

Review



Microplastics in the Aquatic Environment: Occurrence, Persistence, Analysis, and Human Exposure

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Abstract: Microplastics (MP) have recently been considered as emerging contaminants in the water environment. In the last number of years, the number of studies on MP has grown quickly due to the increasing consciousness of the potential risks for human health related to MP exposure. The present review article discusses scientific literature regarding MP occurrence and accumulation on the aquatic compartment (river, lake, wastewater, seafood), the analytical methods used to assess their concentration, their fate and transport to humans, and delineates the urgent areas for future research. To better analogize literature data regarding MP occurrence in the aquatic compartment we subdivided papers based on sampling, analytical methods, and concentration units with the aim to help the reader identify the similarities and differences of the considered research papers, thus making the comparison of literature data easier and the individuation of the most relevant articles for the reader's interests faster. Furthermore, we argued about several ways for MP transport to humans, highlighting some gaps in analytical methods based on the reviewed publications. We suggest improving studies on developing standardized protocols to collect, process, and analyze samples.

Keywords: emerging contaminants; microplastics pollution; aquatic environment; analytical methods

1. Introduction

In recent years, several efforts have been devoted to the detection and removal of those contaminants that are referred to as contaminants of emerging concern (CECs), i.e., any chemical present in water or the environment at very low concentration levels, or only recently detected [1–8]. Microplastics (MP) have all the characteristics to belong to this category especially for their presence in the water environment [9].

Plastic materials play an important role in everyday life and the improvement of human health, referring to disposable medical equipment, food packaging, technology, and so on. However, the greatest exposure to microplastics contamination of the environment and the human body could have awful effects over time. During the COVID-19 pandemic, increasing consumption of single-use plastic, especially personal protective equipment (PPE) such as face masks, gloves, and gowns, that represent one of the safety methods being used in virus prevention, but also plastic used by households to wrap and take foods from supermarkets and restaurants, has been observed [10]. Since the use of PPE is indispensable to the current pandemic scenario, it is important to identify proper

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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses /by/4.0/). ways to dispose of the resulting plastic wastes [11]. In fact, according to a World Wide Fund for Nature (WWF) report of 2020, the improper disposal of just 1% of the masks would result in the dispersion of more than 40,000 kg of plastic in the environment (based on a weight of 4 g/mask and about 10 million face masks per month) [12]. This phenomenon is leading to an increase in plastic pollution and a consequential higher amount of microplastics dispersed in the environment in the future.

As stated by the National Oceanic and Atmospheric Administration (NOAA), plastic particles with a diameter lower than 5 mm are defined as microplastics (MP). Regarding nanoplastics (NP) dimensions, there is still no consensus in the literature [13] and some authors state that NP have a diameter lower than 1 μ m [14–18], while others assert that NP range is below 100 nm [19,20]. Depending on their origin, microplastics can be classified as primary or secondary microplastics. Primary microplastics are intentionally manufactured particles with sizes <5 mm including microbeads in personal care products, cleaning agents, coatings, paints, industrial abrasives for delicate surfaces, and feedstocks used in the manufacturing of plastic materials [21]. Secondary microplastics, much more abundant than primary, are those derived from the deterioration of larger plastics (macro-and meso plastics) during utilization (fragmentation of synthetic fibers in the process of clothes washing, the release of particles from tires) or after discarding (bottles and shopping bags) [14,22,23].

Plastic fragmentation can be induced by chemical and physical aging, especially on land due to higher ambient temperatures, frictional forces, and UV exposure [24], and also via (bio)degradation, the most common process in the aquatic compartment [25,26]. The addition of pro-oxidant compounds (transition metals added in the form of stearates of Fe3+, Mn²⁺, or Co²⁺) leads to the so-called oxo-biodegradable plastics, very attractive for packaging employment thanks to the possibility, claimed by the supplying companies, to have environmentally friendly plastic articles. Even though such additives accelerate polymer oxidation and abiotic (photo-induced) degradation without the influence of light, no improvement of environmental impact with respect to conventional polymers has been observed [27]. On the contrary, they might have a negative effect on the environment due to the release of metals and chemicals such as microplastics, generated by incomplete biodegradation [28]. Such MP particles (derived from waste fragmentation) are found in marine species as a consequence of passive (gill water filtration) or active (ingestion) intake [29]. Furthermore, as particle size decreases and surface area increases, the rate of polymer biodegradation enhances, varying by polymer type and ambient conditions with half-lives ranging from days to centuries [30]. Together with microplastics and nanoplastics, also organic and inorganic additives, unreacted monomers, and other compounds used in the formulation of plastic materials are released into the environment [31]. This represents a risk for human health due to the fact that the majority of additives and plasticizers, such as phthalic acid esters or phthalates (PAEs), alkylphenols (in particular 4-tert-octylphenol and nonylphenol), bisphenol A, di(2-ethylhexyl)adipate (DEHA), and brominated flame retardants (BFRs), have endocrine disruption and carcinogenic properties [32]. Moreover, MP can easily adsorb some organic contaminants such as persistent organic pollutants (POPs), mainly polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and dichlorodiphenyltrichloroethane (DDT), but the role of MP in POPs increment to marine organisms is still not clear and much debated in the literature [33]. Depending on the original form of plastics, the residence time in the environment, and the degradation processes that occurred on the surface, microplastic may have the shapes of fibres, fragments, spheres, beads, films, flakes, pellets, and foam [34]. Size and shape affect the microplastics' potential to cause physical harm to organisms, being taken up by cells or transported in soil [35]. Large microplastics are unlikely to be taken up by most plants and soil organisms, while nanoplastics can be taken into cells, posing an environmental risk [36,37].

Consequently, the complete characterization and the multidisciplinary overview of microplastics contamination in terms of chemical composition, abundance, distribution, uptake, and transport to the human body are of paramount importance to evaluate their impacts and to enable the critical and efficient procedure to avoid clinical repercussions. This review covers microplastics contaminated water environments, describing the analytical methods used for their characterization and quantification, thus discussing the most recent studies on their occurrence, fate, and behavior.

2. Materials and Methods

In this review, the authors attempt to provide a general overview of several implications associated with microplastics occurrence in the aquatic compartment. The methodology of the present work is briefly outlined in the following.

2.1. The Methodological Approach of the Review

Based on the recent scientific literature, this review had three main objectives: (1) to outline and discuss the presence of different analytical methods used to assess MP concentration, technical considerations and limitations; (2) to summarize contamination and accumulation of MP in the aquatic compartments and propose future research directions according to the existing literature; (3) to define the urgency and seriousness of microplastics in water by emphasizing the various ways for microplastics transport to the human body.

2.2. Data Sources Assimilation and Analysis

The keywords "emerging contaminants", "microplastics pollution", "aquatic environment", "analytical methods" were selected individually or jointly to search for relevant information on Web of Science, Scopus, and Google Scholar. Key literature published between 2004 and 2020 (up to December) was assimilated and analyzed.

3. Microplastics in the Aquatic Environment

3.1. Occurrence and Abundance of Microplastics in Water

Microplastics research is mainly focused on the water compartment [38], firstly marine environment [22,39–42], and recently moved also to wastewater [43–46], rivers, and lakes [47–50]. The first evidence of microplastics particles in the marine environment date back to the 1970s, when pellets (2.5–5.0 mm) on the Sargasso Sea Surface [51], spherules (0.1–2.0 mm) in the coastal waters of southern New England [52], spherules and disks (0.2–4.9 mm) in the surface waters of the Atlantic Ocean [53] and pellets (1–5 mm) in the surface waters of the Pacific Ocean [54] were detected.

