



# A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment



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## ABSTRACT

Plastics can be found in food packaging, shopping bags, and household items, such as toothbrushes and pens, and facial cleansers. Due to the high disposability and low recovery of discharged materials, plastics materials have become debris accumulating in the environment. Microplastics have a dimension <5 mm and possess physico-chemical properties (e.g., size, density, color and chemical composition) that are key contributors to their bioavailability to organisms. This review addresses the analytical approaches to characterization and quantification of microplastics in the environment and discusses recent studies on their occurrence, fate, and behavior. This critical overview includes a general assessment of sampling and sample handling, and compares methods for morphological and physical classification, and methodologies for chemical characterization and quantification of the microplastics. Finally, this review addresses the advantages and the disadvantages of these techniques, and comments on future applications and potential research interest within this field.

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## 1. Introduction

The global annual production of plastics is around 280 million tons, the vast majority being for disposable use [1,2]. Due to the high disposability, low recovery of such waste materials, high durability, accidental release, and indiscriminate discards, plastics have become debris accumulating at an uncontrolled rate in the environment, where they are subjected to erosion by wind and rain, and to river-driven transport, mostly reaching coastal areas [1,3]. Generally, these items accumulate as debris in the environment, including the seas and regulated landfill sites [1]. The polymers most widely

produced as plastics and encountered as plastics debris are polypropylene, polyethylene, polystyrene, polyethylene terephthalate, and polyvinylchloride [3,4].

Research on environmental impacts of plastics debris has gained a new dimension due to the study of microplastics and their impacts on terrestrial ecosystems and marine environment [1,5]. The National Oceanic and Atmospheric Administration (NOAA) defined microplastics as particles less than 5 mm in size [3]. Since a positive relationship between microplastics abundance and human population density has been established, the increase in human population would probably lead to an increase in the prevalence of microplastics in the environment [6,7]. In order to understand the potential impacts of microplastics in the environment, their identification and characterization have been attempted in relation to seawater, sandy and muddy sediments, plankton samples,

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marine-sewage disposal, sewage effluent, washing-machine effluent, facial cleansers, and vertebrate and invertebrate ingestion [8–10]. The physico-chemical properties of microplastics, such as size, shape, density, color and chemical composition, greatly affect their transport in the environment and their bioavailability [3]. The toxicological effects on marine organisms are also influenced by the physico-chemical characteristics of microplastics [3].

Taking this into consideration, the complete characterization of microplastics in terms of abundance, distribution and chemical composition is of paramount importance in order to characterize their environmental impacts and to enable large-scale spatial and temporal comparisons. This critical overview therefore covers the different analytical approaches used for characterization and quantification of microplastics in the environment and discusses the most recent studies on their occurrence, fate and behavior.

## 2. Sampling and sample handling

Microplastics can directly enter the environment, whereas large items are continuously fragmenting until they attain micrometric dimensions in size [8]. They can reach the soil and aquatic systems via the effluents of wastewater-treatment plants but they can also be resuspended by wind from landfills and become airborne until the atmospheric conditions allow entry into the ecosystems by deposition. There is also a pattern of accumulation along the shorelines, mostly due to port activities and discharges from river mouths [9]. Based on research on the Web of Knowledge, microplastics have been studied more intensively in the marine environment and on shorelines. Concerning the inland environment, there are no studies in soils or atmospheric aerosols. Sewage effluents, washing-machine effluents and facial cleansers have been studied as examples of microplastics spreading into the environment [9,10].

Sampling microplastics in the water column can be achieved by:

- (1) using a trawl along a transect, such as manta net and Neuston net for surface waters [11]
- (2) using bongo nets for mid-water levels [5];
- (3) benthic trawls for seabed [5]; and,
- (4) vessels for surface and mid-water levels [12,13].

Typically, 333–335- $\mu\text{m}$  mesh apertures are the most used for the net [11,14] and, when a different size aperture is used, it can produce large variations in the quantity of microplastics collected [5].

