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Sources, Transport, Measurement and Impact of Nano and Microplastics in Urban Watersheds

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Abstract

The growing and pervasive presence of plastic pollution has attracted considerable interest in recent years, especially small ($< 5\text{ mm}$ and $> 100\text{ nm}$) plastic particles known as ‘microplastics’ (MPs). These particles are thought to pose greater risks than larger plastic debris as they are more likely to be ingested by many species. Their widespread presence may pose a threat to marine organisms globally. Contamination of freshwater and terrestrial systems also has been reported, and more recent studies indicate the presence of even smaller ($\leq 100\text{ nm}$) plastic particles, termed ‘nanoplastics’ (NPIs), that may have unique risks due to their nanoscale. Most of the nano and microplastic (N&MP) pollution in marine environments is assumed to originate from land-based sources, but their sources, transport routes, and transformations are uncertain. Information on freshwater and terrestrial systems is lacking, and data on NPI pollution is particularly sparse. The shortage of systematic studies of freshwater and terrestrial systems is a critical research gap because estimates of plastic release into these systems are much higher than those for oceans. As most plastic pollution originates in urban environments, studies of urban watersheds, particularly those with high population densities and industrial activities, are especially relevant with respect to source apportionment. Released plastic debris is transported in water, soil, and air. It can be exchanged between environmental compartments, adsorb toxic chemicals, and ultimately be carried long distances, with potential to cause both physical and chemical harm to a multitude of species. Unfortunately, these processes are not well understood. Measurement challenges and a lack of standardized methods has slowed progress in determining the environmental prevalence and impacts of N&MPs.

An overall aim of this project is to report the sources and abundances of N&MPs in urban watersheds. Their fate, transport, transformations, and potential impacts are discussed. Monitoring methods and their limitations also are reviewed, knowing that identifying N&MPs

and their urban/industrial sources is necessary to reduce their presence in all environments. A wide variety of sample collection, preparation, and analysis procedures have been employed to monitor N&MPs. Method standardization is a critical research need. In this work, stable carbon isotope ratio mass spectrometry, attenuated total reflectance Fourier transform infrared spectroscopy, and optical microscopy coupled with Raman spectroscopy were investigated as complementary techniques for characterization of common plastics from different sources. Isotope ratio mass spectrometry (IRMS) has been applied to study the origins of organic matter in the environment. Our results show a significant difference between the IRMS results for plant-derived and petroleum-based polymers. Differences also were found between plastic items composed of the same polymer, both for items from different countries and the same country. The IRMS results for recycled materials were either comparable to nonrecycled products or much higher. In addition, the isotopic abundances of some plastics changed after exposure to UV light. Results of the three techniques and their advantages and limitations are discussed.

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1. Introduction

The growing environmental burden and risks of plastic pollution has been a topic of considerable interest in recent years, especially small plastic particles known as microplastics (>100 nm and <5 mm) and nanoplastics (<100 nm). The potential risks of small plastic particles are closely tied to the broader problem of growing plastic production and waste because much of this pollution results from the breakdown of macroplastic items. The total production of primary plastics from 1950 through 2017 was estimated at 8,300 million metric tons (Mt). As of 2015, about 6,300 Mt of plastic waste was generated, 79% of which was discarded in landfills or the environment (Geyer et al 2017). Nano and microplastics (N&MPs) are generally perceived as posing greater risks than larger plastic debris because they are often invisible and more likely to be ingested by a much wider range of species (SAPEA 2019). Numerous studies have shown the widespread presence of MPs in the marine environment, reportedly posing a threat to marine organisms globally (e.g., Hidalgo-Ruz et al. 2012, GESAMP 2015). Widespread contamination of freshwater and terrestrial systems also has been reported (Wagner et al. 2014; Dris et al. 2015a, 2015b, 2016, 2017; Horton et al. 2017a, 2017b; Gasperi et al. 2018; Allen 2019; Strungaru et al. 2019; Triebkorn et al. 2019). More recent studies have indicated the environmental presence of nanoplastics (NPIs), which may pose different risks than MPs due to their small size. They have much higher specific surface area for sorption of environmental contaminants and can translocate to other body tissues after ingestion. These and other issues on this emerging topic are discussed in following sections.