Since 2004, when Thompson quantified the abundance of microplastics by collecting sediment from beaches, estuarine and subtidal sediments in the UK [16], an increasing amount of researchers have investigated microplastics occurrence, fate, and transport in the marine environment covering the shorelines of all continents [55,56], big and small islands [57–59], passing from Atlantic [60–63], Pacific [64,65], and Arctic ocean [66], and North [67,68], Adriatic [69,70], Baltic [71,72], and Mediterranean sea [73–80] and even in deep-sea habitats [81].

A small fraction of microplastics found in the ocean environment is related to marine activities such as the fishing industry (employing plastic equipment), while the majority of them (around 80%), has at its source, plastic litter derived from land [39,40]. The indiscriminate plastics disposal on beaches and coastal areas [82,83], terrestrial river input to the sea [21,84], stormwater runoff [85], passive uptake in marine species [86–90], wastewater discharge, and deposition of atmospheric microplastics constitute the main contribution to the microplastic occurrence in the marine compartment. Microplastic debris is transported widespread across large distances by ocean currents [22,57,91], winds, river outflow, and drift [92,93] with a consequent spatial and temporal mutability of their abundance [83,94] as

highlighted by the occurrence of this litter in remote and uncontaminated areas [95] such as the poles [96], the ocean depths, and mid-ocean islands [59].

Different sampling techniques have been employed to assess microplastics abundance in water compartments such as sediment recovery from the seafloor, beaches and estuaries, beachcombing to analyze plastic litter on the shoreline, observation of marine visible plastic debris performed by divers, use of marine trawls to collect particles within the water column, and examination of plastic fragments ingested by marine organisms [22,34].

In Table 1 are summarized the most relevant works regarding microplastics abundance in the marine environment subdividing them by matrix sampled (costal beach and deep sediments, surface, and sub-surface water and water column), sampling, and analytical methods, emphasizing both MP concentration, size range, and polymer type. Works that employed recovery of coastal beach and deep sediments principally expressed microplastics concentration as particles/kg of dry sediment (average of 5×10^2 , range $0.6-6.6 \times 10^3$ particles/kg) and particles/m² (average of 3×10^2 and range $1.5-6.3 \times 10^4$ particles/m²).

Table 1. Microplastics abundance in the marine environment.

Matrix	Sampling	Analytical	Ab	oundance	Size	Polymer	D.C
Sampled	Method	Method	Avera	age (Range)	Range	Туре	Kef.
		FTIR				PE, PP, PS,	
			particles	(0-4)	>1.6 µm	ABS, PVA,	[97]
						NY	
		МО	particlos/	(30–800)	-	-	[98]
			particles/	1.51	-	-	[99]
			1112	(12–1300)	-	-	[100]
			particles/ kg	(1.3–36.3)	-	PS	[101]
				(36–228)	1–5 mm	PE, PP, PET	[102]
			particles/ m²	(0-62,800)	1–5 mm	PS, PE, PP	[103]
	Recovery from beach			46.6 (2–178)	0.3–4.75 mm	PE, PP, PS, NY	[104]
Sealment				(16.67–489.7)	1–4.75 mm	PS, PP, PE	[105]
				39 (25–53)	0.1–5 mm	PES, EPM	[106]
				284 (2–1258)	-	PE, PET, NY	[107]
		MO and				PE, PP, TPU,	
		FTIR		12.1 (5.99–21.6)	-	NY, PS, PET,	[80]
			narticles/			PVC	
			ko	(0.58-2116)	_	PE, PP, PET,	[108]
			~8	(0.00 2110)		PA, PS, PES	[100]
				(60–300)	-	PET, RY	[109]
				(33–439)	0.5–3 (average 1.96) mm	PE, PP, NY, PS, PES	[110]

			160 (76–295)	0.20–0.94 (average	PP, PE, PS	[111]
	MO and RMS	particles/ kg	236 (72–1512)	0.51) mm <1 mm (55%)	PP, PE, PES	[112]
	MO and TDS-Pyr- GC-MS	particles/ kg	1.8 (1.3–2.3)	0.100–1.000 mm	PP, PE, PET, PVC, PS, PA	[67]
			(2.5–87.5)	-	PE, PP, NY, TPU, EVOH, LLDP/Oct	[70]
	MO and	particles/	119 (49–390)	0.038–1 mm	PE, PP, PS, PVA, NY PES, PVA	[113]
Van Veen	FIIR	kg	15 (0–27)	0.1–4 mm		[106]
Grab			15 (7–25)	average 1.29 mm	PET, PP, PE, PA, CPH, PVC, PS	[114]
	MO and	particles/	0.98	0.2–5 mm	PE, PP, PS	[115]
	RMS	kg	(54–506)	-	PE, PP, NY, PVC	[116]
		particles	62	0.25–5 mm	PA, PET, PP, acrylic	[117]
			4356 (42–6595)	<0.150 mm	PTFE, CPE, PA, PP, NR	[118]
			(672–2175)	0.030–0.500 mm	PE, PP, PS, PVA, PVC, PA, PAN, PES	[119]
Box corer	MO and FTIR	particles/ kg	134 (60–240)	0.06–1 mm	PE, PET, PES, CPH, cellulose,	[120]
			(560–4205)	0.05–5 mm	PP, PE, PS, PET, NY	[121]
				0.10-4.76		
			(5.30–68.88)	mm (average 1.63)	PP, PET, RY	[122]
Recovery by divers	МО	particles/ kg	900	-	-	[123]
	MO and FTIR	particles/ kg	10	-	RY, PP	[124]

	Recovery	MO and	particles/	129 7 (102 9–		PE PEVA PP	
	with a ring	FTIR	ko	163.3)	-	PET PS ALK	[125]
	Rotating	FTIR	particles	(0-2)	>1.6 µm	PE, PP, PS	[97]
	PE bottles	МО	particles/ m ³	152.000– 2,420,000	<1 mm	-	[68]
	Metal screen	MO and FTIR	particles/ m³	88,000	0.2–1 mm	ALK, PR, PS, PP, PE, PAS, PES, SR	[126]
	Steel sampler	RMS	particles/ m ³	5000–34,000	-	PE, PVC, PP, NY	[116]
	Stainless- steel hydrophore	MO and FTIR	particles/ m ³	46 (20–120)	average 1.29 mm	PET, PP, PE, PA, PVAC	[114]
				0.13 (0.01–0.42)	-	-	[74]
		МО		(0.011-0.285)	-	-	[127]
Surface	Manta Trawl or other nets		particles/ m ²	0.406 (0.012– 2.66)	average 2.69 mm	-	[69]
				0.112 (0.006–1)	0.4–5.2 mm (average 1.48)	-	[77]
Water		MO and	particles/	0.006-0.095	0.3–2.5 mm	PP, PE, PS, PET, PMMA	[128]
		RMS	m ²	0.0006-0.042	0.5–5 mm	PE, PP, NY	[129]
				(0.021–0.578)	average 1.95 mm	PE, PP, PS, PA	[76]
		FTIR	m ²	(0.0156–0.618)	average 1.3 mm	PP, PE, PS	[71]
				(0.043–1.46)	0.5–5 mm	PE, PP, PET	[102]
			particles/ m ²	(0.021–6.553)	-	-	[130]
		MO	particlos/	0.01–0.35	-	-	[73]
	Manta Trawl		m ³	7.68 (0.94–69.6)	0.3–5 mm	-	[131]
	or other nets		1110	(600–1200)	0.2–5 mm	-	[132]
	equipped			(0.03–491.0)	>0.3 mm	-	[133]
	with a flowmeter	MO and FTIR	particles/ m³	0.34 (0–1.13)	0.25–7.71 mm (average 1.93)	PES, PA, PE, PVC, acrylic, cellulose	[66]
				0.01-0.09	0.3–5 mm		[134]

