



Review

Microplastics in the environment: Challenges in analytical chemistry - A review



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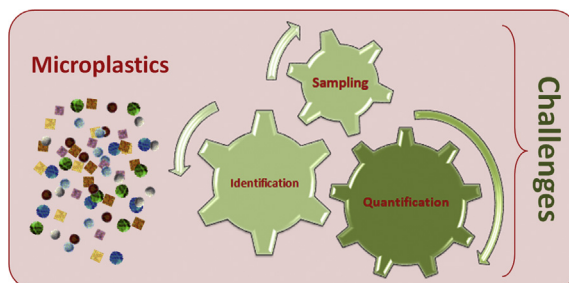
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HIGHLIGHTS

- Microplastics have been identified as environmental pollutants.
- The sampling, sample handling, identification and quantification of microplastics were discussed.
- The validation of analytical methods and use of reference materials for the microplastics quantification were highlighted.
- The current challenges in these issues are identified.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics can be present in the environment as manufactured microplastics (known as primary microplastics) or resulting from the continuous weathering of plastic litter, which yields progressively smaller plastic fragments (known as secondary microplastics). Herein, we discuss the numerous issues associated with the analysis of microplastics, and to a less extent of nanoplastics, in environmental samples (water, sediments, and biological tissues), from their sampling and sample handling to their identification and quantification. The analytical quality control and quality assurance associated with the validation of analytical methods and use of reference materials for the quantification of microplastics are also discussed, as well as the current challenges within this field of research and possible routes to overcome such limitations.

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1. Introduction

Over the last few decades, plastic contamination has become a major cause of concern among scientists, politicians, and the public. World production of plastic surpassed the 320 million tons mark in 2016, most of which is intended for packaging, i.e., for immediate disposal [1]. Consequently, these materials greatly contribute to the generation of waste and it is estimated that between 5 and 13 million tons leaks into the World's oceans every year [2]. When inappropriately dumped or mismanaged, plastic waste can accumulate in both terrestrial and marine environments [3,4] and, once released, it may be subjected to degradation by several agents or routes, such as solar radiation, mechanical forces, and microbial action [5]. This leads to fragmentation and breakdown of those larger materials into microplastics, defined as plastic particles less than 5 mm and, eventually, nanoplastics, which range from 1 to 100 nm, though the latter has only been recently identified as potentially deleterious towards the environment and research is currently underway. In addition, these particles can be intentionally produced with micro- and nano-sizes and disposed directly into the environment [5].

Although their presence and associated dangers have long been reported [6,7], the ubiquity of microplastics in the oceans has become of increasing concern. Consequently, numerous attempts have been made to assess their potential effects not only to the environment, but specifically to biota and, ultimately, to humans. Due to their small size, these particles can be ingested by several marine species, leading to direct physical damage and potential toxicity effects [8]. Microplastics may also leach plastic additives, including persistent organic pollutants (POPs) and potentially toxic elements that are adsorbed in higher concentrations than those found in the surrounding environment [9–11]. These pollutants may transfer and accumulate in different tissues of organisms, possibly undergoing biomagnification along the food chain [9,12]. Hence, consumption of contaminated seafood poses a route for human exposure to microplastics, POPs, and potentially toxic elements [12]. POPs including polychlorinated biphenyl (PCBs) and polycyclic aromatic hydrocarbon (PAHs) have also been shown to accumulate on microplastics, thus enhancing their potential toxic effect in the environment [13–15]. Such dangers have been demonstrated for numerous organisms, such as blue mussels, in which von Moos et al. [16] verified that microplastics, namely, high-density polyethylene (PE), ranging from 0 to 80 μm were ingested and taken up into the cells and tissues of these organisms. Microplastic particles were drawn into the gills, transported into the stomach and into the digestive gland, where they accumulated in the lysosomal system after 3 h of exposure [16]. More recently, Jovanović [17] reported potential negative effects of the ingestion of microplastics and nanoplastics by fish, including possible translocation of microplastics to the liver and intestinal blockage, yielding not only physical damage, but also histopathological

alterations in the intestines and modification in lipid metabolism. It should be noted, however, that, despite demonstrating the potential fate and effects of microplastics on biota, these studies, as well as other numerous reports described in the scientific literature, focus on experiments on the use of polymeric particles at concentrations that far exceed those determined in the environment, thus not accurately simulating natural settings regarding composition, morphology, and concentration [5,18].

A large amount of information concerning microplastics has been made available, including some disparate arguments regarding their prevalence, which may stem from the inherent difficulties in assessing and monitoring microplastics in the environment, as well as the lack of consistency in field studies [5]. In order to determine the real hazards of microplastics, there is a pressing need to develop and implement standardized protocols for sampling, quantification, and characterization of microplastics, including data treatment and visualization, which will allow for the subsequent comparison between different studies [5,18].

Since 2012, some reviews have focused on some of the issues associated with the analysis of microplastics, particularly microplastics found in seawater [9,19–21] and freshwater [21,22], as well as in sediments [14,19,23]. Their potential toxicological effects have also been thoroughly examined through the evaluation of the uptake of these materials by living organisms [9,20,21,25,26]. In terms of the assessment of the presence of microplastics in environmental compartments, some authors have focused on a particular methodological step, such as the extraction of these materials from their matrices [23] or their identification [27]. Hong et al. [28] studied the quantitative and qualitative measurement of chemicals retained in plastic marine debris and microplastics and Hanvey et al. [24] considered the quality assurance/quality control of the analytical process of microplastics in sediments.

In this paper, the analytical techniques for sampling, sample handling, identification, and quantification of microplastics, and to a less extent of nanoplastics, in different environmental samples, namely, water, sediments, and biological tissues are described, with a special focus on recent works, dating from 2015 to the present. Special attention has also been given to the analytical quality control and quality assurance products associated with the validation of analytical methods and use of reference materials for the quantification of microplastics in such environmental samples. The challenges in the sampling and quantification of microplastics are also highlighted and potential routes to overcome such hurdles are also discussed.

2. Sampling and sample handling

Microplastics can be found throughout the water column, in numerous types of sediments and across various tissues and cells of multiple organisms from aquatic environment [8,10,16,29]. There are, therefore, several methodologies that may be used for their

Table 1
Sampling pieces of equipment/processes of microplastics from various matrices [9,20].

Matrix	Equipment/Process
Water	
Surface water	Collection with a trawl with a rectangular opening and a net connected with a collecting bag/neuston net/catamaran; “grab” sampling (bottles)
Mid-water level	Collection with bongo nets
Sediments	
Bottom samples	Collection with a box corer
Surface samples	Collection with iron spoons or non-plastic sampling spades
Seabed samples	Collection with core or bottom trawl
Biological tissue	Dissection (all marine animals), egestion, and regurgitation (seabirds)

collection from environmental samples and they are described in Table 1.

Fig. 1 shows the numerous types of equipment used for sampling of microplastics in seawater. The net mesh sizes vary widely, ranging from 53 μm to 3 mm, thus influencing the volume and nature of the microplastics obtained from samples [30].

Specifically, for the marine environment (sea surface, water column, sediment, and biota), sampling methods may be categorized according to the classification proposed by Hidalgo-Ruz et al. [19]:

- 1) selective sampling, where the samples (usually sediment) are collected by direct extraction, as they are identifiable to the naked eye (particles between 1 and 5 mm \emptyset);
- 2) bulk sampling, where the volume of the sample (water or sediment) is entirely collected without reduction of the sample; and,
- 3) volume-reduced sampling, which reduces the volume of bulk sample (water or sediment), only preserving the portion of interest.

During sampling and sample handling, it is also important to identify potential sources of contamination of the plastic samples, mainly those associated with airborne contamination, such as synthetic fibres stemming from clothing, gear, and atmospheric fallout [26]. For mitigating these cross-contaminating risks, the sources of contamination should be eliminated by cleaning all equipment prior to sampling, covering samples and equipment

between use, wearing polymer-free clothing or cotton coveralls and gloves. Alternatively and/or complementary, contamination sources can be suitably quantified by using protective environmental filters or procedural blanks [26]. Specifically, for marine sediment pollution, the abundance of microfibrils could also be minimized by 90% using a rigorous methodology to sample sediment, extract, and characterize microfibrils based on a forensic science approach, thus ensuring minimum possible post-sampling contamination, as well described in the work of Woodal et al. [31].

After sampling, microplastics from liquid samples are often separated by density flotation through salt addition (usually NaCl and NaI) and floatation, filtration through size fractionation or sieving through size exclusion [9]. In sediments, the most commonly used approach is density separation based on the differences in density between plastic and sediment particles and on the agitation of the sediment sample in concentrated NaCl solution [23,24]. Although NaCl is an inexpensive and eco-friendly salt, its density (1.2 g cm^{-3}) is too low to allow the flotation of all polymers and thus NaI (density of $1.6\text{--}1.8 \text{ g cm}^{-3}$) and ZnCl_2 (density of $1.5\text{--}1.7 \text{ g cm}^{-3}$) solutions could be advantageous for the separation of polymers [9,23,24].

It is important to refer that, for marine waters, the US National Oceanic and Atmospheric Administration (NOAA) has recently published a technical memorandum containing numerous suggestions and procedural advices on the laboratorial methods for the analysis of microplastics in the marine environment, with specific recommendations for quantifying synthetic particles in both waters and sediments [32]. Fig. 2 shows the suggested sequential steps for

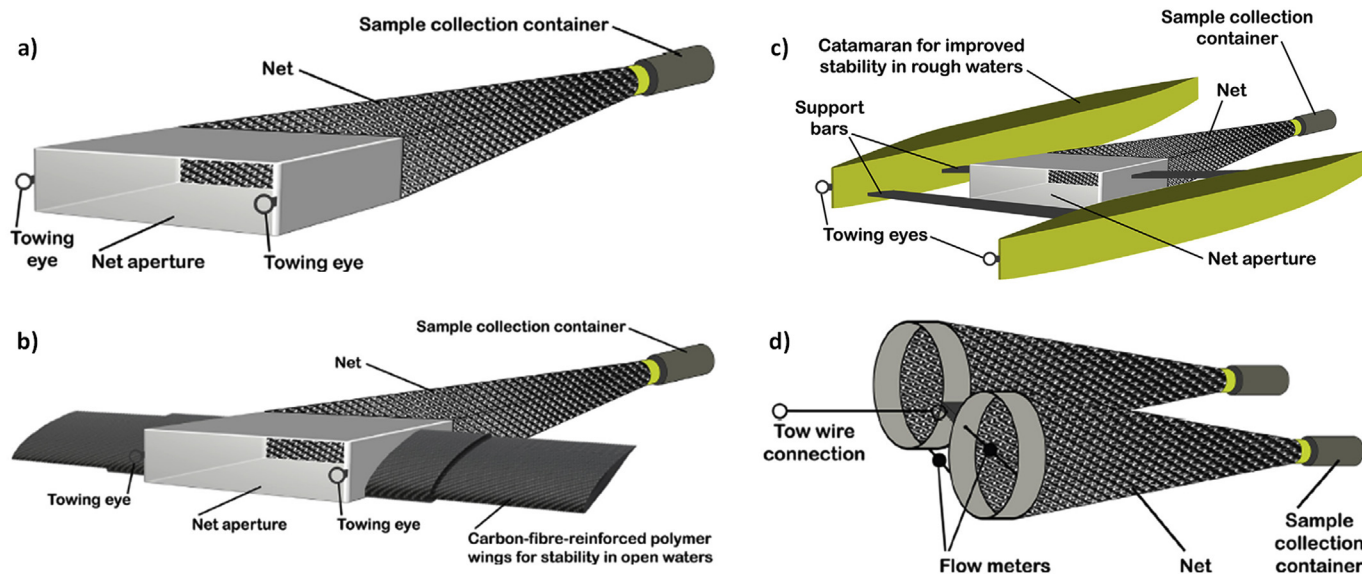


Fig. 1. Types of equipment for sampling microplastics in surface seawater: a) neuston net; b) manta trawl; and, c) catamaran, as well as in mid-water level: d) bongo nets (Reprinted from Crawford and Quinn [30], Copyright (2017), with permission from Elsevier).