Sampling sediments can allow the benthic material from beaches, estuaries and seafloor to be assessed for the presence of microplastics [5,15]. Using stainless-steel spoons or spatula [16,17] and box-corer [18] usually collects superficial sediments or sand from the beaches and estuaries, while seabed sediments are collected using core [19] and bottom trawl [20]. There is a general lack of specific sampling protocols for collection of microplastics in sediments and water.

Biological sampling involves examining microplastics in marine organisms through dissection of marine animals, and investigation of regurgitation in seabirds and analysis for the presence of microplastics in their gut contents [5].

Separation of microplastics from samples has been done by density flotation, filtration and sieving [9,10,12,13,15–29], as shown in Table 1. The specific density of plastics particles varies considerably, since it depends on the type of polymer and the manufacturing process, in the range 0.8–1.4  $\text{g cm}^{-3}$  [29]. These values do not take into account the effect of adding several additives that might be incorporated into the production process, so they refer only to the virgin resin, biofouling and weathering [29].

Since sand or sediments have densities around 2.65  $\text{g cm}^{-3}$ , the difference in density can be used to separate the lighter microplastics from the heavier sand or sediments. A salt-saturated solution (usually

**Table 1**

Methods for the separation of microplastics [9,10,12,13,15–29]

Method	Mechanism	Size range	Complementary technique
Filtration	Size fractionation	Down to 2 $\mu\text{m}$	Optical microscopy
Density flotation	Salt addition (usually NaCl or NaI) and floatation	<sup>a</sup>	
Sieving	Size-exclusion	0.038–4.75 $\mu\text{m}$	

<sup>a</sup> Not applied.

NaCl or NaI) is added to sand or sediments and mixed by shaking or using a vortex. After mixing, the sediment will settle to the bottom, while the microplastics will remain in suspension or float to the surface of the solution. The supernatant is then extracted for further processing of the microplastics particles.

The Marine Strategy Framework Directive (MSFD) Technical Subgroup on Marine Litter recommended the use of NaCl for the separation of microplastics by density flotation [23,30], since it is an inexpensive, eco-friendly salt. However, the use of saturated solution of NaCl (1.2  $\text{g cm}^{-3}$ ) or tap water may lead to underestimation of the microplastics content in sediments because the solution density is too low to enable the flotation of all polymers, principally those containing additives [23]. Instead, an NaI-saturated solution density (1.6  $\text{g cm}^{-3}$ ) is enough to separate the polymers containing additives, so it is preferable to use NaI.

Cauwenberghe et al. [19], in an attempt to investigate the presence of microplastics in deep sea, collected sediments in a surface area of 25  $\text{m}^2$ . After recovery, cores were cut into horizontal slices by extruding them and slicing the sediment with a metal plate. The cores were cut into 1-cm-thick slices and the top centimeter of the sediment cores was wet sieved, first on a 1-mm mesh sieve and subsequently on a 35- $\mu\text{m}$  mesh sieve. The fraction remaining on the 35- $\mu\text{m}$  mesh sieve was used for further separation based on density flotation by adding a solution of NaI. The solids were then transferred to a centrifuge tube followed by shaking and centrifugation. After centrifugation the top layer containing the microplastics was vacuum filtered. As in this example, the microplastics are usually separated from the supernatant, resulting from the density flotation by filtration, either vacuum assisted or under normal pressure.

Microplastics can also be optically identified in filter cakes using techniques, such as binocular microscopy [24,25]. Finally, particles that are optically identified as microplastics can be separated and singled out using tweezers.

Microplastics can also be separated from samples using sieves of several mesh sizes, which allow classification into different size categories. The sieves used are usually made of stainless steel [12] and copper [13]. Desforges et al. [13], aboard a research cruise vessel, collected seawater at 4.5 m below the surface using the seawater-intake system of the vessel. A flow meter measured the volume of water pumped for 10–20 min from the seawater intake and the readings were converted to cubic meters of water filtered. Water was first filtered through a 5-mm filter to remove large debris and organisms before entering the intake system and then running through a series of copper sieves of diminishing pore size: 250  $\mu\text{m}$ , 125  $\mu\text{m}$  and 62.5  $\mu\text{m}$ . The material on each sieve was rinsed with seawater into glass vials and stored.