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				1.00	0.2–20 mm	PE, PP, PA, NY, EP, PVC, PS, PVA, PET	[78]
				0.33 (0.01–1.23)	0.3–5 mm	PE, PP, PS, PET	[135]
				0.06	0.3–5 mm	PE, PP	[136]
				0.71 (0–3)	0.125–15.98 mm	PP, PE, PS, PA, CPH	[137]
		MO and RMS	particles/ m ³	0.24	0.335–5 mm	PE, PP, PS	[115]
					0.068–5.810		
Sub- surface Water	Water intake	МО	particles/ m ³	(8–9180)	mm (average	-	[64]
	system				0.606)		
	equipped with a flow- meter /pump	MO and RMS	particles/ m ³	2.46	1.25–2.5 mm	-	[63]
		MO and FTIR	particles/ m ³	2.68 (0–11.5)	0.25–7.71 mm (average 1.93)	PES, PA, PE, PVC, acrylic, cellulose	[66]
	Multi-level trawl		particles/ m ³	0.68	0.5–5 mm		[138]
	Sea-bird CDT	MO and FTIR	particles/ m ³	70.8	0.4–8.3 mm	PES, PET	[139]
Water Column	Bongo net equipped	MO and	Q and particles/	4.8–18.4	0.335–5 mm	CPH, ALK, PVA, PE	[140]
	with a flow- meter /pump	FTIR	m ³	(0.045–2569)	0.02–5 mm	PES, PE, PP, ALK, PCL, PS PEA, PU	[141]

Abbreviations: FTIR: Fourier-Transform Infrared Spectroscopy; RMS: Raman Spectroscopy; MO: microscope; PE: polyethylene; PP: polypropylene; PS: polystyrene; PET: polyethylene terephthalate; ABS: acrylonitrile butadiene styrene; PVA: polyvinyl alcohol; PVAC: polyvinyl acetate; PVC: polyvinyl chloride; PES: polyester; PAN: polyacrylonitrile; PA: polyamide; PTFE: polytetrafluoroethylene; PU: polyurethane; NY: Nylon; RY: Rayon; ALK: alkyd resin; CPE: chlorinated polyethylene; NR: nitrile rubber; LLDP/Oct: linear low-density polyethylene-octene copolymer; EVOH: ethylene vinyl alcohol copolymer; TPU: thermoplastic polyurethane; PVS: polyvinyl sulfate; PAS: polyacrylate-styrene; SR: synthetic rubber; PR: phenoxy resin; PCL: polycaprolactone; PEA: polyethylacrylate; PEVA: polyethylene-vinyl acetate; EPM: polyethylene-propylene; EP: epoxy resin; CPH: cellophane.

Together with the simple recovery of sediment from the beach, more elaborate sampling methods regard the use of Van Veen Grab [113] or Box Corer [119] to collect deeper sediment. Van Veen Grab is a clamshell bucket that collects sample sediments in water environments and has a sampling surface of 0.1 m² and has a depth of 20 cm. The Box Corer is a marine sampling for soft sediments in lakes or oceans. It is designed for the minimum disturbance of the surface and the penetration depth is 0.5 m. For surface, subsurface and deep water (along the water column) matrix, the concentration unit depends on the sampling method. In particular, particles/m³ (average of 6.3 × 10⁴ particles/m³ ranging from 0.03 to 2.4×10^6 particles/m³) is employed when a known water volume is

sampled using a pump [63,141] or a net equipped with a digital flow meter [132], while particles/m² (average of 0.55 ranging from 0.0006 to 6.6 particles/m²) is preferred as unit when the net surface was considered [42,142,143]. The number of particles recovered strongly depends on the trawl type used, as reported by Di Mauro et al. [140] that discover total microplastic concentrations 10,000 times higher using Niskin bottles, that allow collecting smaller plastic particles, with respect to bongo and neuston nets (110,000 in respect to 9.1 particles/m³). Moreover, as shown by Isobe [133,144] and Cózar [145] microplastics amount exponentially increases as their particle size decreases due to the degradation of a large plastic particle to multiple small pieces, while keeping its original volume and weight. Consequently, particle amount and microplastic concentration in all the measurement units used are affected by particle sizes considered. Thus, in some cases, an apparent low contamination could be reported if only high particle sizes are detected, while on the other hand, bigger concentrations might be disclosed when also lower particle sizes are sampled and analyzed.

Regarding microplastics abundance during the time, Law [146] observed no relevant difference in over 10 years in the North Atlantic gyre, with a concentration of 0.02 particles/m², while Claessens [113] revealed an increase of microplastic amount (from 55 to 156 particles/kg of dry sediment) in sediment sampled along the Belgian coast in 15 years. Notwithstanding several studies have demonstrated that low-density microplastics are mainly found in the sea-surface microlayer [82,147], their position in the water column can differ such as in estuarine habitats and they can sink if marine organisms stick to them [16,82,92,148]. For instance, on average of 0.68 particles/m³ at the ocean surface compared to 0.02 particles/m³ in the bottom was detected by Kooi [138], while Oztekin [149] discovered a microparticle density of 2.7 particles/m³ for sea surface and 24 particles/m³ for the water column. Moreover, microplastics can be redistributed throughout the water column after a storm that can re-suspend litter on the seabed [150].

More recently, microplastic particles have been documented in estuaries [21,38,151] and freshwater systems [47–50,152–155] such as lakes [156] and rivers [157]. In Table 2 are summarized almost all works concerning microplastics abundance in the freshwater environment and estuary subdividing them by matrix sampled (sediments and water), sampling, and analytical methods, highlighting both MP concentration, size range, and polymer type. As in the case of the marine environment, works that employed recovery of shoreline and deep sediments mainly expressed microplastic concentration as particles/kg of dry sediment (range $0.5-75 \times 10^3$ particles/kg) and particles/m² (range $0.2-26 \times 10^4$ particles/m²) while, for water matrix sampled, the concentration units mostly used are particles/m³ (ranging from 0.0005 to 1.1×10^6 particles/m³) when the water volume is known and particles/m² (ranging from 0.01 to 1.4×10^4 particles/m²) when the water surface was considered.

Matrix	Sampling	Analytical	Ab	oundance	Size	Polymer	D (
Sampled	Method	Method	Aver	age (Range)	Range	Туре	Kef.
				Rivers			
				(786–1368)	0.06–5 mm	PE, PP, EPDM, PS, PET, PVC	[158]
				(178–544)	-	PE, PP	[159]
		MO and	particles/	802 (5.3–160)	0.1–5 mm	PP, PES, RY, PR	[160]
		FTIR	kg	210 (99–410)	0.06–5 mm	PET, PE, PP, PS	[161]
				422		PA, PE, PP, PMMA, PES	[162]
	Recovery from			(7–1029)	0.06–5	PES, PA, PE, PP, PS PP, PE,	[163]
	shoreline	MO and RMS	particles/ kg	350 (185–660)	1–4 mm	PMMA, PET, PVC, PS,	[164]
				118 (50–195)	0.045–5 mm	PET, PE, PP, PS, PA	[165]
		MO, SEM and RMS	particles/ kg	414 (307–580)	0.5–5 mm	PS, PE, PET, PP, PA, PVC	[166]
		MO and SEM	particles/ kg	(161–448)	0.06–2.8 mm	-	[167]
Sediment		Visual sorting	particles/ m ²	5595 (16– 258,408)	0.3–5 mm	PS	[168]
		MO and LC-MS/MS	particles/ kg	136–2060	0.01–5 mm	PET, PC	[169]
			particles/ m³	(58–1265) particles/m³	0.055–5 mm	PE, PP, PET, PS, PVA, EVA, PTFE, PMMA	[170]
		MO and		847 (100–3600)	0.01–5 mm	-	[171]
		FTIR		1669 (80–9597)	0.02–5 mm	PE, PP PP, PES, PE,	[172]
	Van Veen Grab		particles/ kg	1971	0.05–5 mm	PA, PVC, ALK, PS, PU, acrylic	[173]
	Grav		0	0.81 (0.46–1.62)	-	PE, PP, PES PS, PTFE, PAC	[174]
				2052	-	PA, PE, PP, PMMA, PES	[162]
		MO and RMS	particles/ kg	82 (25–30)	<5 mm	PS, PP, PE, PC, PVC	[175]
		MO, FTIR, Py-GC-MS	particles/ kg	2080	0.02–5 mm	PE, PP, PS	[176]
	Other grabs	МО	particles/ m ²	13,832	0.04–2mm	PE	[177]