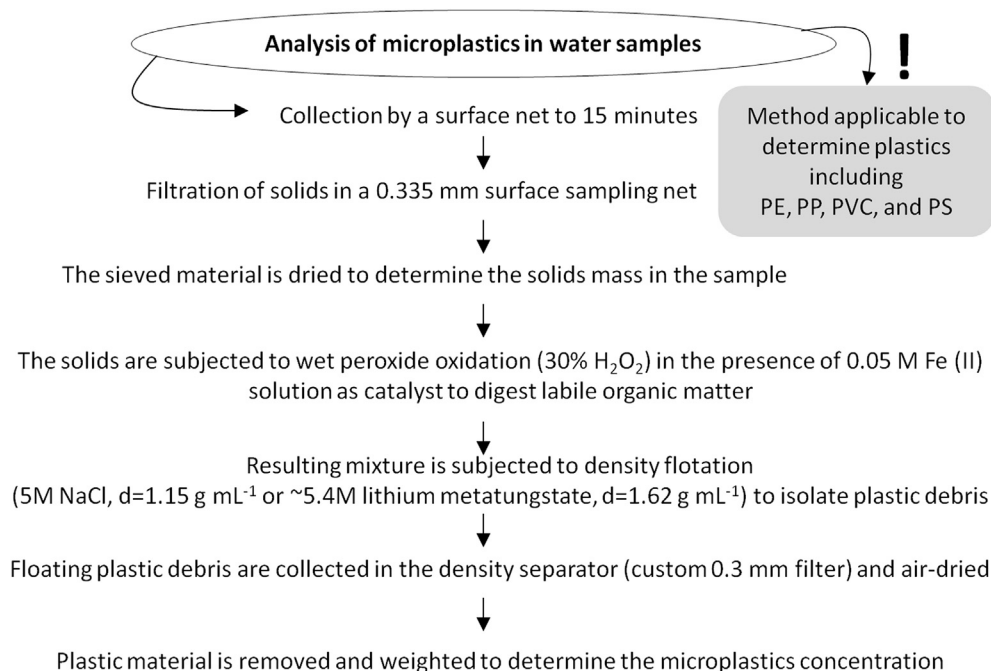


Fig. 2. Sequential steps for the analysis of microplastics in water samples, as suggested by NOAA [32]. PE: polyethylene; PP: polypropylene; PVC: polyvinyl chloride; PS: polystyrene.

the analysis of microplastics in water samples.

It should be noted, nonetheless, that this recommended methodology is applicable to determine several plastics with sizes ranging from 0.3 to 5 mm, including PE ($0.91\text{--}0.97\text{ g mL}^{-1}$), polypropylene (PP) (0.94 g mL^{-1}), polyvinyl chloride (PVC) (1.4 g mL^{-1}), and polystyrene (PS) (1.05 g mL^{-1}), which restricts the scope of the variety of microplastics identified in water samples. However, this technical memorandum may be construed as an initial step towards a highly sought for standardization in sampling and sample handling methodologies for microplastics in water and sediments. The limitation of NOAA methodology is the visual inspection of samples under a dissection microscope, which can be associated with a lack of accuracy due to the high level of false positive and/or false negatives, as discussed in the following section.

3. Identification and quantification of micro- and nanoplastics

After sample preparation, microplastics can be isolated from environmental samples, detected and quantified through several techniques. Although there is no general validated protocol for sampling and quantification of microplastics in the environment, it is possible to summarize the frequently reported techniques in the scientific literature into grouped sampling approaches, including analytical techniques already used and established for other analytes. In Table 2, some parameters for sampling of microplastics in different matrices are identified, as well as the associated analytical techniques for the detection and quantification of microplastic particles.

The most common approach for the detection of microplastics consists in the visual identification of apparent/possible plastic particles followed by confirmation through chemical composition analyses, usually combining optical and spectroscopic techniques [24,51] in order to minimize the occurrence of false positive and/or false negatives [5]. However, such approach has inherent limitations, as highlighted by Erikssen et al. [52], who described the misidentification of approximately 20% of the particles initially

identified as microplastics by visual observation, which were subsequently identified as aluminium silicate from coal ash using scanning electron microscopy (SEM). In other works, 32% of visually counted microplastic particles below $100\text{ }\mu\text{m}$ were not confirmed as microplastics after micro-Raman application [53] and up to 70% of particles was erroneously identified as microplastics after FTIR analysis [19]. Concerning nanoplastics, there are still no established protocols for their identification and quantification in complex samples. Nevertheless, some analytical methods have already been shown to be feasible for this purpose, such as electron microscopy, atomic spectrometry, and light scattering techniques [54]. Recently, a nanoparticle tracking analysis was proposed to determine the particle size distribution of nanoplastics generated during the degradation of a PS disposable coffee cup lid, obtaining an average nanoparticle size of 224 nm [55]. In another work, microbeads (PE, $\approx 2\text{ mm}$) used in consumer products such as scrubs and shampoos were analysed for the identification of nanoplastics, which were confirmed by SEM with sizes ranging from 24 ± 6 to $52 \pm 14\text{ nm}$ and by X-ray photoelectron spectroscopy and FTIR to confirm the PE identified nanoparticles [56].

The authors of a recently published study concluded that, generally, microplastics: a) are mainly collected on beaches (80%) and limitedly from sea surfaces and marine biota; b) can be found in various shapes in marine environment, that is, pellets (70%), fragments (25%), foams, fibres, spheres, and sheets; and, c) the most common polymer types identified in conducted chemical analysis are PE, PP, and PS [28]. In the following subsections, the most prevalent techniques to identify and quantify microplastics and nanoplastics are discussed.

3.1. Optical techniques

The first examination of the sample is frequently performed by visual observation, which can be achieved through simple naked-eye observation or assisted by optical microscopy [57]. In the latter, surface texture and structural information of the particles can be obtained, thus allowing for the identification of ambiguous

Table 2
Parameters used in sampling of microplastics in different environmental matrices and corresponding analytical techniques for their characterization.

Matrix	Sampling			Analytical technique	Reference
	Time elapsed (quantity collected)	Equipment	Extraction		
Water	2 h (between 2.3 and 310 million L/day) 30 min	Stacked Tyler sieves (0.355 mm and 0.125 mm stainless steel mesh)	—	Stereo microscope	[33]
		Manta trawl (rectangular opening 16 cm high by 61 cm wide, 3 m long, 333 μm mesh)	—		
	15–20 min (total of 2 L)	Neuston nets (0.52 \times 0.36 m) of 333 μm mesh	—	Py-GC-MS	[34]
	<10 L 30 min.	Telescopic sampling pole Plankton net (153 μm)	— 32 μm steel-wire sieve and saturated NaCl solution	Micro-FTIR spectroscopy Stereo microscope and ATR-FTIR	[35] [36]
Water, biological tissue	5 min (water, flow rates between 0.11 and 5.04 m s^{-1}), 30 min (biological tissue)	80 μm mesh conical net/seine nets, gillnets, conventional tackle, and minnow traps	10% NaClO, HNO ₃ :NaClO (1:10 v/v)	Stereo microscope	[37]
Water, sediment	—	1, 2, 4 and 10 mm mesh size	—	TED-GC-MS	[38]
Sediment	—	0.25 \times 0.25 m ² , 5 mm sieve	Top layer of sediment (3–6 cm)	ATR-FTIR	[39]
	—	Metal spoon	Directly from the sediment to a depth of 2 cm	Stereo microscope, SEM, Py-GC-MS	[40]
—	—	Stainless steel shovel and 20 \times 20 cm wooden frame	Surface layer (depth of 20 m)	Stereo microscope, micro-FTIR spectroscopy, SEM-EDS, ICP-MS	[41]
—	4–6 L	Sediment cores (diameter of 10 cm to a depth of 5 cm)	Munich plastic sediment separator. Centrifugation tubes with sieves (750 μm mesh)	Micro-Raman spectroscopy	[42]
—	—	Stainless steel scoop (10 cm depth) in order to fill a 1 L glass Kilner jar	Concentrated ZnCl ₂ solution (1.7–1.8 kg L^{-1})	Raman spectroscopy	[43]
—	3 kg	Ekman dredge	Saturated NaCl solution and 30% H ₂ O ₂	Stereo microscope and ATR-FTIR	[36]
Biological tissue	Whenever 3 clams of 40–45 mm were retained Until approximately 50 mussels were collected	0.5 \times 0.5 m ²	69–71% HNO ₃	Stereo microscope	[44]
		Tweezers	30% H ₂ O ₂	Stereo microscope, micro-FTIR spectroscopy, SEM-EDS	[45]
—	Overnight/4 weeks	Gillnets (mesh of 50 mm)/cages	Trituration of dried samples, 15% H ₂ O ₂	Stereo microscope, ATR-FTIR	[46]
—	—	Baka 44/60, 40/60 and GOC 73 trawl gears	Dried samples, NaOH 1 M	Inverted microscope and stereo microscope	[47]
—	—	90 mm GF/A 1.6 μm glass fibre filters and magnetic hot plate stirrer	Digestion solution (KOH 10% solution, 60 $^{\circ}\text{C}$, 24 h)	Py-GC-MS, Raman spectroscopy	[48]
—	—	Gillnet, demersal trawl	SDS, protease, chitinase and H ₂ O ₂ treatment; vacuum dried samples, petroleum ether (60/80)	Py-GC-MS	[49]
—	—	—	Dried fish and excised organs or eviscerated flesh	Micro-Raman spectroscopy, FESEM-EDX	[50]

ATR-FTIR: attenuated total reflectance-Fourier transform infrared spectroscopy; FESEM-EDX: field-emission scanning electron microscopy with energy dispersive X-ray spectroscopy; FTIR: Fourier transform infrared spectroscopy; ICP-MS: inductively coupled plasma mass spectrometry; Py-GC-MS: pyrolysis-gas chromatography-mass spectrometry; SEM: scanning electron microscopy; EDS: energy dispersive X-ray spectroscopy; TED-GC-MS: thermo-extraction and desorption coupled with GC-MS.

particles [27]. Characteristics like colour, shape, surface texture, and any other characteristic that may contribute for distinguishing microplastics from other particles, are used for their separation from the other components of the sample, as shown in Fig. 3. In these results, reported by Zhang et al. [51], sheet and fragment shaped materials corresponded to 27–57% and 22–46% of the total microplastics found, respectively. The latter were dominant in water samples, while sheet constituted the most abundant shape in sediment samples (over 70% of total microplastics). Foams were not observed [51].

Visual identification is a fast, simple, and cheap technique that may be carried out *in situ* for sampling microplastics. Nonetheless, there are several limitations, including the inherent difficulty in distinguishing microplastics from other materials, namely, coloured elements and other small particles [20,57]. Additionally, restricting the analyses of samples solely to visual identification has been shown to be prone to a high frequency in false positives and/or negatives [5,52]. For example, visual microscopic identification of “blue” fibres was confirmed by micro-FTIR as cotton-indigo and

polyacrylic, respectively, as shown in Fig. 4 [58].