In order to prevent the contamination of samples with airborne microplastics during the extraction process, Cauwenberghe et al. [19] used materials previously rinsed with filtered deionized water and in a clean fume hood. Materials, such as containers, must be rinsed onto the filter or sieve, since microplastics may stick to the walls, so losing a proportion of the analyte. During sample collection, cotton clothing should be worn rather than synthetic fabrics to avoid contamination by plastics fibers [9]. Quality-control samples must be used to check whether there has been sample

contamination in order to minimize overestimation or underestimation of sources of contamination.

Visual inspection and sorting of the concentrated samples are the first steps in separation of microplastics from organic debris, such as plant and animal residues, and from other non-plastics anthropogenic debris, such as glass or tar. However, it is often very difficult to distinguish this debris visually from microplastics. Dekiff et al. [27] attempted to test the visual separation of microplastics from other debris by three independent observers, and obtained three different quantitative statements for the same sample extract. Decisions on identification and separation of microplastics often rely on certain features, such as shininess, brightness or unexpected colors, forms and structures, and specific elastic consistency of particles or hardness determined by applying tweezers [24].

Since separation from the sample and identification of microplastics are of paramount importance, there is some urgency to develop new techniques and thus implement standardized protocols and methodologies in order to avoid misidentification and underestimation of microplastics. Cole et al. [31] investigated the efficiency of using acid, alkaline and enzymatic digestion techniques in mineralizing biological material from marine surface trawls in order to avoid damaging any microplastics present. Acid digestion, even using low concentrations of the non-oxidizing, mineral HCl at room temperature, proved to be inconsistent and inefficient, yielding large quantities of material on the filters after digestion. Certain acids, such as H<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub>, were not tried in this study, since they can destroy or damage polymers with low-pH tolerance (e.g., polystyrene).

Cole et al. [31] also verified that the use of 1 M NaOH at room temperature proved 90% effective in digesting marine samples. Increasing molarity and temperature can increase the digestion efficiency; this method was suitable for digesting plankton using 10 M of NaOH at 60°C but proved damaging to microplastics. When applied to marine samples, alkaline digestion can therefore result in an under-representation of pH-sensitive polymers or in mismatching the microplastics color.

Cole et al. [31] developed a rapid method in which the proteolytic enzyme (proteinase-K) treatment was applied to marine samples. The enzymatic method can digest more than 97% (by weight) of the material present in plankton-rich seawater samples without destroying any microplastics debris present.

In another example, Claessens et al. [26] attempted to develop a technique based on the principle of elutriation (the process that separates lighter particles from heavier particles using an upward stream of gas or liquid) for the separation of microplastics from sediments and field organisms. The technique showed extraction efficiency of 93–98% and was preferable to other methodologies requiring visual inspection.

As another example, Imhof et al. [32] developed a method for the separation of plastics particles in sediments of aquatic environments. They improved the density-separation approach by constructing the Munich Plastic Sediment Separator. A ZnCl<sub>2</sub> solution (1.6–1.7 kg/L) was used as separation fluid and the device provided recovery rates of 100% and 95.5% for microplastics particles of 1–5 mm and <1 mm, respectively. The proposed methodologies [26,31,32] were developed in an attempt to establish a reliable, verified and standardized method for separation or extraction of microplastics, which is a crucial pre-requisite for quantifying the increasing contamination of environment with microplastics and therefore for future risk-assessment and management strategies.

### 3. Detection and characterization of microplastics

Microplastics have unique properties, such as size, shape, density, color, and abundance, which are key factors contributing to their

distribution in the environment and bioavailability to organisms. In general, the analysis of microplastics can be separated into two major areas: (1) morphological and physical characterization; and, (2) chemical characterization and quantification.