Studies of the plastic sources and sinks in urban watersheds, and of N&MP transport and fate, are critical to assessing the environmental impact of plastic pollution. A large majority (estimated at 80%) of the N&MP pollution in marine environments is assumed to originate from land-based sources, but the actual sources and transport routes are uncertain (Jambeck et al. 2015, Geyer et al. 2017, Rochman 2018). Information on NPI pollution is particularly sparse

due to the measurement challenges of nanoscale particles (Lehner et al. 2019). The shortage of systematic studies of freshwater and terrestrial systems is alarming, especially because estimates of plastic release into these systems are much higher than those for oceans (4–23 times larger by mass) (Horton et al. 2017a). As most plastic waste originates in urban environments, studies of urban watersheds, particularly those with high population densities and industrial activities, are especially relevant with respect to source apportionment and transport of plastic pollution. Plastic debris/particles can be transported in water, soil, and air. These pollutants can exchange between environmental compartments, sorb toxic chemicals, and ultimately be carried long distances by processes that are not well understood.

An overall aim of this project is to report the sources and abundances of N&MPs in urban watersheds. Their fate, transport, transformations, and potential impacts are discussed. Monitoring methods and their limitations also are reviewed. Overviews of these topics are presented in Sections 1-5. The focus is recent findings of N&MPs in urban watersheds and monitoring methods, knowing that identifying N&MPs and their urban/industrial sources is necessary to reduce their presence in all environments. To this end, a further objective was to evaluate several complementary analytical techniques for characterizing MPs. A wide variety of sample collection, preparation, and analysis procedures have been employed. Method standardization is a critical research need as this gap has slowed advances in our overall knowledge of the potential environmental impacts and human health risks of N&MPs. Methods based on stable carbon isotope ratio mass spectrometry (IRMS), attenuated total reflectance (ATR) Fourier transform infrared (ATR-FTIR) spectroscopy, and optical microscopy coupled with Raman spectroscopy (μ -Raman) were investigated as complementary techniques for characterization of common plastics from different sources. In particular, the utility of IRMS for tracking environmental plastic debris was investigated. Results of these studies are presented in Section 6.

1.1 Growing plastic production and pollutants

Large-scale plastic production began in the 1950s and is increasing exponentially (Geyer et al. 2017). In 2017, about 348 Mt were produced globally. This global production represents trillions of dollars in economic returns, with China being the top producer (29.4%) (PlasticsEurope 2018). Based on current trends in production and waste management, another 12–33 billion metric tons of plastic will occupy landfills or pollute the environment by 2050 (Rochman et al. 2013a, Geyer et al. 2017, Rochman 2018). Of the total plastic waste (6.3 billion metric tons) generated since 1950, 12% was incinerated and only 9% was recycled as of 2015 (Geyer et al. 2017). The remaining 79% accumulated as plastic litter, with oceans being the final sink (Geyer et al. 2017, Rochman et al. 2018). Polyethylene (PE) (high, low, and linear low density: HDPE, LDPE, LLDPE), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC), polyethylene terephthalate (PET), and polyurethane (PUR) resins; and polyester (PES), polyamide, and acrylic (PP&A) fibers represent 92% of all plastics ever made (Geyer et al. 2017). The total amount of non-fiber plastics (i.e., those typically not used for textiles) was estimated at 7,300 Mt, while PP&A fibers contributed 1,000 Mt (Table 1-1). The largest percentages of the total non-fiber and fiber plastics produced were PE (36%) and PES (70%, mainly PET), respectively. Non-fiber plastics were estimated to be 93% polymer resin and 7% additives (e.g., plasticizers, flame retardants, colorants) by mass. The most common types of plastics reported for environmental studies were PE, PP, PS, PVC, and PET (Rochman et al. 2013b, de Haan et al. 2019, Koelmans et al. 2019).