Relatively to Fig. 4a, the prominent vibrations at 3348, 2899, 1428, 1370, 1318, 1160, 1061, 1035, and 889 cm^{-1} have an excellent overlap with those of the cotton reference and the peak at 1630 cm^{-1} may be due to indigo [58]. Relatively to Fig. 4b, the prominent vibrations at 3529, 2938, 2243, 1453, 1372, 1236, and 1068 cm^{-1} have an excellent overlap with those of the polyacrylonitrile reference and the peak at 1738 cm^{-1} suggests the presence of an acrylic resin [58].

Analysing wastewater, Sutton et al. [33] resorted solely to a stereo microscope to remove, enumerate, and categorize microplastics from the collected samples. Davidson and Dudas [44] identified ingested microplastics in wild and cultured Manila clams by examination of flexibility, colour, structure, and lack of biological features of the particles under a stereo microscope (10–40x magnification) and, when needed, individual microplastics fragments were examined under a compound microscope (10–100x). Recently, van der Hal et al. [59] fixed the sampled material (sea surface samples) in 4% formalin for visual inspection thus

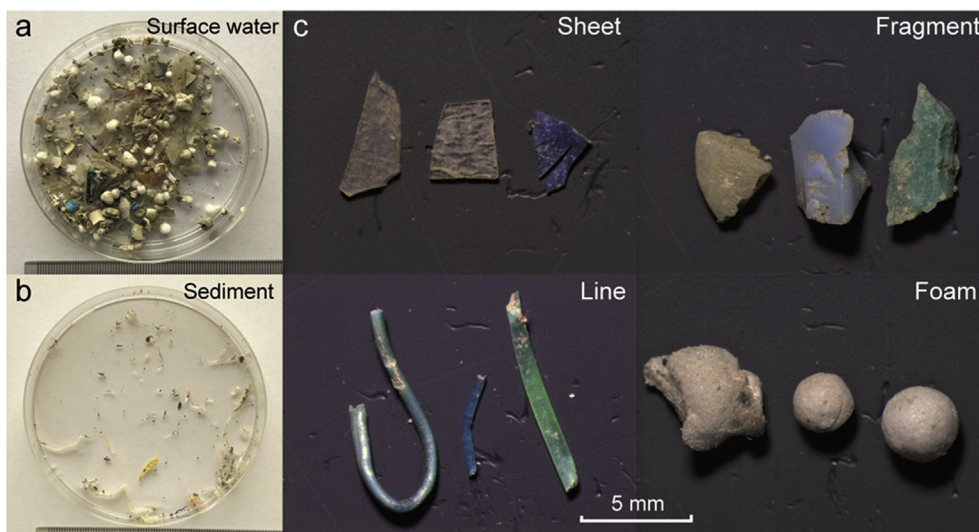


Fig. 3. a) Typical microplastics found in surface water and b) sediment samples; c) Morphology of microplastics (Reprinted with permission from Zhang et al. [51], Copyright (2017) American Chemical Society).

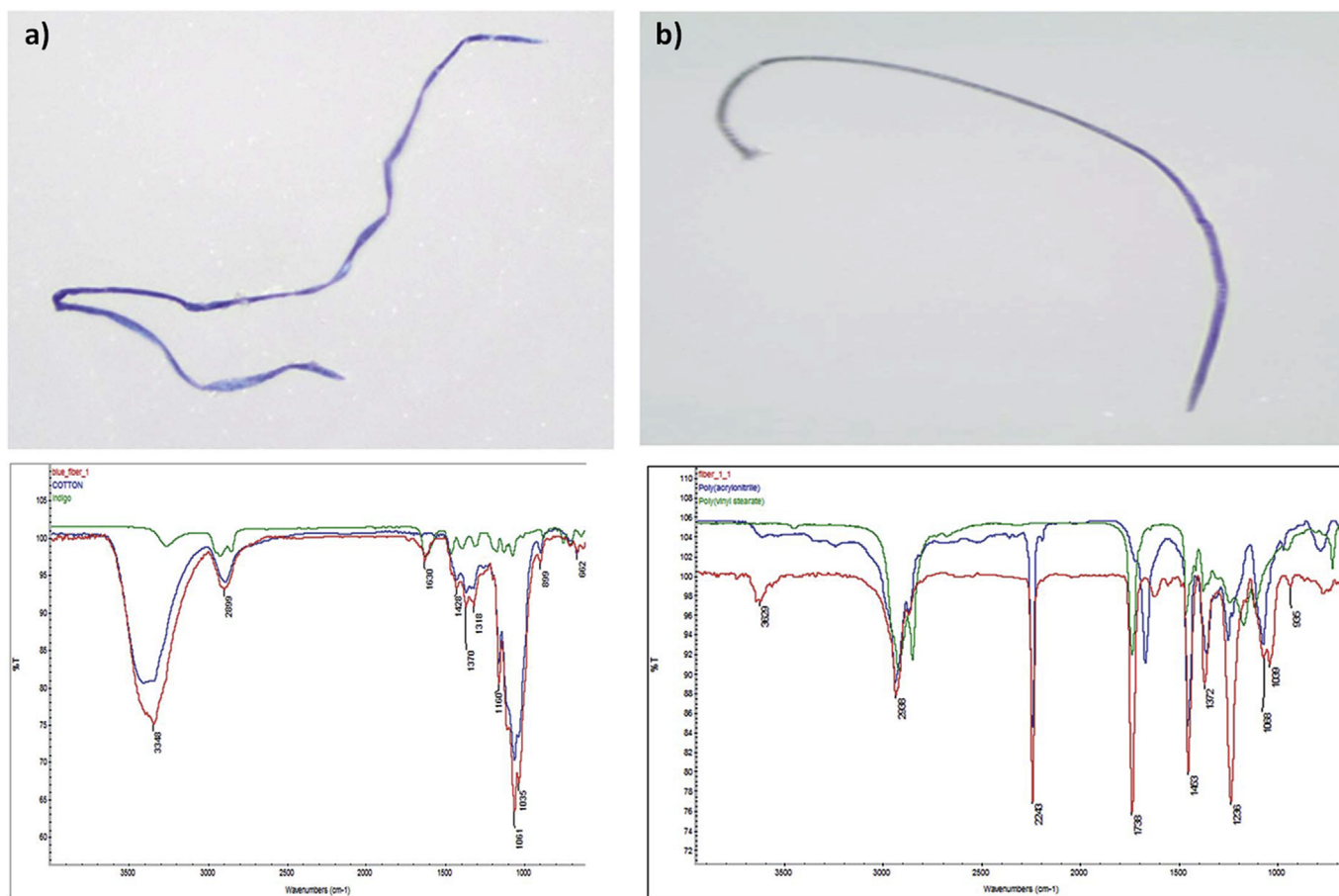


Fig. 4. a) Representative fibre particle confirmed as cotton with the corresponding FTIR spectra; and, b) Representative blue fibre confirmed as polyacrylic with corresponding FTIR spectra (Reproduced from Dyachenko et al. [58] with permission of The Royal Society of Chemistry). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

preserving the biota collected in such samples. The plastic particles were inspected and sorted in the laboratory by a magnifying glass and subsequently by a stereo microscope with adjustable camera to

photograph and examine the particles at higher magnification [59]. However, only by combining these techniques with spectroscopic analyses, such as Fourier transform infrared (FTIR) and Raman

spectroscopies, it is possible to definitively confirm the presence and identity the suspected particles and the polymer type of microplastics, mainly those <1 mm and including those <50 μm [57]. A significantly ($p < 0.05$) greater number of microplastics with fragment morphology was detected by FTIR than by microscopy, which could be due to the transparency or white colour of fragments not detectable in microscopy analysis but identified as synthetic polymers (such as PE and PP) by FTIR analysis [57]. For instance, Li et al. [45] used a stereo microscope to search for microplastics in wild and farmed mussels based on physical characteristics, which were further validated by micro-FTIR and SEM coupled to energy dispersive X-ray spectroscopy (EDS). A large variation in microplastic sizes extracted from mussels of different sites was observed; 17–79% of total microplastics have sizes less than 250 μm [45]. Other studies in beach sediments [39] and fish [46] also reported the initial visual identification of microplastics followed by confirmation through FTIR spectroscopy, which showed an abundance of PP and PE microparticles, as well as nylon and PS. When studied the abundance, size, and polymer composition of marine microplastics in the Atlantic Ocean (23 stations), microplastic concentrations from 13 to 501 particles m^{-3} were found with a majority of particles <40 μm (64%) and 48% of the total microplastics found were PE and PP [60]. In another work [53], the comparison of visual identification of microplastics by optical microscopy and Raman micro-spectroscopy was performed, as shown in Fig. 5.

Lenz et al. [53] verified that a total of 452 fibres and 827 particles were visually identified as plastic of which 75% and 64% were confirmed by Raman spectra, respectively.

While in some cases visual detection is the single technique used to identify microplastics [33,44], when spectroscopic techniques are not possible, other tests can be used. Simply prodding

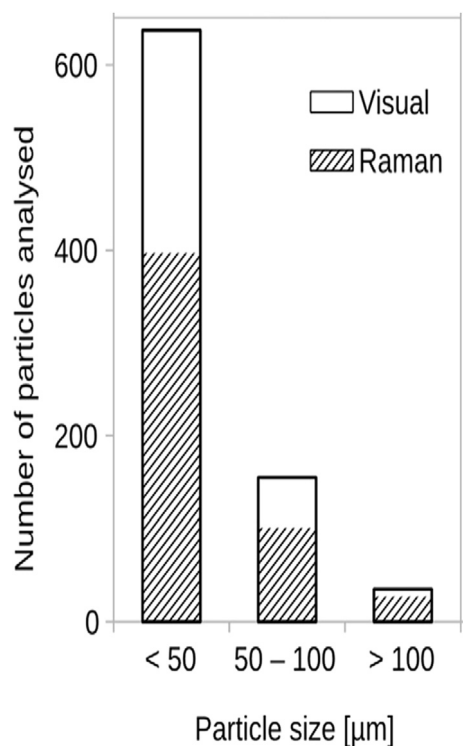


Fig. 5. Microplastic particles identified by visual microscopy (whole bars) compared to Raman spectroscopy (hatched fractions). Number of particles compared in each size class: $n_{<50} = 637$, $n_{50-100} = 155$, $n_{>100} = 35$ (Reprinted from Lenz et al. [53], Copyright (2015), with permission from Elsevier).

larger particles with a needle may suffice to identify particles as microplastics, though this is not feasible for smaller ones [27]. Alternatively, the “hot needle test” can be applied to confirm the plastic nature of suspected materials, as reported by Campbell et al. [37]. This test consists on the use of a heated needle tip to each plastic particle to ascertain whether the suspected particles melt when subject to heat. Nonetheless, this method has the drawback of not allowing for the identification of the polymer in question, although it remains a viable approach, particularly when more expensive equipments, such as spectroscopic analysers, are not available [37].

3.2. Electron microscopy

The use of SEM for identification of microplastics provides extremely clear and high-magnification images of plastic particles, facilitating the discrimination of microplastics from organic particles [61] but it could also have some limitations. When coupled to EDS (SEM-EDS), the elemental composition of plastic particles is obtained, thus discerning carbon-dominant plastics from inorganic particles [27,62]. However, the SEM-EDS is expensive with laborious sample preparation steps, as well as time-consuming for an adequate examination of all samples, hence limiting the number of particles that may be analysed in a given timeframe. Additionally, the colours of the particles cannot be used as identifiers in SEM analyses, and, therefore, this technique is only recommended for specific plastic particles [27]. These constraints may result in inaccuracies on the determination of the microplastics' abundance in a certain environment [62].