		Fluorescenc e MO, FT-IR	particles/ kg	32,947 (18,690– 74,800)	0.02–5 mm	PE,PP,PES,PV C PS, NY,PU,PET	[178]
		MO and RMS	particles/ kg	30	0.05–2 mm	NY, PES	[179]
			-	(10–520)	1–4 mm	РЕ, РА, РР,РЕТ	[180]
		MO and FTIR	particles/ kg	2071 (68– 10,500)	0.01–5 mm	-	[171]
				(0–97)	1–5 mm	PE, PP, PET, PS	[181]
		MO and SEM	particles/ kg	(360–1320)	<5mm	-	[182]
		Fluorescenc e MO	particles/ kg	832 (65–7562)	95%<0.4 mm	-	[157]
	Wooden ruler and frame	MO and FTIR	particles/ kg	(260–11,070)	0.01–0.5 mm	APV, CPE, EPDM, PES, PP	[183]
			particles/ m ²	3408–13,618	0.1–5 mm	PE, PP, PS	[184]
	Bulk sample collected in containers of different	MO and FTIR		100,000 (48,000– 187,000)	0.01–5 mm	-	[171]
				1200 (0–67,500)	0.1–9.6 mm	Cellulose, PET, PES, PVA	[185]
				2724 (379– 7924)	0.02–2 mm	PP, PE, PET	[172]
			m ³	1650 (293– 4760)	0.05–5 mm	PP, PES, PE,PA, PS, PU, PVC, ALK, acrylic	[173]
Surface Water	type			164,790 (86,000– 106,1000)	0.05–1 mm	PE, PET, PP,PS, PAN, PVA, NY	[186]
Water				6186 (1770– 14,330)	0.05–1.5 mm	PE, PS, PP, PVC	[187]
		MO and	particles/ m ³	910 (0–5400)	0.1–0.5 mm	PP, PE, PET, PES	[188]
		RMS	particles/ m ³	5850	0.05–2 mm	NY, PES	[179]
		Fluorescenc e MO	particles/ m ³	120 (0–300)	-	-	[157]
				(2516–2933)	0.05–5 mm	PET, PP, PE, NY, PS	[189]
	Water intake with a pump	MO and FTIR	particles/ m ³	13.79 (3.52– 32.05)	-	PE, PP, PS, PES, PTFE, PAC	[174]
				(0.1–5.6)	1–5 mm	PE, PP, PET, PS	[181]
				(67–11,532)	0.02–0.3 mm	PE, PP, EPDM	[190]

				67.5	0.075–5 mm	PE, PP, PB, PA, DVC, PS, PET	[191]
		MO and	particles/	4703 (1597– 12,611)	<5 mm	PVC, PS, PET PS, PP, PE, PC, PVC	[175]
		RMS	m ³	694 (483–967)	0.045–5 mm	PET, PE, PP, PS, PA	[165]
		MO and SEM	particles/ m ³	(3670–10,700)	<5mm	-	[182]
		МО	particles/ m ³	(4–108)	0.1–5 mmm	-	[192]
				3.5	-	PA, PE, PP, PMMA, PES	[162]
				1.8 (0.1–4.7)	0.5–5 mm	PE, PP, PS	[193]
	Manta Trawl or other nets	MO and FTIR	particles/ m ³	(1.47–43.11)	1–5 mm PE, PP, PET	PE, PP, PS, PET	[194]
				0.6	0.075–5 mm	PE, PP, PB, PA, DVC DS DET	[191]
		MO and	particles/ m ³	2.57 (0-13.7)	0.1–0.5 mm	PP, PE, PET, PES	[188]
		RMS	particles/ m ²	(3–31)	0.1–5 mm	PP, PE, PS, PET	[195]
		MO and Py-GC-MS	particles/ m ³	(0.9–13)	0.5–5 mm	PE, PP, PVC, PS, PU	[196]
		MO and SEM	particles/ m ³	(1.94–17.93)	0.330–2 mm	-	[197]
			particles/ m ²	893	0.3–1 mm	PS, PP, acrylate, PES, PVC	[198]
	Manta Trawl			(58–1265)	0.055–5 mm	PE, PP, PET, PS, PVA, EVA, PTFE, PMMA	[170]
	or other nets equipped with a	MO and FTIR	particles/	450 (140–1960)	0.25–5 mm	PP, PE, PET, PS, PVC, PA, PVA, EVA	[199]
	flowmeter		m	1.6 (0–12)	-	PE, PP, PS	[200]
				11.6 (0.3–58.9)	0.3–5 mm	PES, PA, PE, PP, PS	[163]
				(0.1–4.6)	1–5 mm	PE, PP, PET, PS	[181]
				0.15 (0–3.4)	0.7–5 mm	PE, PS, PP	[201]
		MO, FTIR and Py-GC-MS	particles/ m ³	5.57 (0.88– 13.24)	0.15–5 mm	PE, PP, PS	[176]
Water Column	Water intake with a pump	MO and FTIR	particles/ m ³	846 (360–1273)	0.05–5 mm	PP, PES, PE,PA, PS, PU, PVC, ALK, acrylic	[173]

	Manta trawl	MO and FTIR	particles/ m ³	(0.76–12.56)	1–5 mm	PE, PP, PS, PET	[194]
Bottom Water	Manta trawl	MO and FTIR	particles/ m ³	(1.43–34.63)	1–5 mm	PE, PP, PS, PET	[194]
				Lakes			
		SEM and RMS	particles/ m ²	483–1108	-	PS, PE, PP, PA, PVC	[202]
		RMS	particles	6172	-	PE, PP	[203]
		SEM and	particles/	3209	-	PE, PP, PET	[204]
		FTIR	m ²	1.72 (0.18–8.34)	5-20 mm	PE, PP	[205]
		and SEM	kg	174 (109–266)	0.3–5 mm	-	[206]
	Recovery from	rery n line MO and RMS	particles/	563	<5 mm	PE, PP, PS, PET, PVC	[207]
	shoreline		m ²	(50–1292)	0.1–5 mm	PP, PE, EVA, PVC, NY, PC	[195]
				560 (270–866)	0.5–5 mm	PS, PE, PET, PP, PA, PVC	[166]
			particles/ kg	403 (180–693)	0.05–5 mm	PET, PA, PE,PP PVC,PS,PMM A	[208]
		MO, FTIR and RMS	particles/ m ²	400	-	PES, PET	[156]
Sediment	Box corer	Mo and FTIR	particles	35	0.5–3 mm	PE, PP, nitrocellulose	[203]
		МО	particles/ kg	300	0.5–5 mm	-	[209]
		MO and RMS	particles/ kg	539	0.5–1 mm	PS, PAN, PVC	[210]
		MO and	particles/ m ²	253 (96–496)	<5 mm	PE, PS, PP	[211]
		RMS	particles/ kg	(54–506)	0.05–5 mm	PP, PE, NY, PVC	[116]
	Van Veen grab	MO, SEM and FTIR	particles/ kg	(14–24)	<5 mm	PE, PET, PP, PVC	[212]
	5	MO and	particles/	0.81 (0.46–1.62)	-	PE, PP, PS, PES, PTFE, PAC	[174]
		FIIR	кg	309 (92–604)	0.3–2 mm	NY, PE, PS, PP, PVC	[213]
		MO, FTIR, SEM-EDS	particles/ kg	(11–235)	0.005–5 mm	CPH, PET, PES, PP	[214]
	Other grabs	er grabs MO and	particles/	395.8	<5mm	PA, PS, PU,PET	[215]
		FTIR	kg	372 (8–1070)	>0.125 mm	PS,PE,acryl,N Y	[216]
Surface Water	Bulk sample collected in	MO, FTIR, SEM-EDS	particles/ m ³	(3400–25,800)	0.005–5 mm	CPH, PET, PES, PP	[214]