1.2 Micro and nanoplastic pollutants

Increasing plastic demand and global production of a myriad of plastic types and products is having a cumulative effect on plastic pollution, including a rise in the environmental levels of many types of small plastic particles that may better be viewed as a diverse suite of contaminants (Rochman et al. 2019). Left unchecked, the environmental burdens of N&MPs are expected to rise sharply due to the persistence of plastics and growing size of environmental reservoirs. Microplastics are generally defined as plastic particles < 5 mm but > 100 nm in their largest dimension, while particles ≤ 100 nm are considered NPIs (e.g., GESAMP 2015, Horton et al. 2017a). Particle classification, formation, and potential toxicity are discussed below.

1.2.1 Microplastics

Microplastics have been categorized as primary or secondary. Those manufactured at the μm scale are considered primary MPs, which includes ‘microbeads’ (PE) in cosmetics, plastic particles (acrylic or PES) in industrial abrasives, and pre-production plastic pellets called ‘nurdles.’ In contrast, secondary MPs are formed by the continuous environmental degradation of larger plastic items, through processes that affect polymer structure and reactivity (Andrady 2011, Koelmans et al. 2015, Lambert and Wagner 2016, Gigault et al. 2016, Lehner et al. 2019). The continued weathering/aging of plastics is expected to yield secondary N&MPs. Polymer breakdown can occur by several processes including hydrolysis, photodegradation (ultraviolet [UV] exposure), abrasion, temperature fluctuations, and biological and chemical degradation (e.g., Thompson 2004, Browne et al. 2007, Barnes et al. 2009, Andrady 2011, Browne et al. 2011, Imhof et al. 2012, Lehner et al. 2019). Multiple stressors can act simultaneously. For example, prolonged UV exposure can cause brittleness due to changes in polymer structure (e.g., carbonyl formation), which increases mechanical degradation (Mattsson et al. 2015). Some polymers are more susceptible to certain stressors. In particular,

Table 1-1. Estimates^a of major plastic types and total amounts produced since 1950.

Polymer	Code	Total plastics produced since 1950 (Mt) ^a	Mass % of total nonfiber or fiber since 1950 ^a	Total 2015 primary production (Mt)	% of total produced in 2015	Applications
Nonfiber plastics^b						
Polyethylene: High density (HDPE), Low density (LD), Linear low density (LLDPE)	2 (HD), 4 (LD & LLD)	2628	36	64 (LD and LLD) 52 (HDPE)	17 14	Plastic bags, storage containers
Polypropylene (PP)	5	1533	21	68	18	Rope, bottle caps, gear, strapping
Polyvinylchloride (PVC)	3	876	12	38	10	Pipe, film, containers (69% used in building & construction)
Polystyrene: expanded, extruded (EPS, XPS)	6	<730	<10	25	7	EPS: cooler boxes, cups, floats XPS: containers
Polyethylene Terephthalate (PET, PETE)	1	<730	<10	33	9	Bottles, strapping
Polyurethane (PUR)		<730	<10	27	7	Durable foams (insulation, etc.)
Fiber plastics						
Polyester, polyamide (PA), & acrylic (PP&A)		1000	70% PES, 30% PA&A	59	15	Textiles, nets, ropes

^aData from Geyer et al. 2017. Estimated total production in 2015 was 382 million metric tons (Mt) of primary (manufactured from virgin materials) plastics (407 Mt including additives). Generated plastic waste in 2015 estimated at 302 Mt. ^b42% used for packaging (mainly PE, PP, and PET). ‘Other’ plastics (2015) estimated at 16 Mt (4%) and additives at 25 Mt (9%).