#### 3.1. Morphological and physical characterization

The characterization of the size distribution of microplastics depends on the sampling and separation methods due to sieve and filter-pore sizes. The size categories can be given using a sieve cascade during the separation procedure or by length measurements assisted by optical microscopy. As an example, Desforges et al. [13], during an attempt to study the distribution of microplastics in sub-surface seawater, used three sieves (250 µm, 125 µm and 62.5 µm) and then combined the particles from the three sieves to categorize the particles by size. Size categories were based on length measurements assisted by optical microscopy of the longest dimension of each particle: < 100 µm (62–100 µm), 100–500 µm, 500–1000 µm, or >1000 µm (1000–5000 µm). Lee et al. [11] used laser diffraction to confirm the median diameter obtained by optical analysis for polystyrene microplastics.

Several studies described the morphological characteristics of microplastics by assessing the shape and/or the color [10,13–15,18,24,28], often using optical microscopy, such as stereomicroscopy. Some classifications in terms of color and shape were also made by visual inspection [33]. Scanning electron microscopy (SEM), scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDS) and environmental scanning microscopy-energy dispersive X-ray spectroscopy (ESEM-EDS) were the techniques used for characterizing surface morphology [14,18,19,24] (Table 2). SEM-EDS and ESEM-EDS were used both for characterizing the surface morphology of microplastics and determining the elemental composition of polymers.

#### 3.2. Chemical characterization and quantification

Table 2 shows the analytical techniques used to assess the composition and the concentration of microplastics [9,12,14–19,22–24,27,34,35]. Identification of the polymeric composition of the microparticles is often performed using Fourier-transform infrared (FT-IR) spectroscopy since the IR spectra of an unknown microplastics sample can be compared with the IR spectra of known polymers available from IR spectra libraries.

The use of micro-FT-IR spectroscopy recently increased and allows collection of spectra in attenuated total reflectance (ATR), transmittance or reflectance modes. Micro-FT-IR spectroscopy enables the simultaneous visualization, mapping of samples and collection of spectra. Micro-ATR-FT-IR spectroscopy also facilitates the identification of irregularly shaped microplastics. As such, it is possible to develop protocols based on micro-FT-IR spectroscopy for the spectral visualization of many types of polymer. This technique requires expensive equipment (FT-IR linked to a microscope) and a trained operator, and is time consuming; however, it remains the most widely used, reliable and reproducible method for characterizing microplastics in the environment.

Harrison et al. [16] investigated the applicability of micro-FT-IR and reflectance micro-FT-IR spectroscopy for the determination of microplastics in marine sediments, and proved the ability of such techniques for molecular mapping analysis to detect microplastics in sediments successfully, based on their spectral characteristics, without the need for visual inspection of fragments for characterization. Since micro-FT-IR spectroscopy methodologies depend on the heterogeneity of the materials under analysis, this study [16] also emphasized the urgency of developing efficient, reproducible techniques for collection of samples and separation of microplastics.

**Table 2**

Analytical techniques used to assess the surface morphology, the composition, and the concentration of microplastics

Method	Approach and information obtained	Sample preparation (excluding separation)	Advantages/limitations	Ref.
Scanning electron microscopy (SEM)	Interaction of an electrons beam with the sample to measure the secondary ions producing a sample image	When working at high-vacuum requires coating	High-resolution image. May require coating. Destructive. Charge effects	[19,24]
Fourier Transform Infrared Spectroscopy (FT-IR)	Spectra can be collected in Transmittance, Reflectance or Attenuated Total- Reflectance (ATR) mode.	No sample preparation required (sample clean-up may be needed)	When using a micro FT-IR: - Software enables the visualization of samples, collect spectra and map samples - Fully purgeable infrared beam and sample stage which eliminates the effects of water vapor and carbon dioxide in the spectra and provides a dust free environment for the microscope	[9,12,15–18, 22,27,34,35]
Pyr-GC-MS	Mass spectrometry of microplastics by analyzing their thermal degradation products	Sampler equipped with A thermal desorption system	Able to analyze polymer type and organic plastic additives in one run without the use of solvents thus avoiding background contamination; Destructive	[23,24,27]
Raman spectroscopy	A laser excites lattice and molecular vibrations; Raman spectroscopy informs about bonding within the material, and about molecule and networking structures	No sample preparation requirement (sample clean-up may be needed)	When using a micro-Raman: - Non-contact and non-destructive measuring procedure can be applied to very much different materials - The presence of color in the microplastics interferes with the measures and plastic type and it can be obtained the spectra for pigment instead of plastics	[19]
Scanning electron microscopy – Energy-dispersive X-ray Spectroscopy (SEM-EDS)	Diffraction and reflection of emitted radiation from microplastics surface	No requirement of coating due to work in low vacuum	Chemical and morphological characterization of particles	[14,24]
Environmental scanning electron microscopy -energy dispersive X-ray spectroscopy (ESEM-EDS)	Diffraction and reflection of emitted radiation from microplastics surface	No sample preparation required	Elemental composition and surface morphology of microplastics No charge effects	[18]