	containers of different	MO and RMS	particles/ m ³	(5000–34000)	0.05–5 mm	PP, PE, NY, PVC	[116]
	type	MO and FTIR	particles/ m ³	250 (0–710)	>0.125 mm	PE, PP, PS, PU, PVC, PA, PES	[216]
		MO and	particles/	1660–8925	0.05–5 mm	PET, PP, PE, NY, PS	[189]
	Water intake with a pump	FTIR	m ³	13.79 (3.52–32.05)	-	PE, PP, PS, PES, PTFE, PAC,	[174]
		MO, SEM and RMS	particles/ m ³	(900–4650)	0.05–5 mm	PE, PP, PS, PVC	[217]
		SEM	particles/ m ²	43	0.355–5 mm	-	[218]
			particles/	20 (1–44)	0.355–5 mm	-	[219]
		МО	m ²	0.13 (0.01–0.42)	-	-	[74]
Manta Tra or other ne			particles/ m ³	4.2 (0.05–32)	0.355–4.75 mm	-	[220]
		UV-MO and SEM	particles/ m ³	(0.82–4.42)	0.3–5 mm	-	[206]
	Manta Trawi	MO and SEM-EDS	particles/ m ²	(53–748)	-	-	[221]
		MO and RMS MO and	particles/ m ²	5–758	0.1–5 mm	PP, PE, PS, PET	[195]
			particles/ m ³	0.40 (0.372– 1.29)	>0.125 mm	PE, PP, PS, PU, PVC, PA, PES	[216]
		FTIR		(11.9–61.2)	0.005–5 mm	PP, PE	[222]
			particles/ m ²	0.028 (0.013– 0.054)	0.3–2 mm	Ny, PE, PS, PP, PVC	[213]
	Manta Trawl equipped with a flowmeter	MO, FTIR and Py-GC-MS	particles/ m ²	(0–110)	-	PE, PP, PS, PVC, PET	[223]
			Other	r freshwater		DE DD DEC	
Sediment	Box corer	MO and FTIR	particles/ kg	(672–2175)	0.03–0.5 mm	PE, PP, PES, PS, PAN, PVC, NY	[119]
s of Lagoon	Recovery	MO and	narticles/	(2340–6920)	0.2–5 mm	PP, PE PF PP PFS	[224]
Lagoon	from shoreline	FTIR	kg	(2.9–36.3)	0.025–5 mm	PS, PVC, PTFE, PA	[225]
Surface Water of Antarctic	Net sampler	MO and FTIR	particles/ m ³	0.00095 (0.00047– 0.00143)	0.01–3.5 mm	Acrylic, PES, PTFE	[156]
			Ι	Estuary			
Sediment	Box corer	MO and FTIR	particles/ kg	121(20–340)	0.05–5 mm	RY, PES, acrylic	[226]
Seament	Van Veen grab	MO and SEM	particles/ kg	(0–126)	<5 mm	-	[227]

	Recovery from shoreline	MO, SEM and FTIR	particles/ kg	351 (46–916)	0.3–4.75 mm	PE, PET, PS, PVC, PP	[228]
Surface	Water intake with a pump	МО	particles/ m ³	4137 (50– 10,200)	0.5–5 mm	-	[229]
Water	Bulk sample	MO and FTIR	particles/ m ²	(192.5–11,890)	0.1–5 mm	PE, PP, PS	[184]

Abbreviations: FTIR: Fourier-Transform Infrared Spectroscopy; RMS: Raman Spectroscopy; MO: microscope; PE: polyethylene; PP: polypropylene; PS: polystyrene; PET: polyethylene terephthalate; PVA: polyvinyl alcohol; PVAC: polyvinyl acetate; PVC: polyvinyl chloride; PES: polyester; PAN: polyacrylonitrile; PA: polyamide; PTFE: polyethrafluoroethylene; PU: polyurethane; NY: Nylon; RY: Rayon; ALK: alkyd resin; CPE: chlorinated polyethylene; PR: phenoxy resin; PEVA: polyethylene-vinyl acetate; CPH: cellophane; EPDM: ethylene propylene diene rubber; PMMA: polymethyl methacrylate; APV: acrylates/polyurethane/ varnish cluster; PB: polybutylene. Py-GC-MS: Pyrolysis gas chromatography-mass spectrometry; SEM-EDS: Scanning electron microscopy-Energy-dispersive X-ray Spectroscopy; LC-MS/MS: Liquid Chromatography tandem-Mass.

The occurrence of microplastics in estuaries is higher than that in sea samples (4137 respect to 0.167 particles/m³ [229,230]) due to the input of anthropogenic debris from freshwater systems and beaches and wash-back by marine surface currents [151,231]. Several studies regarding estuaries aim to demonstrate the presence of microplastics in surface waters [21,232,233] and sediments [234–236] mainly coastal beach ones [38]. This acute presence of microplastic pollution in estuaries indicates that terrestrial river input is an important source of microplastics to coastal and marine environments [9,148,151,237,238]. Less than one microplastic particles/m² at the surface of the Rhine, one of the largest European rivers [198], and 7 particles/m³ along the Ofanto river in Southeast Italy [196] were detected, while higher concentrations were determined along the Snake and Lower Columbia rivers [188] and in the surface waters of the Wei River Basin, in northwestern China [182]. One of the most studied lakes is the Great Lake Basin of North America [203,205,223], where the average abundance of microplastics floating on the surface was as high as 0.043 particles/m² [218]. A similar amount (0.048 particles/m²) was detected in Europe, in Lake Geneva, Switzerland [239].

Finally, the occurrence of microplastics in wastewater was recently reported and reviewed [44,45,55]. Sources of microplastics in wastewater are fibers from synthetic textiles [240] industries and the domestic ones released when washing clothes [241], glitter [242], detergents for contact lens, plastic particles used in air blasting [147], and dust formed during plastic items production. The majority of microplastics (>80%) are removed from the wastewater during the primary treatment [243] being captured by sedimentation and skimming processes [244,245] and thus depositing in the sludge fraction. The resulting effluents have a low microplastic concentration ($0-447\cdot10^3$ particles/m³), but the high volume discharged daily results in significant pollution of the water compartment [44,243,245–247] with an estimated median value of 2 × 10⁶ released particles/day [45].

Regarding microplastic form in the water environment, the dominant shapes are fibers, mainly derived from laundry activities and conveyed over wide distances by the marine stream, followed by fragments with polyethylene, polypropylene, and polystyrene as the most abundant polymers.

Although all the works reported suggest a high contamination of the water environment, a full comparison among the various studies is still difficult and often impossible due to the lack of a standardized procedure for water sampling, particle counting, and polymer matrix individuations.

Based on the above reported microplastic contamination all over the world, in Figure 1 we show a map of the distribution of microplastics in the aquatic environment, from sea to freshwaters, according to nations in order to indicate the areas where microplastic research is actually performed, highlighting those of serious pollution and, on the other

hand, places where a lack of microplastic monitoring is observed. Indeed, studies on the occurrence and distribution of microplastics in aquatic environments are mainly focused in the more industrialized areas, such as south-east Asia, especially China, Europe and North America, while Africa, South America and North Asia remain poorly investigated.