As noted by Dehghani et al. [62], SEM can be suitable for accurate detection of microplastic particles of different sizes and shapes (e.g., fibre, spherule, hexagonal, irregular polyhedron) and trace amounts of Al, Na, Ca, Mg, and Si can be detected by EDS, as depicted in Fig. 6, in which the chemical composition signature determined clearly demonstrates the presence of additives of plastic polymers and/or adsorbed debris on the surface of the analysed microplastics [62]. The limitation of such EDS spectra is the no differentiation of elemental signatures between additives of plastic polymers and adsorbed debris on microplastic surface or both of them.

In another work, Li et al. [45] searched for microplastics in wild and farmed mussels, resorting to visual identification of these particles based on their physical characteristics under a stereo microscope. Smooth or irregular surface topographies were observed, as shown in Fig. 7A. Approximately 8.5% of all suspected plastic particles, selected from visually identified particles, were identified by micro-FTIR as diethanolamine and selenious acid, and then some selected plastic particles were identified by SEM-EDS as diatoms (uniform transparent spheres, Fig. 7C) and CaCO_3 (dark blue particles, Fig. 7D), while 7.0% remain unidentified.

Recently, SEM-EDS in conjunction with optical microscopy, was used for the analysis of microplastics retrieved from ocean trawls and fish guts for the determination of size, morphology, and chemical composition [63]. The optical images showed that plastics particles ranged from 70 to 600 μm , and from SEM-EDS, the results indicated that chlorinated plastics, such as PVC, could easily be identified due to their unique elemental signatures (including chlorine), as well as mineral species that were falsely identified as plastics through optical microscopy [63]. On the other hand, particle morphology determined by optical microscopy and SEM suggested that the fish-ingested particles contained both degradation fragments from larger plastic pieces as well as manufactured microplastics [63].

When concurrently used with spectroscopic techniques, as Raman and FTIR spectroscopies, SEM-EDS provide more complete

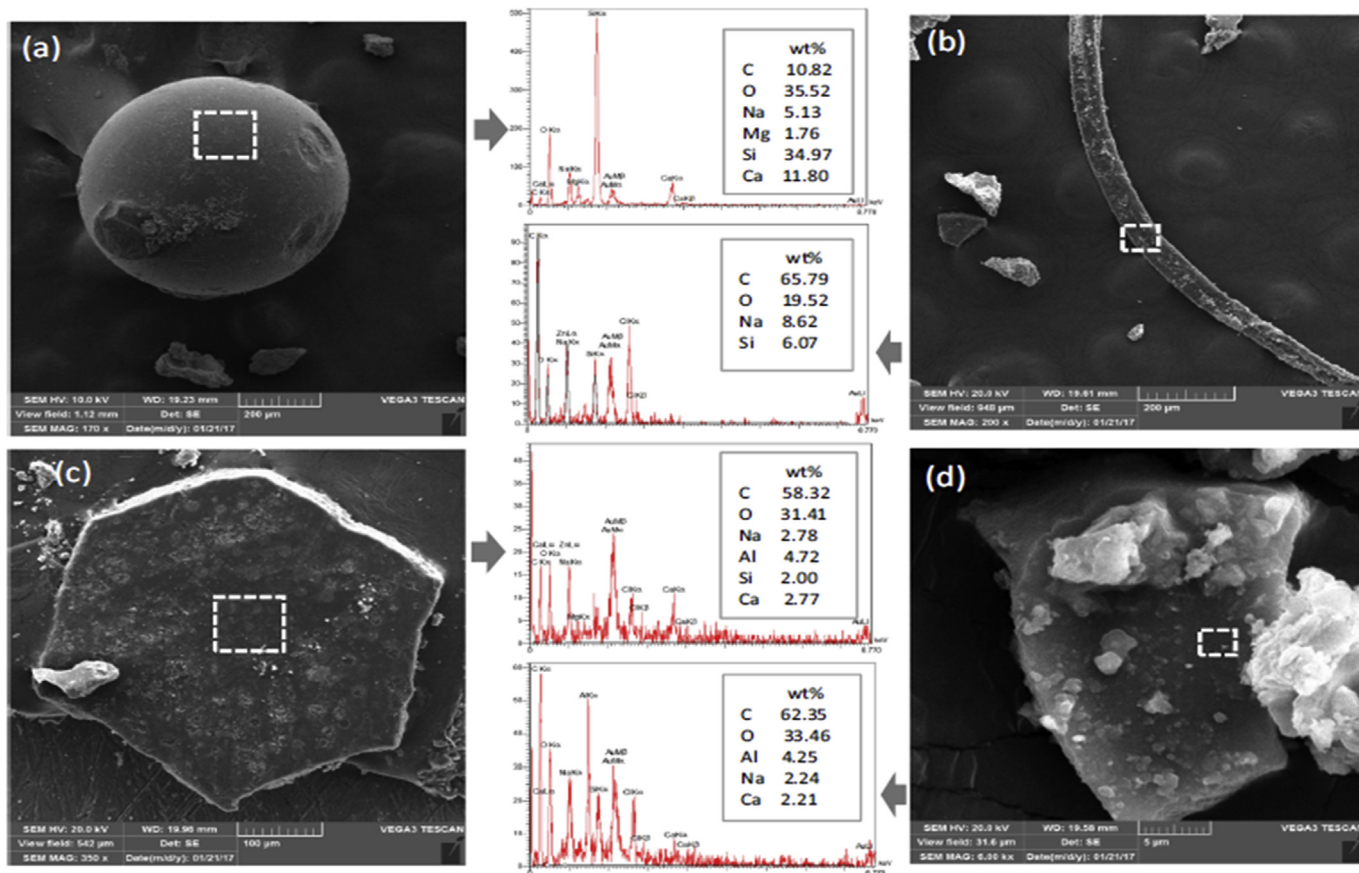


Fig. 6. SEM images and EDX analyses: a) smooth silicate glass spherule with a few pits on its surface and 600 µm diameter; b) microplastic fibre with 2 mm length; c) hexagonal fragment of 500 µm diameter; and, d) microplastic fragment with longest diameter of 30 µm (Reprinted from Dehghani et al. [62], with permission from Springer).

information regarding the studied microplastics. Napper and Thompson [64] studied the release of synthetic microscopic fibres, such as nylon, from different textiles washed under different experimental conditions. Each textile material type was confirmed by micro-FTIR and the fibres recovered from the waste effluent were analysed by SEM for morphological analysis. These authors found that a higher number of microscopic fibres (<5 mm) of acrylic nature was released per wash with low quantity for PE-cotton-based fibres. In a study by Li et al. [45], transparent spheres were identified as aluminium silicate by micro-FTIR; however, SEM analysis revealed that they were, in fact, diatoms. These findings emphasize the need to apply different and complementary methods for an accurate classification of suspected microplastics [45]. Specifically, regarding SEM, this technique can also be used to evidence modifications on the morphology of the microplastics, like cracks and pits, as demonstrated in the study of ter Halle et al. [65] on the degradation of these particles in the environment (Fig. 8). The surface cracking can also lead to embrittlement (Fig. 8D).

3.3. Infrared and Raman spectroscopies

Infrared and Raman spectroscopies are the two most commonly used techniques for the characterization of microplastics and are, in fact, recommended by the Marine Strategy Framework Directive Technical Subgroup on Marine Litter through the Guidance on Monitoring of Marine Litter in European Seas [66]. These spectroscopic techniques required low sample amounts with minimal sample preparation and they are also indicated for the

discrimination of plastics and natural particles for marine and soil samples. Concerning their spatial resolution, Raman spectroscopy is able to assess microplastic samples higher than 1 µm while infrared spectroscopy only could identify microparticles higher than 10–20 µm [67].

FTIR spectroscopy is frequently used for the qualitative analysis of microplastics (>10 µm), as the polymer type can be quickly and directly identified when their spectra is compared with those of known plastics [20]. With FTIR spectroscopy, the functional groups present in microplastics polymers can be identified. Focal plane array (FPA)-reflectance FTIR micro-spectroscopy (FPA-FTIR) was used to identify different microplastics (PE, PP, nylon-6, PVC, and PS) with 150–250 µm from effluents of wastewater treatment facilities using a pre-treatment step of 30% H₂O₂ to remove biogenic material [35]. With FPA-FTIR, a considerable reduction in analysis time was observed, since samples were imaged in less than 9 h when circular filters of 47 mm Ø were used [35]. Recently, attenuated total reflectance (ATR)-FTIR was used to identify polymer type of suspected microplastic particles (total of 240, selected by a stereo microscope) from surface waters and sediments, concluding about the presence of PP (50.9%), low-density PE (18.2%), high-density PE (26.4%), a blend of PP and ethylene propylene (3.6%), as well as styrene acrylonitrile (0.9%) [36]. In another work, the study of microplastics in atmospheric fallout samples was reported, verifying the presence of microplastics with different shapes (fibres, pellets, fragments, and films) by visual inspection and then by a digital microscope, with the identification by micro-FTIR of polymers PE (14%), PP (9%), PS (4%), and cellulose (73%) in randomly selected samples, as shown in Fig. 9 [68].