Other analytical techniques, such as pyrolysis-gas chromatography-mass spectrometry (Pyr-GC-MS), Raman spectroscopy, SEM-EDS and ESEM-EDS, were also used to identify microplastics polymers. SEM-EDS was used by Fries et al. [24] to characterize inorganic additives in microplastics fragments, and they observed the presence of Al, Ti, Ba, S, O, Zn, and C, besides titanium-dioxide nanoparticles (TiO<sub>2</sub>-NPs). The C signal was from the polymer material, while Al and Zn could have been sorbed to marine plastics particles [24]. TiO<sub>2</sub>-NPs could have been added to plastics during manufacture as white pigments or UV blockers [24].

The identification of microparticles is often performed using certain characteristics well defined for known polymers, such as specific density, shape, and color [11]. These methods are more economical, but they can lead to erroneous characterization of microparticles. Using Pyr-GC-MS, Dekiff et al. [27] showed that only 47% of a sub-sample of 32 samples optically identified as potential microplastics could be assigned to a common polymer type. Hidalgo-Ruiz et al. [29] also confirmed that 70% of the visually identified particles are not confirmed as plastics by FT-IR spectroscopy. Chemical identification should therefore confirm the results of identification by visual inspection and optical analysis, which, by themselves, are incomplete and inappropriate for confirmatory analysis.

Quantification of microparticles has been performed in order to obtain spatial and temporal distribution, to determine rates of accumulation, to investigate the organic pollutants and metals sorbed onto microplastics and to assess the impacts of microplastics on marine organisms. The concentration of microplastics is usually given by the number of particles per:

- square meter (particles m<sup>-2</sup>);
- cubic meter (particles m<sup>-3</sup>); and,
- kg of dry sediment.

Usually, particles are counted under the microscope [27,29,36]. Differences in terms of concentration and composition (predominant types) of microplastics exhibited in different studies could be explained by significant methodological differences [27]. Liebszeit and Dubaish [36] quantified microplastics particles in the East Frisian Island of Norderney by counting particles under the microscope, whereas Dekiff et al. [27] quantified microplastics particles from Norderney using both optical and chemical analysis (Pyr-GC-MS). Comparison of the quantitative results obtained by both studies would be very interesting, yet impossible, since Liebszeit and Dubaish [36] counted particles under the microscope but did not perform chemical verification.

#### 4. Occurrence, fate, and behavior in the environment

Microplastics can occur in the environment as primary or secondary microplastics [3]. Primary microplastics are those manufactured for a number of specific purposes, such as those used in cosmetic products. Secondary microplastics are those produced through environmental degradation of larger-sized products. Microplastics can arise from direct sources, such as industrial accident spillages or release of microbeads from cosmetics through wastewaters. Even washing machines can produce secondary microplastics fibers [19] that can enter into the environment through sewage wastewaters.

Microparticles can accumulate in landfills but they can be resuspended and become airborne and spread in the terrestrial ecosystems, namely in soils through deposition processes [1]. They have also been accumulating in the oceans worldwide over the past four decades [3–5] and they can be found in beaches, surface waters, throughout the water column, sediments, and marine organisms. Table 3 shows the spatial distribution and the abundance of microplastics in the marine environment [12–15,17,18,20,22,25,27,36–40].



