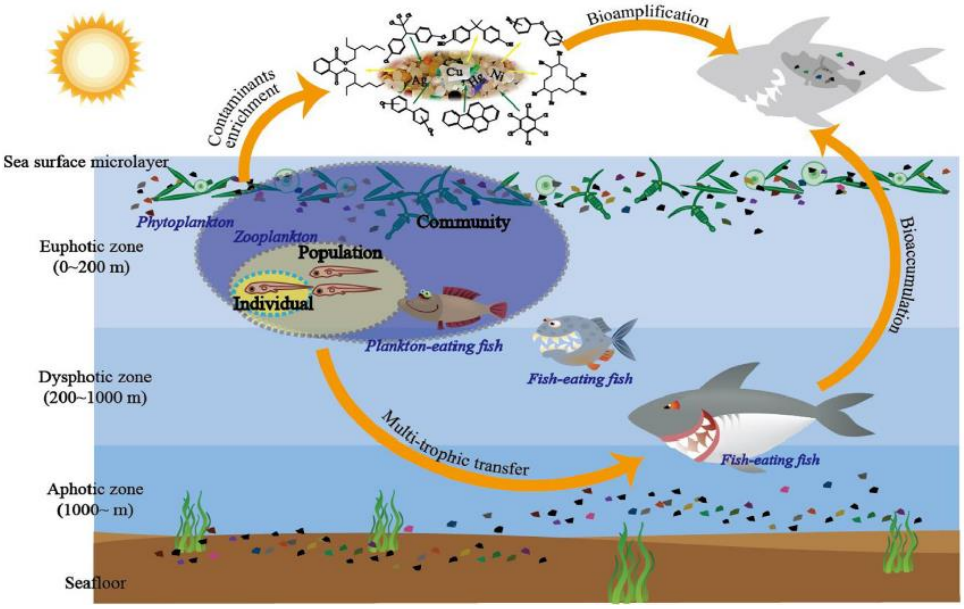

Figure 1. Introduction of Microplastics inside the Tropical Chain of Oceans Obtained from (Ma, H. et al., 2020)