The map was created using all the concentrations reported in Tables 1 and 2, with units of particles/m², particles/m³, and particles/kg distinguished by different shapes and colour from light yellow to dark red according to microparticle densities as shown in Figure 1.

As we can see from this map, the more polluted areas are in China and some European and North American countries, however too many areas remain uninvestigated, so further studies should be performed in these countries in order to give a wider view of microplastic contamination all over the world.



Figure 1. (**a**) Map of distribution of microplastics in aquatic environments (based on data in Tables 1 and 2); (**b**) magnification of Europe; (**c**) magnification of south-east Asia.

Therefore, microplastic pollution in water environments is a severe problem and MP, as other contaminants, could be assessed with the systematic and quantifiable approach in life cycle assessment (LCA). Recently, first methodological approaches for including impacts of the marine litter of microplastics to LCA were tested [248]. The assessment of marine litter impact (derived from the microparticle abundance in the marine environment and their effects on different organisms) in LCA was strongly dependent on the number of microplastic particles produced from the original litter over time. So, the model used for impact assessment within LCA includes the relationship between fragmentation and degradation of microplastics [248]. With this approach, the authors were able to develop an applicable characterization model for LCA that can be refined through further research activities and with the help of additional data in order to reach a better understanding of this issue.

3.2. Analytical Approaches for MP Separation, Identification, and Quantification

Several analytical methods have been developed to measure MP in water and the most important procedures include separation, identification, and quantification.

Separation of microplastics is usually achieved by sieves with mesh sizes ranging from 0.038 to 4.75 mm that are used singly or in a series. Moreover, filters with small mesh sizes (0.02–5 μ m) are also used to separate small microplastics or nanoplastics [50,249]. For plastic particles <1 µm, chromatographic techniques, active, and passive separation, are typically used. Active separations, such as the field flow fractionation (FFF) technique [250], apply external fields into microfluidic environments for separation of dispersed particles; while passive separations, such as hydrodynamic chromatography (HDC) [251], utilize hydrodynamic and surface forces to separate particles in liquid [252]. The extraction process is also referred to the different densities of samples. For example, for low-density water (0.9–2.3 g/cm³), MP can be directly separated using a net in the water or directly filtered by filters or sieves after known volume water is collected, followed by visual sorting [67] while for sediment and soil having a high density (2.6–2.7 g/cm³) [253] flotation method is preferred, wherein MP are extracted by a higher density salt solution such as saturated NaCl (1.2 g/cm³), ZnCl₂ (1.5-1.7 g/cm³), NaI (1.6-1.8 g/cm³), and CaCl₂ (1.5 g/cm³) [254]. In order to separate flotation (plastics and organic matter) from the minerals in the mixture solution after centrifugation, a rubber disc is inserted into the middle of a centrifugal tube. This can prevent mineral particles from being resuspended, substantially reducing separation time [255]. Moreover, a high amount of organic matter could affect separation from MP, for this reason, acid solvents, alkali solvents, or oxidation agents, including KClO (30%), NaOH (56% or 52.5 M), H2SO4 (96%), HNO3 (65% or 22.5 M), and H₂O₂ (30% or 32.6 M), have been used to digest and remove organic matter from flotation [255]. However, a recent study demonstrated that many of these agents decompose, disintegrate or modify the weight, number, and shapes of MP particles [256]. In fact, polyamide (PA), polyester (PET), and polycarbonate (PC) have low resistance to strong acids. PA, polyethylene (PE), and polypropylene (PP) are resistant to 10% KOH, but polycarbonate (PC) and PET are degraded. Treatment with NaOH 40% at T = 60 °C caused deformation of PA fibers, yellowing of PVC granules, and melding of polyethylene particles. Enzymatic digestion is a biological means to hydrolyze proteins and break tissues. Enzymes ensure no loss or degradation of plastics, however enzymatic digestion can be long and expensive [257].

The quantification of MP is still a major challenge, due to their special chemical and physical properties (very high molecular weights, poor solubility in most solvents). MP cannot be analyzed by classical analytical methods such as Gas Chromatography–Mass Spectrometry (GC-MS) and Liquid Chromatography tandem-Mass Spectrometry (LC-MS/MS). Table 3 outlines the main analytical techniques employed for MP detection, advanced visualization, and counting, focusing on operation principles, advantages, and disadvantages of each method.

Basically, two different analytical strategies have been discussed in recent publications [258–261]. The first one is based on the isolation of MP particles by density separation and spectroscopic identification [82,262,263] through Fourier-Transform Infrared Spectroscopy (FTIR, available in transmission, reflectance and attenuated total reflectance, ATR) or Raman Spectroscopy. The reflectance and ATR modes do not require the sample preparation steps for thick and opaque microplastics [264]. These methods deliver information about polymer species, particle numbers, and size distribution [265], important parameters to estimate microplastics' toxicological potential. Raman method spectroscopy (<125 mm and >5 mass%) [266], micro-Raman spectroscopy (>100 µm), micro-FTIR spectroscopy (>100 µm) [267], m-Raman spectroscopy (>1 mm), m-FTIR spectroscopy(>10 mm) [268] macroscopic dimensioned near-infrared (NIR) in combination with chemometrics (>10 mm and 1 mass%) [266] and hyperspectral imaging technology (0.5–5 mm) can be used to identify plastics type, but these techniques are timeconsuming, they can be expensive and result in an uncertain extrapolation of MP quantity [269]. For example, the ATR mode produces stable spectra even from irregular microplastic surfaces, however a lot of microplastics are weathered or made of complex materials, thus an experienced operator is necessary for the acquisition and interpretation of the spectra. Moreover, the probe used in ATR is made of germanium and can be easily damaged by hard and sharp particles remaining on filter papers [264].

The Raman signal is suitable for particles below 20 μ m, but the spectra are heavily influenced by dyes, additives, organic, and inorganic substances, besides some materials exhibit fluorescence masking the vibrational information [270].

The second strategy is based upon thermal decomposition coupled to mass spectrometry, which is more suitable for MP quantification. Unice et al. [253] developed a method for polymers of tires (sample amount of 0.5 mg) using pyrolysis-gas chromatography mass spectrometry (pyr-GC-MS). Quantification was based upon specific pyrolysis products and deuterated internal standards. The method needed only a minor sample preparation and yielded a limit of detection of 14 μ g/g of tire tread in soil and sediments. For the quantification of thermoplastics, Duemichen et al. [254] described a combination of thermal extraction with thermogravimetric analysis and subsequent analysis with thermal desorption gas chromatography mass spectrometry (TDS-GC-MS), that required a sample quantity of more than 20 mg. After pyrolysis at 600 °C and enrichment of the degradation products on solid-phase absorbers, plastic species were quantified by GC-MS analysis detecting polymer-specific decomposition products [271]. The deficiencies of these methods are the expense of the equipment and the complicated operation process.

Visualization is important for identifying microplastics in sample materials or debris (e.g., shell fragments, algae, sand, and glass) [34]. Large microplastics (1–5 mm) are identified with the aid of an optical microscope or using the naked eyes. By evaluating the color, shape, and light transmission of plastic particles, MP are distinguishable from non-plastic particles [272]. For example, polypropylene (PP) fibers were found to be typically blue or red [119]. The features of milky white color, slight shine, and amorphousness make expanded polystyrene (PS) very easy to identify by naked eyes [272], which sometimes could obfuscate PS particles with minerals such as perlite. White pellets dominate the majority of the debris, followed by transparent and colored pellets [273]. An optical microscope is used for smaller microplastics particles. A stereomicroscopy, which has two separated optical paths, allows the acquisition of three-dimensional images instead of the flat surfaces typical of the traditional optical microscopies. Polarized light microscopy was used to obtain a stacked image at different sample heights and estimate the number of microplastics in synthetic water samples [274].