**Table 3**  
Spatial distribution and abundance of microplastics from selected references

Location	Maximum concentration observed	Ref.
Waters around Australia	839 particles km <sup>-2</sup>	[12]
NE Pacific Ocean	9 180 particles m <sup>-3</sup>	[13]
USA, Laurentian Great Lakes	466 000 particles km <sup>-2</sup>	[14]
Belgium, Coastal zone	213 particles kg <sup>-1</sup>	[15]
Singapore, Coastal mangrove ecosystems	63 particles kg <sup>-1</sup>	[17]
Venice Lagoon, Italy	2 175 particles kg <sup>-1</sup>	[18]
Belgium, Coastline	18 particles kg <sup>-1</sup>	[20]
Portugal, Beach	218 particles m <sup>-2</sup>	[22]
North Western Mediterranean Sea	1 particles m <sup>-2</sup>	[25]
Germany, Beach, Norderney Island	4 particles kg <sup>-1</sup>	[27]
Germany, Beach, East Frisian Islands	621 particles 10g <sup>-1</sup>	[36]
USA, California, Coastal waters	3 particles m <sup>-3</sup>	[37]
Lanzarote Beach, Canary Islands	109 gL <sup>-1</sup>	[38]
La Graciosa Beach, Canary Islands	90 gL <sup>-1</sup>	[38]
Sub-alpine Lake Garda, Italy	1108 particles m <sup>-2</sup>	[39]
Tamar Estuary, Southwest England	0.040 particle m <sup>-3</sup>	[40]

Eriksen et al. [14] collected Neuston samples at 21 stations during a 1300-km expedition in the Laurentian Great Lakes of the USA and found an average abundance of 43,000 microplastics particles km<sup>-2</sup>. However, in a station downstream from two major cities (Detroit and Cleveland), they [14] found 466,000 microplastics particles km<sup>-2</sup>, a value much higher than all the other stations combined. The results of this study [14] posed several questions about the sources of microplastics particles, since they have physical characteristics similar to microbeads used in two primary commercial applications: sandblasting media and abrasives in consumer products. Sandblasting media was excluded since it would include polymeric materials using acrylic with a density of 1.19 g cm<sup>-3</sup> and amino thermoset plastics (1.47–1.52 g cm<sup>-3</sup>), which would be negatively buoyant in freshwater and therefore not likely present in the samples obtained in the Great Lakes. Based on dense urban populations adjacent to the lakes that employ combined sewage overflow, and the convergence of the lake currents near sampling sites, the spherical microplastics could be linked to consumer products, such as facial cleansers, and other personal products, since they contained spherical microbeads labelled on the product as polyethylene (0.91–0.96 g cm<sup>-3</sup>) or polypropylene (0.91 g cm<sup>-3</sup>), which would float in freshwater systems.

The evaluation of links between hydrodynamic factors (e.g., current velocity, turbidity, turbulence, density of water mass, and residual circulation), anthropic factors (e.g., urban and industrial activities), meteorological factors (e.g., temperature, wind) and geographical conditions (e.g., slope, canyons, bays, capes, and beaches) are important, since they determine the behavior of microplastics that varies according to nature, size and composition in the aquatic environment and can improve understanding of the transport dynamics and the accumulation zones.

There is evidence that the abundance of microplastics is increasing in the marine environment [13,37]. Since microplastics are in the same size range as sediments and some planktonic organisms, they are potentially bioavailable by ingestion to a wide range of marine organisms, such as invertebrates, birds, and fish [41–44]. They may therefore accumulate within organisms, resulting in physical harm, such as internal abrasions and blockages [3]. Toxicity can also arise from leaching constituent contaminants, such as monomers of plastics additives, capable of causing carcinogenesis [3].

It is of paramount importance to understand the fate and the effects of microplastics-related compounds (e.g., phthalates, bisphenol A, and polybrominated diphenyl ethers) in marine organisms in order to establish the environmental consequences of microplastics. Rochman et al. [45] found that *Myctophidae* fish sampled at stations with greater plastics densities have

significantly higher concentrations of polybrominated diphenyl ethers (PBDEs), BDEs 183–209 in their tissues suggesting that higher brominated congeners of PBDEs, added to plastics as flame retardants, may be suitable indicators of plastics contamination in the marine environment. Moreover, microplastics can concentrate hydrophobic persistent organic pollutants (POPs) [46–51], which can be sorbed on microplastics up to six orders magnitude greater than in seawater [3] – a possible route of exposure to marine organisms with consequent bioaccumulation and biomagnification through the food chain.