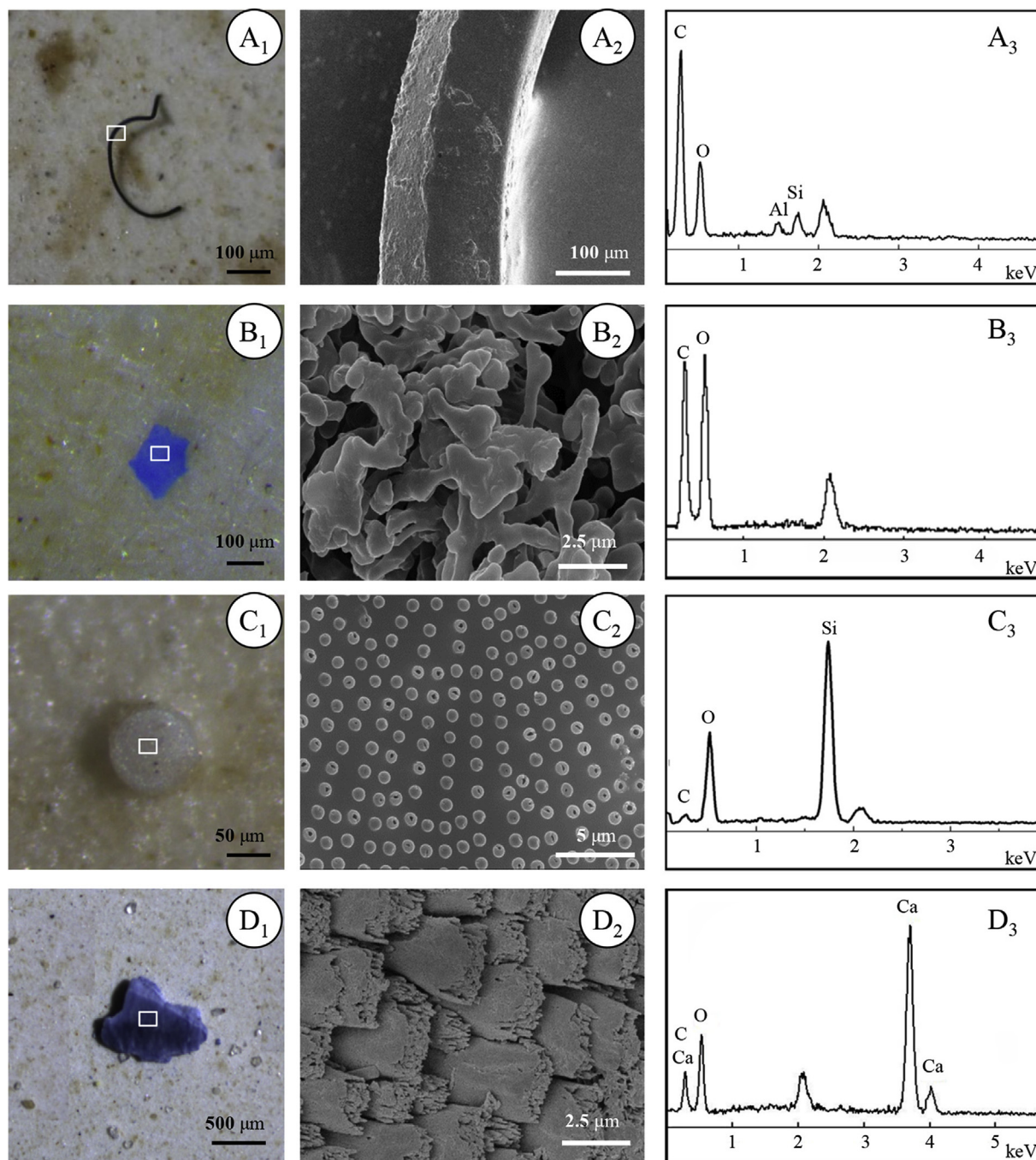


Fig. 7. Identification of microplastics with SEM-EDS. The left photos were taken under microscopes, the middle photographs were taken under SEM for the white box areas of the left ones, and the right photographs were the spectra of EDS for particles in the middle photographs. Some particles were identified as microplastics (A, B), and the others were identified as non-plastics such as diatoms (C) and CaCO_3 (D) (Reprinted from Li et al. [45], Copyright (2016), with permission from Elsevier).

Fig. 9 shows FTIR spectra of identified polymers in comparison to standard spectra, with the presence of a new absorption peak at about 1715 cm^{-1} in PE and PP spectra, and at 3300 cm^{-1} in PE spectrum, which are attributed to carbonyl and hydroxyl groups, respectively [68]. That could be due to the exposure of polymers to sunlight, having high oxygen availability and thus chemically weathered [68].

Raman spectroscopy is a non-destructive technique frequently applied to the characterization of microplastics, even those less than $1\text{ }\mu\text{m}$ [67]. One of the main limitations in Raman spectra analysis is the sample degradation by UV exposure, as noted, for

example, for PVC, which spectrum after photo-degradation shows a simultaneous intensity reduction of peaks at 693 and 637 cm^{-1} , corresponding to the characteristic C-Cl bonds of the polymer [53]. Thus, the analysis of spectra of degraded polymers at different stages should be included in reference databases in order to obtain a more correct identification of polymers in microplastics [67]. In addition, poor Raman signal quality is attributed to fluorescence and then measurement conditions (for example, integration time and number of scans) should be optimized, sometimes leading to longer analysis procedures [53]. Compared to FTIR, Raman spectroscopy provides a better response of non-polar, symmetric bonds,

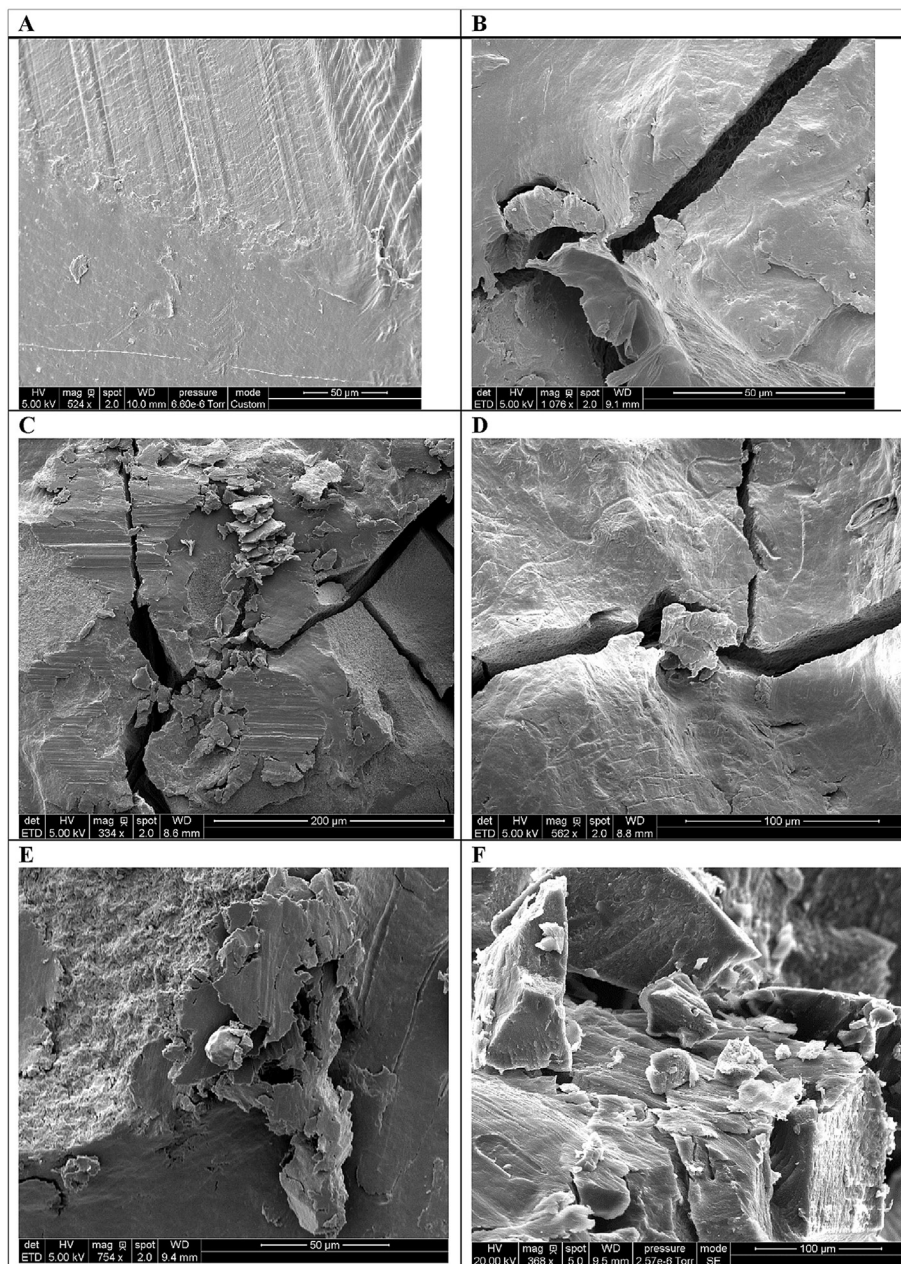


Fig. 8. SEM images of A) a virgin pellet (high-density PE), as there were no cracks visible on the virgin pellets; and, B-F) SEM images of 5 microplastics in PE previously washed with sodium hydroxide solution (1 M) (Reprinted from ter Halle et al. [65], with permission from Elsevier).

while FTIR allows for a clearer identification of polar groups [53], rendering these techniques complementary. In addition, Raman provides wider spectral coverage, better resolution, and lower water interference when compared to FTIR [67]. Micro-Raman analysis of microplastics retrieved from commercial dried fish has recently been allowed to examine the composition of approximately 87% of the isolated particles, where 59% of these particles were identified as microplastics (i.e., particles confirmed as plastic polymer or plastic polymer plus pigment), while the remainder included pigments, cellulose, and actinolite [50]. The most abundant plastic polymers were PP (47%), PE (42%), PS (6%), polyethylene terephthalate (PET) (3%), and nylon-6 (3%) and Fig. 10 shows the Raman spectrum of a PE particle containing phthalocyanine, showing the similarity between peaks of the PE particle + phthalocyanine and reference materials [50].

Additionally, the same authors hypothesized that the unidentified particles could be microplastics whose spectra did not match pure materials due to degradation of the constituent polymers, underlying the need to develop specific dedicated libraries of spectra of such materials subject to degradation [50].

In a recent study, researchers verified that for a marine sample containing particles under 400 μm, FTIR imaging lead to significant underestimation (about 35%) of microplastics compared to Raman imaging, especially with sizes lower than 20 μm, when the number, size and type of detectable microplastics, as well as spectra quality, measurement time and handling were compared [41]. Fig. 11 shows a comparison of the spectra obtained by FTIR and Raman spectroscopies for the two particles identified as PE (Fig. 11c1) and PP (Fig. 11c2). The two particles were selected from optical images taken by the Raman microscope (left, a and b) and FTIR microscope

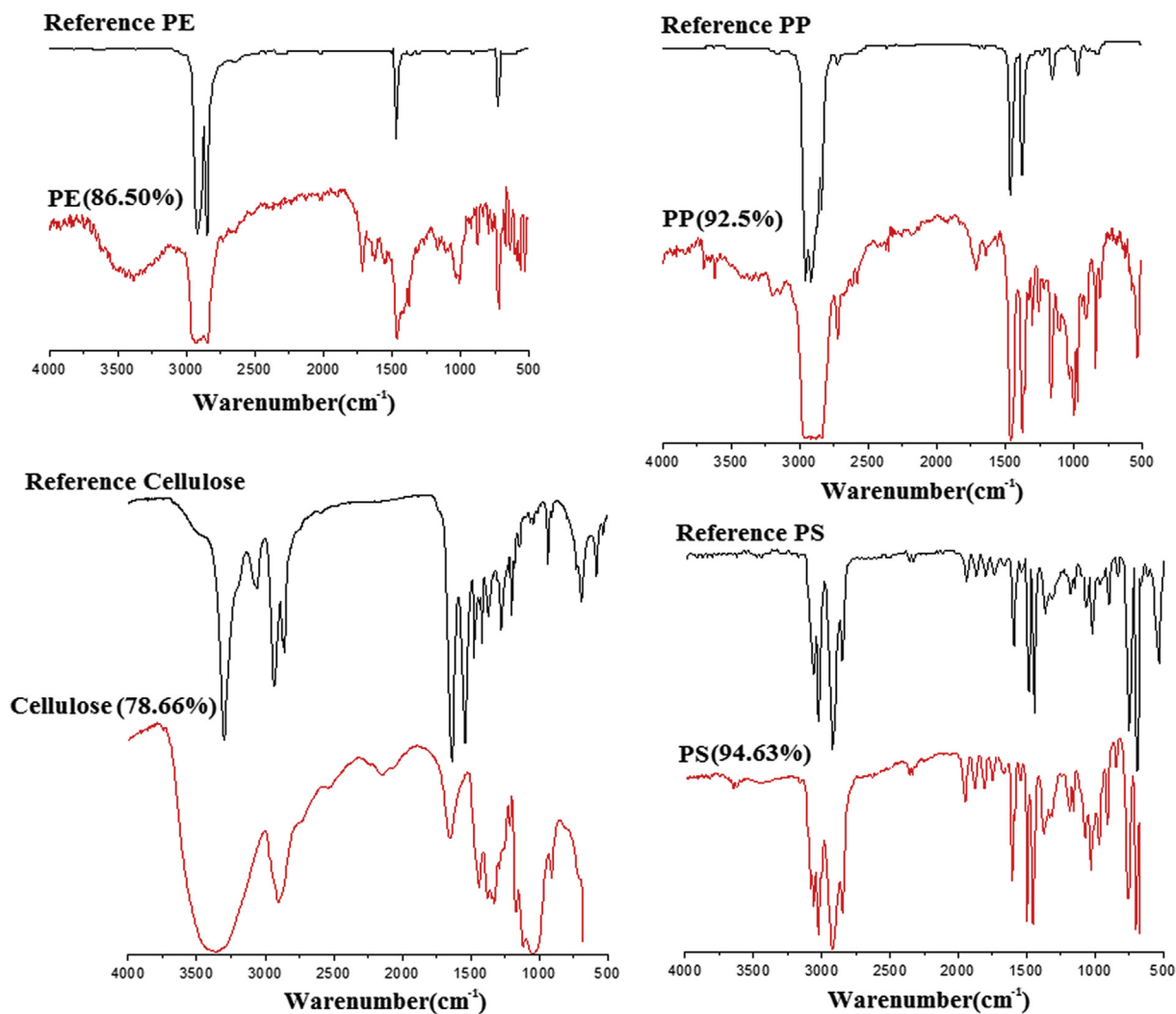


Fig. 9. Spectra of identified polymers and the match degrees with the standard spectra (Reprinted by permission from Springer Nature, Cai et al. [68] [Copyright] (2017)).