PS and common polyolefins such as PE and PP are more prone to UV breakdown (Feldman 1984, Gijssman et al. 1996, Song et al. 2017). Polymer weathering can produce large amounts of secondary MPs: up to 10^6 MP particles/km² were found in subtropical gyres (Law et al. 2010, 2014). Secondary MPs mainly consist of many polymers, while primary MPs typically contain PE, PS, and PP (Browne et al. 2007).

1.2.2 Nanoplastics

In addition to MPs, the risks of NPLs is a growing concern (Andrady 2011, Cozar et al. 2014, Mattsson et al. 2015, Ter Halle et al. 2016, Jahnke et al. 2017, Alimi et al. 2018, Lehner et al. 2019). Two definitions of NPLs have been proposed, based on the upper size limit: one at 1,000 nm (e.g., Gigault et al. 2018) and another at 100 nm (Cole et al. 2015, Cole and Galloway 2015, Crawford and Quinn, 2017, da Costa et al. 2016, Koelmans et al. 2015, Ter Halle et al. 2017). The lower cutoff follows an official EU definition of nanomaterials (EC 2018).¹ However, the relevance of a 100-nm cutoff to plastics is questionable. Gigault et al. (2018) proposed the following definition of NPLs: “plastic particles in a size range of 1–1,000 nm that can exhibit colloidal behavior and result from degradation of industrial plastics, as manufacturing byproducts or from use of a plastic object” (Bouwmeester et al. 2015). Like nanomaterials, NPLs can be generated by both intentional (manufactured) and unintentional (byproducts) processes, but the latter definition applies only to degradation products.

And as with nanomaterials, concerns have been raised about NPLs in consumer products, and a lack of regulations on their use/presence (Pekka 2008, Kessler 2011, Hansen and Baun 2012). Nanoplastics are used in coatings, paints, pharmaceuticals, electronics, and research (Koelmans et al. 2015, Hernandez et al 2017b). And manufacturing processes such as thermal

¹ “A natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range of 1–100 nm.”

cutting of PS foam and 3D printing are known to release NPLs (Zhang et al. 2012, Stephens et al. 2013, Stefaniak et al. 2018). Over time, environmental concentrations of NPLs are expected to increase due to their use in a variety of products (Hernandez et al. 2017b), release as by-products during manufacturing (Stephens et al. 2013, Zhang et al. 2012), and the enormous potential for degradation of macroplastic and MP debris (Koelmans et al. 2015, Lee et al. 2013, Song et al. 2017, Besseling et al. 2019).

While studies of MPs in freshwater systems are increasingly being reported (Browne et al. 2010, 2011; Dris et al. 2015a, 2015b, 2016; Eerkes-Medrano et al. 2015; Rillig et al. 2017a, 2017b), environmental studies of NPLs are lacking (Koelmans et al. 2015, Chae and An 2017, Hernandez 2017b, Alimi et al. 2018, Besseling et al. 2019, Lehner et al. 2019). The presence of NPLs in the environment is generally assumed, but data are lacking because their determination in environmental matrices poses difficult measurement challenges. No studies of NPLs in aquatic or soil systems were reported in recent reviews (Alimi et al. 2018, Lehner et al. 2019). A lack of studies on environmental NPLs mainly relates to difficulties in their recovery and detection in complex matrices (Koelmans et al. 2015, Chae and An 2017, Revel et al. 2018, Nguyen et al. 2019).

Although data on environmental NPL loads are not yet available, their presence has been widely hypothesized (Kessler 2011, Chae and An 2017, Mattsson et al. 2015, Horton et al. 2017a, da Costa et al. 2016), and several laboratory studies indicate significant environmental releases (Koelmans et al. 2015, Hernandez et al. 2017b, da Costa et al. 2018). For example, Hernandez et al. (2017b) reported that consumer products could be an important source of NPLs in water and soil. Products containing microbeads (e.g., scrubs, shampoos) are processed by high-shear mixers. Microbeads (PE) were intentionally added to the products tested (three facial scrubs), but the NPLs were unintentional. Although their source was not determined, degradation of MPs by high-shear mixing during product manufacture was hypothesized,

and/or a broad size distribution of the added bulk material (Hernandez et al. 2017b). Particles in the scrubs were sequentially filtered and characterized by electron microscopy and spectroscopic techniques. Analyses confirmed large amounts of PE NPLs: at least 300 billion NPLs/g.