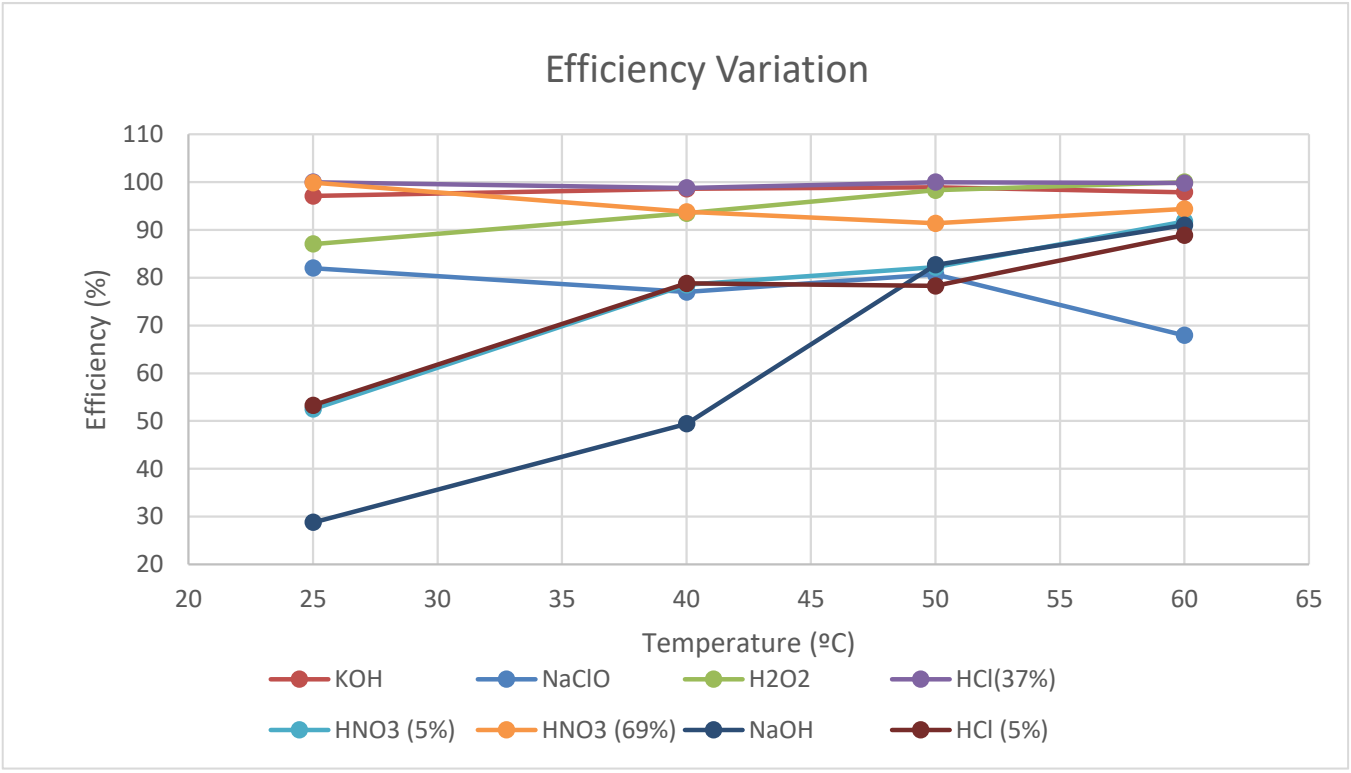

Figure 2. Variation in the removal efficiency of organic matter from microplastic as a function of temperature and digestion. Own elaboration of the seed according to the data of Karami et al. (2017).

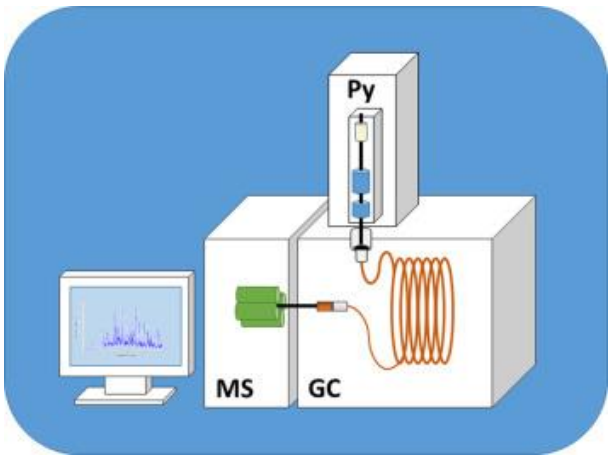

Figure 3. Scheme of the instrumentation in Py-GC-MS. From Picó et al. 2020.