Analytical Techniques Advantages		Limitations							
¥1	Methods for MP detection								
- Identification (non-destructive) of									
polymer matrix									
Fourier Transform Infrared Spectroscopy (FTIR) Spectra collected in Transmittance, Reflectance or Attenuated Total-Reflectance (ATR) mode	 Selective and reproducible Small sample amounts Limited sample preparation Quantification of different types of particles (with sizes ≤2 μm) 	 Not totally reliable on weathered samples, degraded samples or mixed polymer samples. Combination with microscope is expensive 							
	- Localization of debris in living								
Raman spectroscopy	 Identification (non-destructive and non-contact) of polymer matrix Selective and reproducible Small sample amounts Limited sample preparation Identification and quantification of individual sub-micron particles Localization of debris in living 	 Not totally reliable on weathered, colored, and degraded samples or mixed polymer samples Auto-fluorescence mask the signal Samples may be damaged Time consuming for wide area imaging Combination with microscope is 							
	organisms	expensive							
Pyrolysis gas chromatography-mass spectrometry (Py-GC-MS) MS analysis of microplastics thermal degradation products	 Identification of polymer matrix and organic additives Quantification of small amounts in mass concentration (<0.5 mg) Limited sample pre-treatment 	 Require an expert operator and a sampler equipped with a thermal desorption system Time-consuming Particles size major than fractions or 							
	- Suitable for biological matrices and environmental screening	mm - No data on particle number, size, and shape							
Time-of-Flight Secondary Ion Mass Spectrometry (ToFSIMS)	 Identification of polymer matrix High spatial resolution imaging Suitable for mixtures of particles Particles size in the low μm range Information on inorganic and organic chemical contaminants 	 Complex to use and expensive Relative quantification of different polymers is usually not possible Identification complicated by weathering or by surface contaminants Sample must be vacuum compatible 							
	Advanced methods for MP visualization	F							
Fluorescent tagging with Nile Red (labelling of microplastics when irradiated with blue light)	 Detects and quantifies particles (20 μm–1 mm) Low cost and semi-automated 	 Require sample purification Not reliable with weathered samples Organic matter in the matrix may produce false positives Not able to identify polymer types Dye fluorescence may prevent identification by Raman spectroscopy Require coating for working at high 							
Scanning electron microscopy (SEM)	- Provides high-resolution image	 vacuum Destructive Charge effects 							
Scanning electron microscopy-Energy- dispersive X-ray Spectroscopy (SEM-EDS)	 Chemical and morphological characterization of particles No requirement of coating due to work in low vacuum Environmental-SEM-EDS gives elemental composition and surface morphology of microplastics with no charge effects 	 Complex to use Expensive 							

Table 3. Comparison of main analytical methods for MP detection and visualization.

Though visual sorting and identification are feasible for large microplastics with disguisable color or morphologies, particles without identifiable color or shape are challenging for visual sorting with the naked eyes. Thus, electron microscopy providing magnified images is critical for identifying ambiguous plastic-like particles. Scanning electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM-EDS) is widely used for characterizing the morphology of ultra-small materials and determining their elemental compositions [275]. SEM could provide high-resolution topography images of objects and facilitate the differentiation of the microplastics from other plasticlike particles [276]. EDS provides the elemental information of the samples via detecting the characteristic X-rays emitted from the elements within the sample by the electron beam, which enables certain capabilities of microplastic identification in sample matrices. However, as most of the microplastics are non-conductive, SEM-EDS requires substantial sample preparation and analysis time (e.g., sample drying, sample deposition, and carbon or gold coating), which may introduce artifacts and interfere with the morphological analysis for ultra-small microplastics. Moreover, due to exposure to the high-energy electron beam, some plastic particles such as polyvinyl alcohol (melting point 35-38 °C) and polyvinyl chloride (melting point 115–120 °C) that are thermally unstable may be softened or burned during the SEM examination.

Recently, new advanced techniques have been proposed for the characterization of microplastics, sometimes microplastics were produced in the laboratory to obtain standard reference materials. Monteleone et al. [277], used fluorescence lifetime imaging microscopy (FLIM) to characterize microplastics. Several kinds of microplastics produced with a cryogenic swilling mill, with and without heat treatment were subjected to FLIM with excitation wavelengths of 470 nm and 440 nm. The fluorescence lifetimes of each microplastics were evaluated and compared and no significant differences emerged in the fluorescence lifetimes of PET samples coming from different countries, therefore the lifetimes measured do not depend on the origin of the microplastics.

Berto et al. [278] reported a preliminary characterization of carbon stable isotopes ($^{13}\delta$) of different plastic polymers. Stable isotope analysis is a technique that measures the relative abundance of stable isotopes giving an isotopic ratio that can be used as a research tool. It is widely used in food analysis [279,280], in forensic cases [281], and in medical diagnostics [282] for the monitoring of air quality indoor and outdoor [283,284] and the characterization of commercial cleaning products [285]. This technique has been applied only rarely to assess the presence of microplastics. A proof-of-concept study was performed with fully labeled ¹³C polyethylene to follow its fate across the aquatic microbial-animal interface. It emerged that the biodegradation of PE-MP (<100 µm) occurs in natural waters, becoming part of nutritionally valuable biomolecules for aquatic organisms [286].

In [278,287], standard petroleum and plant derived polymers were analyzed to estimate the carbon isotope ratio. The ¹³ δ values of several polymers span over a wide range: PTFE, silicon and ABS show ¹³ δ values between –41% and –35%, petroleum polymers have ¹³ δ values between –34% and –24%, PLA (a biodegradable polyester derived from the fermentation of starch and condensation of lactic acid) has a value of -14%. Thus, this technique shows an interesting perspective for the discrimination between petroleum and plant-derived polymers in aquatic environments. Furthermore, unlike vibrational spectroscopy techniques such as Raman, it is not sensitive to variables such as colors.

Another analytical method includes high temperature gel-permeation chromatography (HT-GPC) coupled with an IR detector. HT-GPC-IR can assess polyolefin microbeads in aqueous environments and more complex matrices such as personal-care products [288] and also gives the distribution of molecular masses.

In conclusion, FTIR and Raman spectroscopy are commonly used for identifying the polymeric composition of microplastics, whereas mass spectrometry-based methodologies, although require a lower amount of sample, are more expensive and trickier to operate. The same can be said by comparing optical microscopy, the most used technique for microplastics visualization, with the most advanced techniques such as SEM-EDS. Consequently, interlaboratory analyses should be performed to provide comparability of data regarding chemical characterization and quantification of microplastics. In fact, the use of one technique only can lead to the overestimation or underestimation of microplastics. Fragmented microplastics were underestimated and fibers were overestimated using stereomicroscopy compared to FTIR in samples coming from sea surface microlayers and beach sand. The total abundance by FT-IR was higher than by microscope both in the sea surface microlayers and beach sand samples. The stereomicroscopy allowed mainly for the identification of 50–100 μ m fragmented microplastics, while FTIR also identified lower sizes [289].

Therefore, in Scheme 1 we reported a possible simple protocol for the identification of microplastics in aqueous samples that can include a first preparation step of the sample (identification of organic matter presence with preliminary treatment of degradation), separation with sieves with different mesh sizes, a visualization step with a microscope to count and estimate the sizes of MP, and in the end, a screening with FT-IR or Raman to identify the chemical composition of MP.



Scheme 1. Flowchart for the procedure of MP sample data analysis for full detection, identification and quantification.

Although new accurate and sophisticated analytical methods have been reported for microplastic detection and characterization, FT-IR and Raman Spectroscopy remain those commonly used for identifying the polymeric composition of microplastics due to their inexpensiveness and user-friendliness.