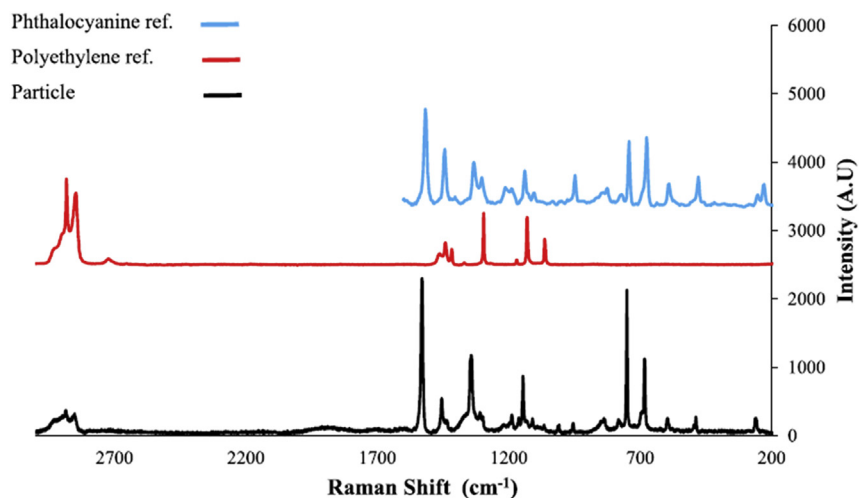


Fig. 10. Raman spectrum of a particle identified as polyethylene + phthalocyanine and spectra of the reference materials (Reprinted by permission from Springer Nature, Karami et al. [50], [Copyright] (2017)).

(right, a and b).

Further automation of FTIR and Raman analysis is highly

required in order to provide even faster and complete analysis of microplastic samples. The automation in terms of identification and

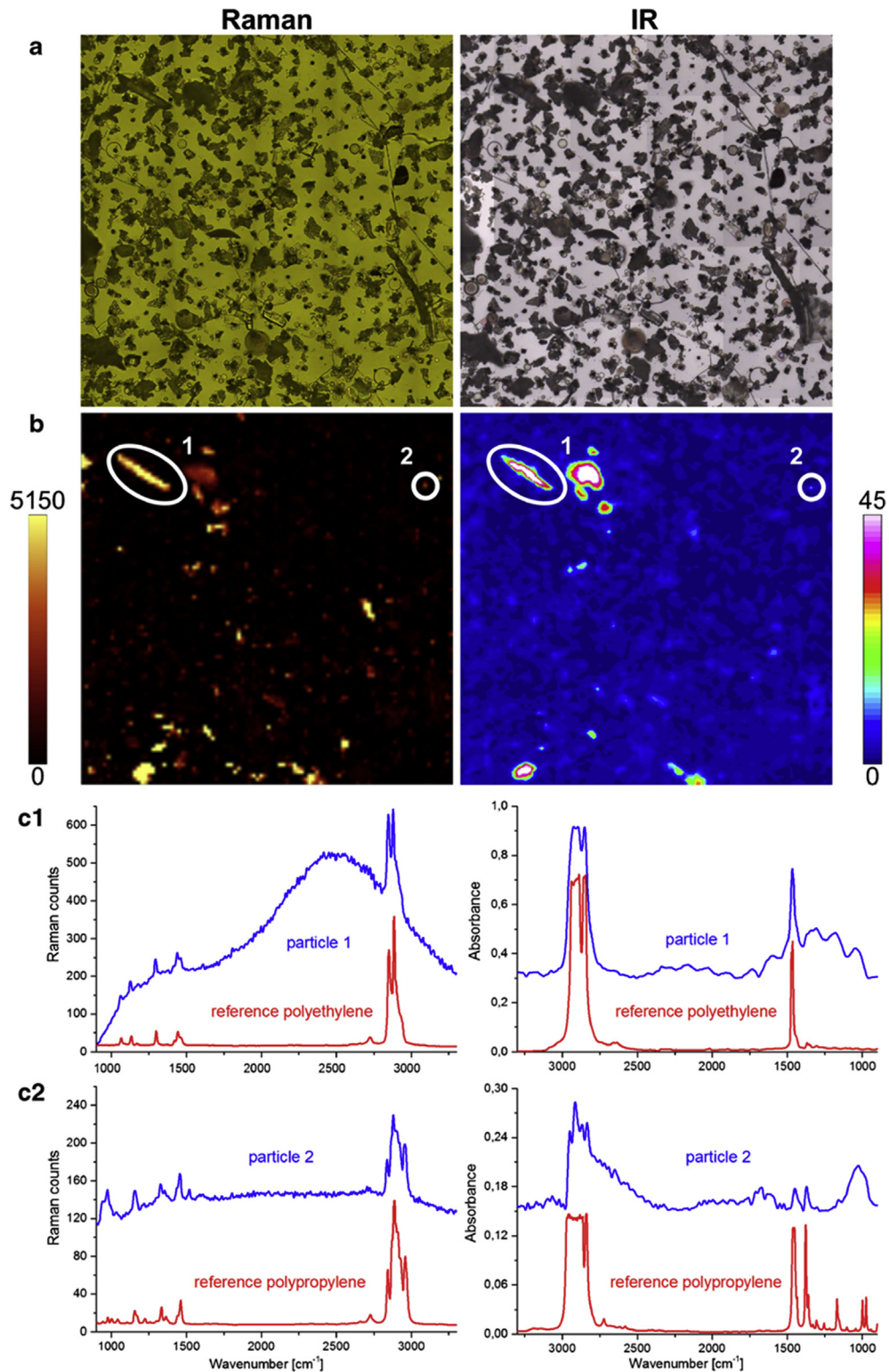


Fig. 11. a) Optical microscopic image taken by the Raman microscope (left) and by the FTIR microscope (right) of the same selected area sized $1000 \times 1000 \mu\text{m}$ of a marine microplastics sample $<400 \mu\text{m}$; b) Raman image (left) and IR image (right) of the selected sample area by choosing a spectral range of $2780\text{--}2980 \text{cm}^{-1}$. The colour scale bar represents the intensity of the integrated spectral band (arbitrary units); and, c) Complete Raman spectra (left) and IR transmission spectra (right) of particle 1 and 2 in comparison with a reference of PE or PP, respectively (Reprinted from K ppler et al. [41], with permission from Springer). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

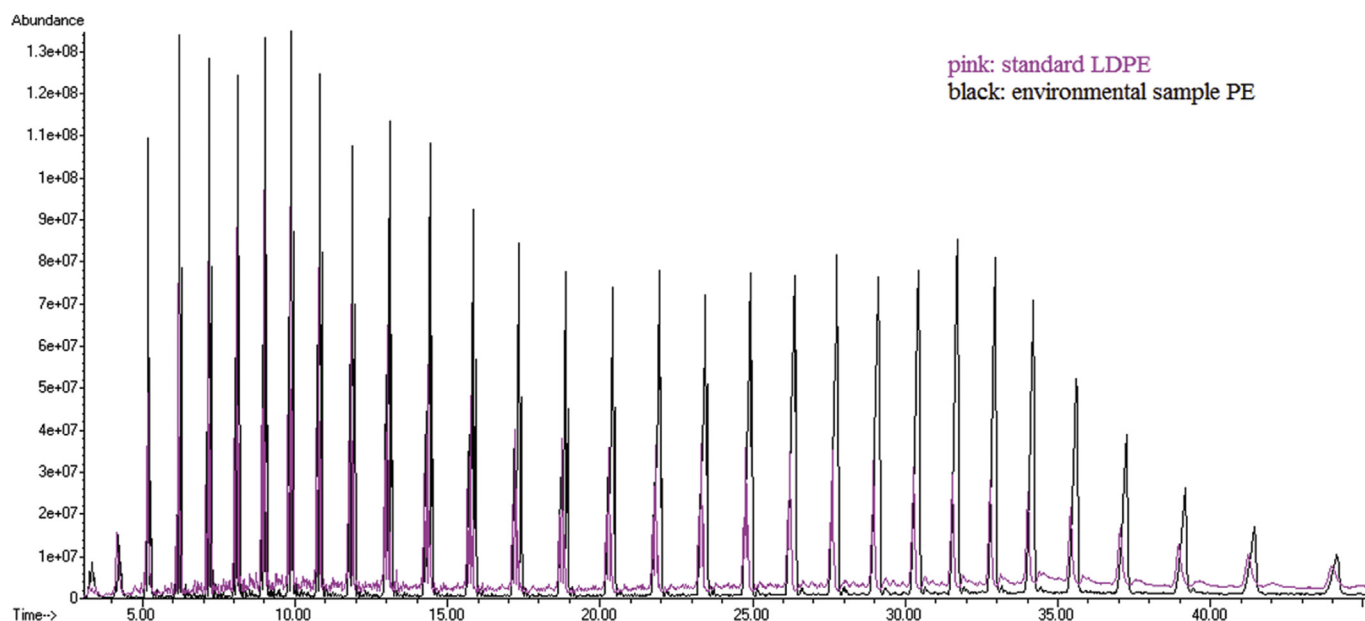


Fig. 12. Pyrogram of PE (black) found in the environmental microplastic overlaid by the pyrogram of a PE standard (pink) (© 2013, Fries et al. [40]. Originally published in "Identification of polymer types and additives in marine microplastic particles using pyrolysis-GC/MS and scanning electron microscopy" under Creative Commons 3.0 license). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

measurements of particles was also reported in some works. For example, Löder et al. [69] proposed a FPA-micro FTIR system where a FTIR microscope was equipped with an automated xyz-stage, which facilitates the placement of a gold coated mirror for reflectance measurements or the insertion of round CaF₂ sample filter plates for transmittance measurements, leading to the fast analysis of whole sample filters for microplastics imaging [69]. A semi-automated Raman micro-spectroscopy method was also proposed for morphological and chemical characterization of microplastics (collected at sea surface) being time effective (<3 h), reproducible, and requiring minimum operator intervention [70]. In this work, the semi-automation allows the thorough analysis of large quantities of environmental samples for microplastic characterization; 71% of the identified particles was microplastics and found as PS (with 50% in 2–5 mm range), PE (with 40% in 1–2 mm range), and PP (with 2% in 0.335–1 mm range). Recently, an automated approach for microplastic analysis using FPA-FTIR was put forward to reduce the time for data analysis and increasing the data quality [71]. When compared to manual analysis, seven-fold increase in number of polymer particles was found with the automated analysis; an underestimation of PP and PVC was also recorded by manual analysis since mainly small particles were missed [71].