Products containing microbeads (5- μm to 1-mm diameter) have been banned in the United States (US), Canada, and the European Union (Burton 2015, Rochman et al. 2015b) but widely available elsewhere. Given the continued use, their impact may persist for some time (Burton 2015). The finding of NPLs in products with added microbeads (Hernandez et al. 2017b) is noteworthy as it demonstrates, in general, the possibility of NPLs in products manufactured by processes that degrade plastics. Other laboratory studies have demonstrated NPL formation by different mechanisms. For example, a study of PS (cup lid) weathering in a simulated marine environment demonstrated NPL and MP (30 to 2000 nm) releases (Lambert and Wagner 2016). Another possible source may be marine species that ingest MPs. For example, evidence of digestive formation of “NPLs” (using the $< 1 \mu\text{m}$ definition) from MPs (31.5 μm) by a planktonic crustacean has been reported (Dawson et al. 2018). If significant uptake by biota occurs (Hermsen et al., 2018), biota may have a major impact on N&MP transport and transformation. The role of ingestion, migration, and egestion in the environmental plastic burden is currently unknown.

Ultimately, direct releases and degradation of many types of plastics produces diverse N&MP pollutants (Bouwmeester et al. 2015, Cozar et al. 2014), with different environmental impacts for NPLs (Velzeboer et al. 2014). Although environmental levels are not yet known, NPLs may be as pervasive as MPs and larger plastic particles (Alimi et al. 2018). Some studies indicate that the average size of environmental MPs appears to be decreasing (Rocha-Santos and Duarte 2015). The concentrations of small MPs and NPLs are expected to increase, which may pose unique risks due to their small size.

2. Ecotoxicity and human exposure risks

While the environmental impact of plastic waste has received considerable attention, relatively little is known about the extent and impacts of trophic transfer of N&MPs. Similarly, the extent of N&MP contamination in food and beverages, and the human health implications are not yet known. Ingested particles have the potential to cause direct physical harm to a wide variety of species due to particle effects, and indirectly through bioaccumulation of associated toxins. An overview of these issues is provided below.

2.1 Ecotoxicity

Wang et al. (2019) reviewed the ecotoxicological effects of MPs on the aquatic food web and suggested that their pervasiveness may cause adverse effects in a wide range of aquatic biota. However, most laboratory studies have focused on ingestion by aquatic fauna at concentrations that greatly exceeded environmental levels (de Sá et al. 2018, Triebkorn et al. 2019). Fang et al. (2018) conducted a study of benthic organisms in Arctic and sub-Arctic regions. They reported MP sizes from 0.10–1.50 mm, and that fibers were dominant (87%) in trophic transfer in the benthic food web. Microplastic ingestion by riverine macroinvertebrates also has been reported (Windsor et al. 2019). Prata et al. (2019a) reviewed the impacts of MPs on microalgae. They reported negative effects at the low parts per million range (ppm) range, including inhibited growth, lowered chlorophyll and photosynthesis, and oxidative stress, but that microalgae appeared to recover by adaptive responses, and that no harm was expected at current environmental levels. However, MPs could disrupt microalgae populations by reducing absorption of available nutrients or the population of predator species. Possible effects depend on polymer type and properties that are not well understood, though smaller size and positive surface charge are associated with higher toxicity. Besseling et al. (2019) performed a ‘proof of concept’ risk assessment on the ecological risks of aquatic N&MPs, accounting for diverse

materials. They reviewed current knowledge of the aquatic environment, on occurrence, measurement, modeling, fate, exposure, effects, and effect thresholds. They concluded that organisms in near-shore surface waters could be at risk, and that the increasing concentrations of N&MPs may eventually pose more widespread risks, especially for small particles. As an example, based on degradation of 5-mm MP spheres into 100-nm spheres, the authors estimated future NPI number concentrations 17 orders of magnitude higher than those of MPs.