3.3. Transport of MP to Humans

Microplastics detected in the water environment can reach the human body through ingestion [290,291] of sea products and also drinking water [292] and related beverages.

Several papers considered the occurrence of microplastics in marine organisms and their ingestion, sorption, bioaccumulation, and translocation, identifying the presence of plastics in the gastrointestinal tract of marine animals [293,294], causing concern worldwide to MP transport to humans [87–90]. Microplastic particle uptake by fish can be passive (e.g., gill water filtration) and active through ingestion both of particles themselves or contaminated prey [295].

The principal works that gave microplastics concentration as the number of particles/g or particles/individual are reported in Table 4, with a focus on marine species, analytical method used, size range, and polymer type.

Marine Species	Analytical Method	Abundance Average (Range)	Size Range	Polymer Type	Ref.
Whales	MO and FTIR	-	0.3–7 mm	RY, PES, acrylic, PP, PE	[296]
Atlantic Herring, Sprat, Common Dab, and Whiting	MO and FTIR	-	0.300– 0.400 mm	PMMA	[297]
European anchovies	MO and RMS	-	0.124– 0.438 mm	PE, styrene/acryl onitrile	[298]
Bivalves (Mytilus edulis and Crassostrea gigas)	MO and RMS	0.36–0.47 particles/g	>0.005 mm	-	[290]
Mussels (Mytilus edulis) and lugworms (Arenicola marina)	MO and RMS	0.2–1.2 particles/g	0.015–1 mm	-	[293]
Dogfish, hake, red mullet	МО	1.56 particles/individ ual	0.38–3.1 mm	-	[299]
Semipelagic fish	МО	3.75 (2.47–4.89) particles/individ ual	0.5 mm	-	[300]
Pelagic and demersal fish	MO and FTIR	1.90 particles/individ ual	0.13–14.3 mm	PA, cellulose, RY	[301]
Benthic and pelagic fish	MO and FTIR	0.27 particles/individ ual	0.217–4.81 (average 2.11) mm	PP, PE, ALK, RY, PES, NY	[302]
Different fish species	MO and FTIR	2.36 particles/individ ual	average 0.656 mm	polystyrene: isoprene, PE, PP	[75]
Red mullet (Mullus surmuletus)	MO and FTIR	(0.32–0.68) particles/individ ual	-	PET, CPH, Polyacrylate, PAN	[303]
Deep benthic invertebrates	MO and FTIR	1.582 particles/g	0.023–6.25 (average 1.191) mm	ALK, PES	[139]

Table 4. Occurrence and quantification of microplastics in marine species.

Benthic	MO and	(1.7-47.0)	0.05–5	PP, PE, PS,	[121]
organisms	FIIK	0 9–4 6	mm	PEI, NI	
Mussels (Mytilus edulis)	MO and FTIR	particles/individ ual 1.5–7.6 particles/g	0.033–4.7 mm	CPH, PET, PES	[304]
Thamnaconus septentrional	MO and FTIR	1.1–7.2 particles/individ ual 0.2–17.2 particles/g 2.5	0.04–5 mm	CPH, PET, PES	[305]
Different fish species	MO and FTIR	particles/individ	0.2–5 mm	PE, PP	[136]
Bivalve (oyster, mussel, Manila clam and scallop)	MO and FTIR	0.97 (0–2.8) particles/individ ual 0.15 (0–1.8) particles/g	0.1–0.2 mm	PE, PP, PS, PES, PEVA, PET, PU	[306]
Deep-sea fish	MO and FTIR	Stomach: 1.96 particles/individ ual and 1.56 particles/g; Intestine: 1.77 particles/individ ual and 4.89 particles/g 0.39	<1mm	CPH, PA, PET,	[307]
Indian white shrimps	MO and FTIR	particles/individ ual 0.04 particles/g	0.157– 2.785 mm	PA, PES, PE, PP	[308]

Abbreviations: FTIR: Fourier-Transform Infrared Spectroscopy; RMS: Raman Spectroscopy; MO: optical microscope; PE: polyethylene; PP: polypropylene; PS: polystyrene; PET: polyethylene terephthalate; ABS: acrylonitrile butadiene styrene; PVA: polyvinyl alcohol; PVAC: polyvinyl acetate; PVC: polyvinyl chloride; PES: polyester; PAN: polyacrylonitrile; PA: polyamide; PTFE: polyetrafluoroethylene; PU: polyurethane; NY: Nylon; RY: Rayon; ALK: alkyd resin; CPE: chlorinated polyethylene; NR: nitrile rubber; LLDP/Oct: linear low-density polyethylene-octene copolymer; EVOH: ethylene vinyl alcohol copolymer; TPU: thermoplastic polyurethane; PVS: polyvinyl sulfate; PAS: polyacrylate-styrene; SR: synthetic rubber; PR: phenoxy resin; PCL: polycaprolactone; PEA: polyethylacrylate; PEVA: polyethylene-vinyl acetate; EPM: polyethylene-propylene; EP: epoxy resin; CPH: cellophane.

Researches have shown that mussels [304], shellfish (including crustaceans and bivalves), commercial fish [302], and also cultured organisms [309] are often contaminated with microplastics. Moreover, several papers identify potable water, bottled water and beverages [310–314] as potential sources of MP. Recently, annual microplastics consumption, expressed as particles/person, was estimated to be 11,000 from shellfish [290], 37–1000 from sea salt [315]. In addition, Cox et al. [292] evaluated the total annual microplastics consumption based on microplastic presence in commonly eaten food and their daily intake in the American diet. The annual estimated intake of microplastics depends on age and sex, ranging from 39,000 to 52,000 particles/person. Moreover, a great increase in the amount of microplastics intake is observed in individuals who drink water only from plastic bottles with respect to those who consume only tap water (additional 90,000 annual microplastics particles compared to 4000).

Notwithstanding the above discussed works assert an elevated microplastics intake, a deeper investigation on the translocation and accumulation of MP in the human body is needed to better characterize their potential to harm humans. In fact, MP occurrence and detection in fluids of the human body remains a poorly investigated field, but it could be very useful to assess the interaction of plastic particles with human apparatus.

4. Conclusions

Plastic microparticles accumulated over the environment increase stress for the marine, freshwater, and terrestrial ecosystems. In this review, we analyzed the most recent literature related to microplastics in the aquatic environment, highlighting contamination of water (river, lake, wastewater, seafood), and the strength and limitations of current analytical methods employed in microplastics detection and their transport to humans.

Based on our investigation of literature data concerning microplastic pollution in the water environment, some conclusions could be made:

- As inferred from the data reported above, a standard procedure for sampling, counting and confirmming the presence of microplastic is still not present in the literature. These aspects result in concentrations expressed in different units and also diverse data obtained from the same authors depending on the sampling method. Consequently, the comparison between MP abundance values reported in various works regarding the water compartment is sometimes difficult or impossible. We suggest improving studies in developing standardized protocols to collect, process, and analyze samples in order to obtain data useful for assessing a real contamination issue.
- Although FT-IR and Raman Spectroscopy are useful techniques for identifying the
 polymeric composition of MP and Pyr-GC-MS and other MS-based methodologies
 may be applied to monitor microplastics and to indicate the type of polymer, we
 think that interlaboratory analyses should be performed to ensure comparability of
 chemical characterization and quantification data and suggest the more proper
 analytical method based on the matrix sampled and particle sizes.
- Most of the studies showed evidence of nano and microplastics accumulation in sea products, which are part of the human diet, and recently also in drinking water and related beverages. However, in our opinion, a deeper investigation of the transport and accumulation of MP in humans, and the consequent effects on human health, is needed.

In conclusion, we believe that in this field future research efforts should focus on developing standardized and reproducible methodologies and analytical strategies capable of counting and characterizing MP in all environmental matrices, especially fluids of the human body, which are poorly investigated, to better characterize their risk for humans.

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