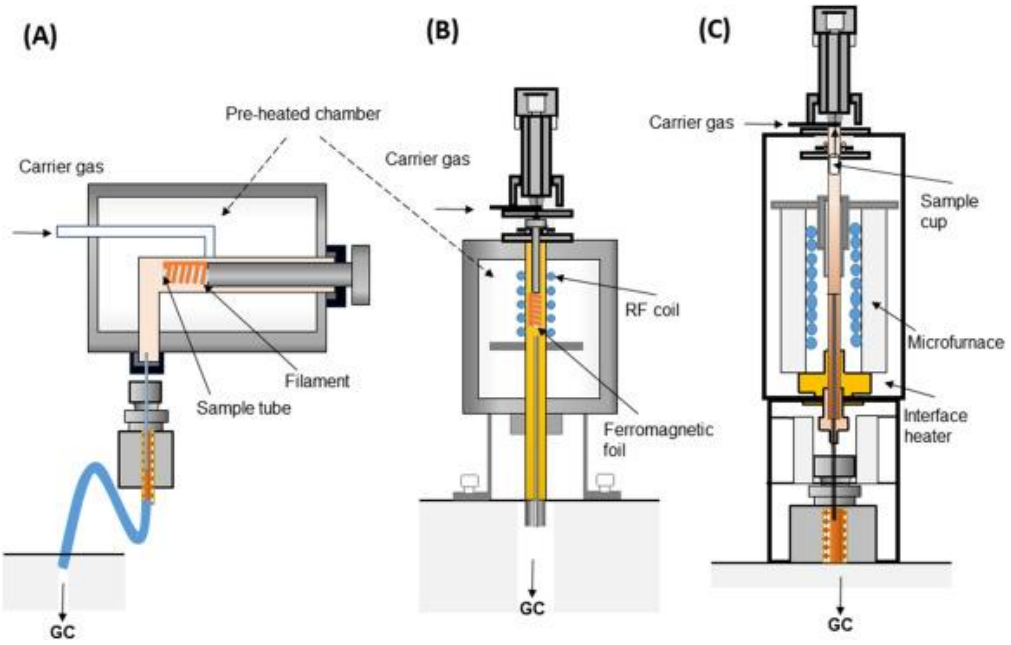

Figure 4. Scheme of the different types of pyrolyzers currently in used. From Picó et al. 2020.

Table 1. Density of some commercially typical polymers. (Data obtained from Agranoff, 1998).

Polymer	Density (g/cm^3)
Low-density polyethylene (LDPE)	0.92-0.93
High-density polyethylene (HDPE)	0.95-0.96
Vinyl polychloride (PVC)	1.30-1.58
Polypropylene (PP)	0.90-0.91
Polistyrene (PS)	1.04-1.05
Polimetylmetacrylate (PMMA)	1.17-1.20
Poliethylenthereftalate (PET)	1.29-1.40

Table 2. Density of typical solutions used in density separation. (Data obtained from Quinn et al., 2016).

Solution	Density (g/cm^3)
Water	1.00
Salty water	1.03
NaCl supersaturated solution	1.20
Solution of NaI	1.80
Solution of ZnCl	1.50-1.70
Solution of $ZnBr_2$	1.70

Table 3. Selection by density of the solution for each material that guarantees its separation. Own elaboration from data of table 1 and 2.

Solution	LDPE	HDPE	PVC	PP	PS	PMMA	PET	Sediments
Water	Yes	Yes	No	Si	No	No	No	No
Salty water	Yes	Yes	No	Yes	No	No	No	No
Solution of NaCl	Yes	Yes	No	Yes	Yes	Yes	No	No
Solution of NaI	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Solution of ZnCl	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Solution of $ZnBr_2$	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No

Table 4. Type of digestion used for the separation of organic material from the microplastic and the normally used chemical agent.

Type of digestion	Agent	References
Alkali	KOH	(Thiele et al. 2019), (Kühn et al. 2017), (Karami et al. 2017), (Enders et al. 2016)
	NaOH	(Karami et al. 2017)
Acid	HNO_3	(Karami et al. 2017)
	HCl	(Karami et al. 2017)
	NaClO	(Karami et al. 2017), (Enders et al. 2016)
Oxidizing	H_2O_2	(Karami et al. 2017), (Xu, Z. et al. 2020)
Enzymatic	K-Proteinase,	(Karlsson et al. 2017)

	Trypsin	(Courtene-Jones et al. 2016)

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502 *Table 5. Conditions for the selection of the sampling type and the obtained sample.*

Sampling		
Selective	**Bulk**	**Volumen reduction**
Visible Microplastics	Non-viscous microplastics	Large area of study
Beaches	Low concentration of MPs	-
-	Beaches	-
Samples		
Sediments	Water	Water
-	Sediments	Sediments

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505 *Table 6. Comparison of advantages and disadvantages for every identification technique.*

Advantages	Drawbacks
Identification and optical microscopy	
Low cost	Errors due to human factors
Easy Manipulation	Difficult Identification of Smaller Pieces
Electronic microscopy	
High-resolution	High cost
Identification to the nanometric scale	Minucious preparation of the samples
Substances Differentiation	Vacuum Conditions
-	Cualified Emplolyees

FTIR	
Identification of Microplastic Type	High cost
Precise identification of materials	Time
Quantification	Generation of nonsimple data to analyze
Raman spectroscopy	
Identification of MP type	Precise
Identification of other materials	-
Quantification	-
Identification of MPs with sizes close to the micra (micro-Raman)	-

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