3.4. Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS)

Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS) is a destructive technique that has also been described for the characterization of microplastics in terms of identification of polymer type, by analysing their thermal degradation products [20]. This technique eliminates the need of pre-treatment of sample since it directly examines the solid polymer sample; in addition, only a small quantity of sample is analysed in one measurement (5–200 µg) [72]. Fig. 12 shows a typical Py-GC-MS chromatogram (pyrogram) of PE found in the environmental microplastic overlaid by that of a standard PE [40].

Py-GC-MS can also be used to simultaneously identify polymer types and associated organic plastic additives [40]. From marine sediment samples, particles of PE, PP, PS, PA, chlorinated PE, and

chlorosulfonated PE were identified together with polymers containing diethylhexyl phthalate, dibutyl phthalate, diethyl phthalate, diisobutyl phthalate, dimethyl phthalate, benzaldehyde, and 2,4-di-*tert*-butylphenol [40].

Recently, McCormick et al. [34] examined microplastics collected from wastewater treatment effluent samples, which were retrieved and counted under a stereo microscope and the respective polymer type was assessed by Py-GC-MS. In this case, Py-GC-MS served only as a complementary technique to characterize the suspected microplastics sorted by visual techniques. Pellets, fibres, and fragments were the most common microplastic types found and the samples consisted on PE, low-density PP, PS, and ethylene/propylene rubber (EPDM). When testing the impact of digestion protocols on the integrity of known microplastics, Dehaut et al. [48] verified that Py-GC-MS was reliable for the identification of the polymer type, although it was not possible to establish differences of polymer subtypes (e.g., low-density PE vs. high-density PE).

The use of Py-GC-MS by itself does not allow to determine the number, type or morphology of microplastics, as it only provides the mass of polymer per sample [24], thus requiring pre-selection of microplastics by optical techniques [34,40]. This leads to the use of Py-GC-MS solely as a strategy for the verification of the composition of suspected microplastics [34,48]. Moreover, while in some cases the small quantities required may be an advantage, this limited quantity may compromise the representativeness of the sample composition when complex environmental samples are analysed, as it may not be homogenous on a small scale [38,73]. In this context, variants of this technique have been used to develop new methods, such as thermo-extraction and desorption coupled with GC-MS (TED-GC-MS) [38,73]. TED-GC-MS combines thermogravimetric analysis (TGA) and thermal desorption gas chromatography mass spectrometry (TD-GC-MS), allowing fast analysis and quantification of high quantities of microplastics of 5 common polymers (PE, PP, PS, polyamide 6, and PET) in environmental samples, assuring its composition representativeness, and without pre-selection of microplastics in the samples [38,73]. Fig. 13 shows an example of the results obtained from a TED-GC-MS analysis, where the highlighted fragment ion ($m/z = 55$) was chosen as it is

known to be present in all aliphatic compounds with high response [73].

Usually, in Py-GC-MS measurements, the pyrograms show a number of groups with three to five peaks. As shown in Fig. 13, for PE, triple peaks were observed and the second peak shows the highest response in each group. The first peaks of the groups were identified as dialkenes with two double bonds at the ends; the second peak was identified as monoalkene with one double bond mainly at one end, and the third was considered as a saturated alkane [73]. Even though the information about size and morphology of the plastics is lost, this method provides fast measurements, which can be useful for routine analyses [38]. The same authors have recently applied this method to samples obtained from rivers and from a biogas plant; mainly PP, PE, and PS were identified for the samples from the biogas plant and PE and PS from the rivers [38]. Similarly, Fischer and Scholz-Böttcher [49] developed a method based on Curie-Point Py-GC-MS and thermochemolysis, which allows for simultaneous identification and quantification of microplastics of 8 common polymers (PE, PP, PS, PET, PVC, poly(methyl methacrylate), polycarbonate, and polyamide 6) in environmental samples, again without the need for mechanical or visual pre-selection. This method was tested in fish samples spiked with known polymers, whose recovery proved to

be successful [49].

3.5. Development of analytical methods fit for purpose

New and improved techniques were recently developed to identify and characterize microplastics with different sample treatment when compared to traditional techniques. For example, Fuller and Gautam [74] developed a method based on pressurized fluid extraction to better quantify microplastics in complex environmental samples, such as municipal waste material and soil samples. This method consisted in two ensuing extractions; in the initial extraction, methanol at 100 °C was used to remove semi-volatile organic compounds, as fats and oils, and, in the second extraction (pressurized fluid extraction), dichloromethane was used to recover the microplastic fraction. The collected dichloromethane extracts were evaporated to dryness and they were measured gravimetrically. This method was initially developed by recovering 101–111% of spiked plastic (high density PE, PS, PVC, PET, and PP obtained from various plastic packaging materials and containers) onto glass beads (4 mm), where approximately 40 g of glass beads and 10–20 mg of plastic material was used, and then applied to a composted municipal waste sample (used for method validation) with spiked recoveries ranging from 84 to 94% [74]. The

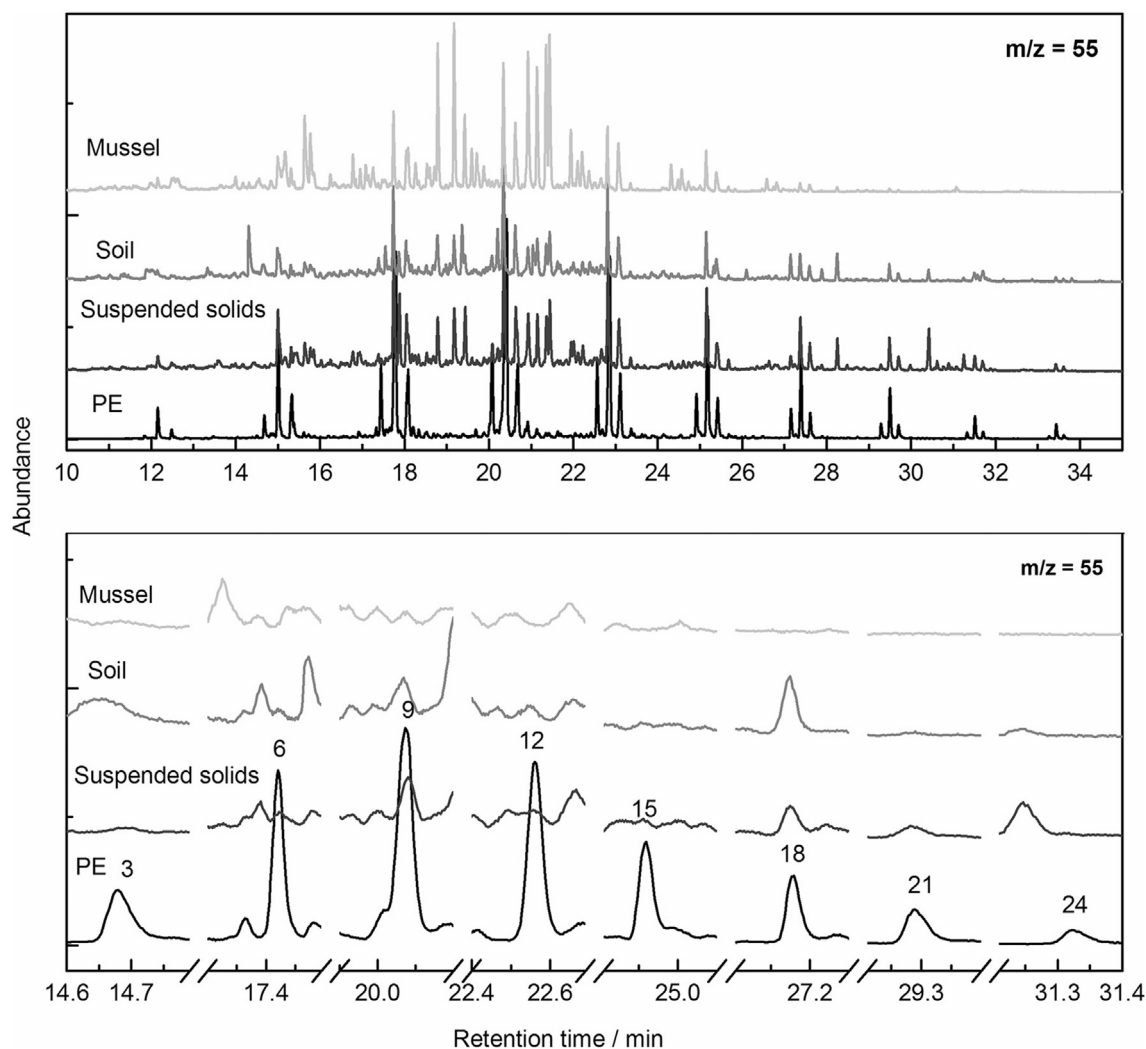


Fig. 13. Top: Overlap of the ion chromatograms of the $m/z=55$ of PE and the environmental samples; Bottom: detailed view of the dialkenes of PE in comparison to the environmental samples (peak 3: 1,11-dodecadiene; peak 6: 1,12-tridecadiene; peak 9: 1,13-tetradecadiene; peak 12: 1,14-pentadecadiene; peak 15: 1,15-hexadecadiene; peak 18: 1,16-heptadecadiene; peak 21: 1,17-octadecadiene; and, peak 24: 1,18-nonadecadiene) (Reprinted from Dümichen et al. [73], Copyright (2015), with permission from Elsevier).

residues obtained from the municipal waste sample were typically found to be amorphous and homogeneous materials that can break up into flakes [74]. The validated method was then used in a case study where soil samples from around an industrial area were tested. The results obtained from FTIR analysis showed that up to 6.7% of the samples are microplastic and PVC as the major component (>80%) in most of the soil samples. The advantages of this method are its simplicity, cost, speed, and uniformity in reporting concentration results, as well as the possibility of automation of the extraction component, minimizing operator skill requirements and associated errors [74]. Recently, Karlsson et al. [75] reported an improved sediment extraction method based on density separation and an adapted enzymatic digestion protocol (using proteinase K and CaCl_2). In order to improve recovery rates, one drop of olive oil was added to the salt solution in the glass beaker prior to stirring, which allowed for the plastic particles to gather in the oil and collected on the filter, rather than sticking to the glass walls [75]. With this optimized method, an increase of 18% in recovery was found for the spiked sediment. Also for mussels analysis, 97% of recovery of spiked plastic particles (low-density PE, high-density PE, PP, and expanded PS) was observed using the same enzymatic digestion protocol with no observed degradation effects on the plastics in subsequent Raman analysis.

Coppock et al. [76] described a new, small-scale and portable unit for extracting microplastics in a single step from marine sediments, using the principle of density separation. When tested by spiking sediments samples with known quantities of microplastics (PE, PVC, and nylon), a mean efficiency of 96% was obtained and the method was considered as simple and cheap, with the added benefit of portability, a highly desirable attribute in field research, namely, aboard research vessels [76].

In another recent study, a rapid screening approach for detection and quantification of microplastics in marine sediment samples based on selective fluorescent tagging using the lipophilic fluorescent dye Nile Red, followed by density-based extraction and filtration, was detailed [77]. In this procedure, the Nile Red adsorbs onto plastic surfaces and renders them fluorescent when irradiated with blue light and the image analysis allows for the identification and counting of fluorescent plastic particles (>100 μm) [77]. In addition, the categorization of plastic nature can be performed based on surface polarity characteristics of the identified particles, due to the solvatochromic nature of Nile Red, i.e., its ability to change colour due to a change in solvent polarity. Thus, it is possible to distinct polar polymers (nylon and PET) and hydrophobic polymers (PE, PP, and PS), but further validation of “colour typing” is required in order to optimize the method for identification of polymers of the same type but with different densities.