Farrel and Nelson [52] investigated the trophic transfer of microplastics from mussels [*Mytilus edulis* (L.)] to crabs [*Carcinus maenas* (L.)]. After exposing mussels to polystyrene microspheres, the mussels were fed to crabs. The number of microspheres in the hemolymph decreased from 24 h to 21 days after ingestion, and the maximum amount of microspheres in the hemolymph was 0.04% of the amount to which mussels were exposed. Microspheres were also found in crabs' stomachs, ovaries and gills. The trophic transfer of microplastics from mussels to crabs was demonstrated and increased concern about the potential of microplastics to reach higher trophic levels and for the health of animals and humans [52].

Wright et al. [3] reviewed the physical impacts of microplastics on marine organisms, focusing their attention on marine invertebrates. In this review, characteristics, such as size, shape, color, density, and abundance likely to influence the physical impacts of microplastics on invertebrates, were thoroughly discussed. Lee et al. [21] found size-dependent effects of polystyrene microbeads on survival, development and fecundity of copepod *Tigriopus japonicus* using acute and chronic toxicity tests. The beads were fluorescently labeled and ingested by copepods, which were observed under a fluorescent microscope.

Ingestion and entanglement of microplastics may become mortality factors for many marine species, although there is a lack of knowledge of effects at the population and community levels. Furthermore, the ecological impact of litter on marine organisms and ecosystems will need to relate quantities of ingested microplastics to lethal or sub-lethal effects and to model these effects under different environmental conditions [30].

Risk assessments of microplastics require analytical methods to monitor the processes that control the fate of the microplastics and their interactions with other contaminants in the environment.

No data were found on concentration levels of microplastics in environmental compartments, such as soils and airborne and effects on related organisms.

## 5. Conclusions and future trends

Microplastics are widely spread in the environment, leading to concern regarding their potential impact on environmental health. It is known that substantial amounts of microplastics are in the marine environment, so causing further concern regarding the risks to marine organisms. Moreover, microplastics may sorb and consequently enhance contamination of aquatic organisms by persistent organic pollutants.

There are still no studies that have considered trends in microplastics abundance over time, and the main difficulties are due to the lack of standardization of sampling methodologies and separation of plastics microparticles. Further research therefore needs to consider sampling design in terms of the number and the size of replicates, the spatial area and the frequency of coverage, and the methodology used for sampling (i.e., type of net for aquatic samples or core for sediment samples) and methods used for identification of microplastics. These studies should take into consideration the relationships between sampling effort and variability. Moreover, the identification and recommendation of protocols to provide consistent, reliable, easily obtainable data on spatial and

temporal scales are needed to improve understanding of the persistence and the fate of particles, and the sources and the consequences of microplastics in the environment.

Although FT-IR proved a useful technique for identifying the polymeric composition of the microparticles, there is still a lack of analytical methods capable of characterizing and quantifying the chemical compounds derived from microplastics in real environmental samples and consequently assessing their concentration. Certain methods, such as Pyr-GC-MS and other MS-based methodologies, may be applied to monitor microplastics and to indicate the type of polymer used, but further interlaboratory comparison exercises should be performed in order to ensure the comparability of data collection, separation, and chemical characterization and quantification. Furthermore, there is also a need for studies on the evaluation of rates of degradation of different types of microplastics and the related leachability of pollutants, and understanding of the fate and the effects of microplastics-related compounds (e.g., phthalates and bisphenol A) in marine organisms.

In the near future, this field should focus research efforts on developing new tools and analytical strategies capable of adequately counting and characterizing microplastics particles (i.e., detectors of polymers) in the laboratory. Microelectronics and nanoelectronics, with their potential for miniaturization and coupling to automated samplers, can become valuable tools for developing new methods and sensors suitable for analysis of microplastics, especially *in-situ* analysis in a wide range of environmental compartments.

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