4. Identification and quantification of chemicals accumulated by microplastics

The chemicals accumulated by microplastics in different environmental matrices also need to be identified and quantified, as this association may ultimately enhance the toxicity of the particles, which hence become both the source and the sink of pollutants. This is of utmost importance for future research since POPs such as PCBs and PAHs have been shown to accumulate on microplastics [13,15]. These compounds, in fact, can accumulate to concentrations up to 6 orders of magnitude (apparent adsorption coefficient: 10^5 – 10^6) greater than those in seawater, as demonstrated by Mato et al. [78], for some organic compounds, namely, PCBs, dichlorodiphenyldichloroethylene (DDE), and nonylphenols (NPs). The sorption of PCB 77, an analytical standard, by PP microplastics in simulated seawater was recently described [79]. The sorption experiments were performed

through several times, ranging from 1 to 24 h, and with several variations in particle size, temperature and solution environment. The compound was extracted by partitioning and its concentration was assessed by gas chromatography equipped with ECD. The results showed that equilibrium sorption time was of about 8 h and sorption capacity increased with decreasing particle size and temperature.

Recently, Liu et al. [13] reported the distribution coefficients for sorption of PAH onto PS nanoplastics (70 nm) in a freshwater simulated system. Polyoxymethylene (POM) passive samplers were used to determine PAHs aqueous phase concentrations, eliminating the need to separate nano-PS from the water. PAHs were extracted from POM sheets with methanol using accelerated solvent extraction and were further analysed by high-performance liquid chromatography (HPLC). The high and nonlinear sorption was explained due to π - π interactions between the planar PAHs and the surface of the aromatic polymer PS, which was higher than for micro-PS. In another work, the bioavailability of particle-associated hydrophobic organic contaminants (PCB from PP microplastic) was investigated by comparing three different biological and physicochemical measurements, that is, equilibrium solid-water distribution coefficients, *in vitro* gut fluid solubilisation, and *in vivo* bioaccumulation using sediment invertebrate worms as test systems [14]. It was observed that biouptake in worms was lower than 76% when PCB were associated with PP compared to natural sediments and the presence of microplastics in sediments has an overall impact of reducing bioavailability [14].

Also, metals (i.e., Ag, Cd, Co, Cr, Cu, Hg, Ni, Pb, and Zn) can be accumulated in microplastics in freshwater (pH ~6.5) and the formation of complexes of modified organic surface from microplastics with metals ions and hydrous oxides is suggested as the basis of this accumulation [80]. A more specific study stated that metals accumulated in five plastic types (PET, high-density PE, PVC, low-density PE, and PP) in different patterns, depending on space and time [81]. To extract the metals from microplastics, the samples were digested with 20% of Aqua Regia ($\text{HCl}:\text{HNO}_3$ at 3:1) and subsequently analysed for Al, Cr, Mn, Ir, Co, Ni, Zn, Cd, and Pb by inductively coupled plasma-mass spectrometry (ICP-MS). As sampling periods varied from 1 to 12 months, the authors concluded that concentrations of all metals increased over time, which suggests that plastic debris may accumulate greater concentrations of metals the longer these remain at sea [81].

More recently, Brennecke et al. [11] examined the adsorption of Cu and Zn from antifouling paint to PS beads and aged PVC fragments in a simulated marine environment. A modified Aqua Regia (12 M HCl and 16 M HNO_3 at 3:1) extraction was performed to isolate the metals from the microplastics surface and their concentration in both the water and microplastics was determined by flame atomic absorption spectrometry. The accumulation of Cu in PVC fragments was significantly higher than in PS beads, possibly due to the higher surface area and reactivity (polarity) of PVC with partition coefficients between pellets and surrounding water ranging between 650 and 850 for Cu on PS and PVC, respectively. Furthermore, the concentrations of Zn and Cu in microplastics increased over time and, in the case of PVC, this accumulation did not reach the equilibrium during the duration of the experiment (14 days) [11].

According to a review by Hong et al. [28], the most frequent analytical methods applied to the analysis of chemicals accumulated in microplastics in the marine environment include GC-ECD and GC-MS. However, other techniques, such as ICP-MS, gas chromatography-ion trap mass spectrometry (GC-IT-MS), liquid chromatography-mass spectrometry (LC-MS), X-ray fluorescence (XRF) and SEM-EDS are also sometimes used for this purpose [28].

5. Analytical quality control/quality assurance

For quality control, the use of validated and standardized methods is crucial for the comparison of results of the applied analytical technique in order to demonstrate its fitness for purpose. The standardization of analytical methods for the detection, identification, and quantification of microplastics in environment is in its beginning and validation methods are yet scarce.

Before the identification and quantification of microplastics in different environmental matrices, representative sampling is crucial and, unfortunately, there is a current lack in global research for its standardization, which can have evident impacts in inter-laboratory studies. For example, for the sampling of microplastics in sediments, rigorous reporting of sampling details including depth, weight or volume, density and water content of sediments sampled should be mentioned [24], although this is not frequently the case.

Contamination, overestimation, and underestimation of microplastics from environmental samples occur due to the lack of optimized analytical methods for their quantification, as validation studies and blanks should be included for the reliability assessment of the method [24]. Recently, 43 research studies were evaluated for the quantification of microplastics in sediments, and only seven conducted laboratory control sample or validation trials [24]. In the same assessment, it was determined that: a) the size range of spiked plastics varied greatly and higher recoveries were reported for the larger spiked plastics compared to the smaller size fraction and, b) laboratory blanks were used in three of the studies evaluated, which allowed to determine whether contamination from the laboratory or clothing of scientists was effectively results [24].

Procedural blanks, replicate samples, spiked blank samples, and matrix spiked samples have been performed, but they are hampered by the lack of certified reference materials with known concentrations of target, which are important for method validation, measurement uncertainty estimations, internal quality control, external proficiency tests, and inter-laboratory studies. The accuracy and validity of analytical data related to microplastics could be improved by developing more certified reference materials with chemical groups including both absorbed and additive chemicals and polymer types (e.g., PE, PP, PS, PET, and PVC) since at this moment, only PE and PVC certified references containing bisphenol A and phthalates are available [28]. This inexistence of standards is not exclusive to polymeric materials with additives, but also to the different polymers when subject to biological, chemical and physical degradation phenomena, which may hamper the identification of such microplastics in environmental samples, as previously noted [50].

The recovery of microplastics is usually determined by spiking of clean sediments or collected marine waters with synthetic polymers. For the extraction of microplastics from sediments, Claessens et al. [82] developed a device based on the principle of elutriation, followed by density separation using a high density NaI solution (3.3 M, with a density of approximately 1.6 g cm^{-3}). For the validation of the methodology, clean sediment was spiked with known concentrations of fibres and granules (PVC or PE) and the sediment was subjected to extraction in order to determine its efficiency. The extraction efficiency for PVC particles was 100% after one extraction in the fluidized sand-bath followed by three subsequent extractions with 10 mL of NaI solution. For fibres, a 98% (49 out of 50 fibres) recovery was obtained after one extraction in the elutriation tube, followed by three subsequent NaI extractions.

For the analysis of microplastics from wastewater, Tagg et al. [35] proposed a pre-treatment step using 30% H_2O_2 to remove biogenic material, and FPA-based reflectance FTIR imaging to successfully image and identify different microplastics types (PE, PP, nylon-6, PVC, and PS). Microplastics-spiked wastewater samples were used to validate the methodology, resulting in a robust protocol which was non-selective and reproducible, with an overall success identification rate of 98%.

Other analytical parameters, such as precision, trueness, selectivity, specificity, limit of detection and sensitivity, which are established in the European Commission Decision 2002/657/EC [83] and required for the validation of analytical methods used for the determination of environmental contaminants, are currently not established for the validation protocols of applied analytical quantification techniques of microplastics. This compromises the quality assurance on the development of such analytical techniques. In many cases, the basis for this is the lack of replicates, which is, to some extent, understandable for larger studies, such as those encompassing high-sea cruises with manta trawls and catamarans [84]. Nonetheless, for adequately assessing the presence of these materials in the oceans, such statistical significance is of the utmost relevance.

As stated by Hanvey et al. [24], the key difference between chemical quantification and plastics quantification is the diversity of polymers in terms of type, size, colour, and morphology combined with the lack of homogeneity within environmental samples, which could affect every stage of the analytical process (sampling, extraction and quantification).

6. Key challenges and road map for future research

There are numerous challenges in the methodological processes of sampling, identification and quantification of microplastics and nanoplastics in different environmental matrices. There is, for example, the need to develop efficient and detailed sampling strategies, as sampling is crucial for the accurate assessment of the prevalence of these particles. The inexistence of such standardized protocols has led to a profusion of reports detailing the presence of micro-, and, to a lesser extent, nanoplastics, in the environments that are not susceptible of direct comparison, due to the use of different units (e.g., mass per volume; number per volume). Additionally, sampling of these particles does not encompass seasonal or inter-annual variants of environmental parameters. Conversely, short spatial and/or temporal (hours, meters) are not considered either, in spite of their ecological relevance, for example, at the benthic level [85]. For marine waters, the recently published technical memorandum by NOAA concerning the laboratory methods for the analysis of microplastics [32] could be important for future research on standardization of the analytical methods for the quantification of microplastics. Perhaps as a corollary of the discrepancies of the reported values regarding the quantification of these materials in the environment, there is a lack of consistency between such concentrations and those used in laboratory experiments. Currently, the concentrations of microplastics used are considerably higher than those observed in environmental matrices. Therefore, some of the potential effects of these materials already described may be environmentally irrelevant. In addition, the types of polymers tested for their ecotoxicological impacts are often limited to one or two [86]. And, because there are intrinsic difficulties in gathering samples isolated from environmental samples, micro- and nanoplastics in laboratorial experiments are often commercially acquired. This frequently

results in the use of rather uniform plastics, not only in terms of size and shape, but colour as well. Additionally, in order to avoid their aggregation, these materials also sometimes include anti-aggregation or dispersing agents.

There is also a profound lack of knowledge regarding the modifications that plastics, and, more precisely, micro- and nanoplastics undergo once subject to the elements. As noted by Fotopoulou and Karapanagioti [87], the infrared spectra of different polymers vary according to their interaction with the environment, reflecting the modifications taking place at their surface as the result of the creation of new functional groups. Hence, a comprehensive effort should be made to develop databases that contain the different spectra of the polymeric materials when subjected to some degree of (bio)degradation, thus ensuring that all particles are accounted for when analysing environmental samples.

Also, there is a need to increase awareness that contamination, i.e., the presence of alien elements, is not a synonym of pollution, meaning that these alien elements exert biological effects on the biomes of affected habitats. Long-term fate and behaviour of microplastics on water column and soils could lead to important conclusions at global scale concerning the effects of microplastics to ecosystems, further providing thresholds for regulatory guidelines and protection of environmental quality.

Ultimately, these considerations may lead to the implementation of standardized methodologies for sampling and quantification of micro- and nanoplastics in the environment. Only then, collected data will allow for a thorough assessment of the potential ecotoxicological effects of these materials, actively contributing to the fill these knowledge gaps.

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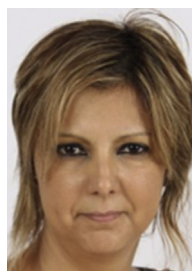
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