2.2 Biological effects

Inhalation/ingestion exposure to N&MPs may result in inflammation, chemical leaching, and/or particle translocation to other tissues, depending on particle size, shape, and composition. Small plastic particles may accumulate in tissues, and release monomers, toxic additives, and pollutants sequestered from the environment (e.g., heavy metals, persistent organic pollutants [POPs]) (Rochman et al. 2014, Koelmans et al. 2016, Ziajahromi et al. 2016, Wang J. et al. 2017, Wright and Kelly 2017, Li et al.). Inflammatory responses to plastic dusts have been observed in animal models but the relevance of these studies to humans is unclear. Evidence of inflammatory effects in workers exposed to plastic fibers exists; however, how these studies relate to chronic, relatively low-dose exposures is uncertain. Further, the potential impact of particle shape (fibrous vs. nonfibrous) and size on toxicity is not yet known (SAPEA 2019). As with other High-Aspect Ratio Materials (HARMs, e.g., asbestos and asbestos-like materials, carbon nanotubes and nanofibers), there is concern that N&MP fibers may have adverse effects, depending on dimensions, dose, and durability.

Small MPs and NPIs can translocate from the gut to the blood, potentially affecting a wide range of organisms. (Browne et al. 2008). Studies of rodents and humans have shown that PVC and PS particles < 150 µm can translocate from the gut to the lymph and circulatory systems, especially smaller particles (Browne et al. 2008). Similar findings were obtained in a

study of mussels exposed to small (3.0 or 9.6 μm) PS particles in seawater, which translocated from the gut to the circulatory system within 3 days and persisted 48 days. Particle size influenced translocation, with over 60% more smaller (3.0 μm) MPs in the circulatory fluid than larger (9.6 μm) MPs, indicating that as particle size decreases, the potential for accumulation in tissue increases. The study results also are consistent with *in vitro* studies using colonic tissue of rats, which showed fast translocation of 14 nm PS particles relative to larger (415 and 1000 nm) PS particles (Browne et al. 2008). The short-term, pulse exposure caused no significant biological effects, but plastics are persistent and diverse pollutants. Studies on a wide range of organisms, polymers, particles sizes, and exposure durations were recommended to assess potential biological effects of N&MPs (Browne et al. 2008, Wright and Kelly 2017), especially for NPLs. The unique risks of NPLs are discussed below (2.2.1).

2.2.1 Nanoplastics

Research on nanomaterials has shown that nanoscale particles can have markedly different properties than macroscale (bulk) forms of the same material. The cytotoxic effects (e.g., Lewinski et al. 2008, AshaRani et al. 2009) and potential environmental impacts (e.g., Klaine et al. 2008, Lead et al. 2018) of nanomaterials are well documented. Similarly, NPLs may pose greater risks than their MP counterparts. Small size, combined with hydrophobicity, may result in cell entry and cytotoxicity (Hoet et al. 2004, Oberdörster et al. 2005, Bothun 2008, Rasch et al. 2010, Yousefi 2016a, 2016b, Lehner et al. 2019).

Based on the definition of ‘nanoparticle,’ manufactured nanoscale (≤ 100 nm diameter) plastics have been used in ecotoxicological studies, polystyrene latex (PSL) beads in particular (Lu et al. 2016, Bergami et al. 2017, Chen et al. 2017, Nolte et al. 2017, Rist et al. 2017, Lehner et al. 2019). These studies may be relevant to PSL nanoparticles, but their relevance to environmental NPLs is unclear (e.g., Phuong et al. 2016, Lehner et al. 2019). Nanomaterials are

produced differently and not representative of environmental NPLs. They have specific properties and sizes by design (Colvin 2003, Ju-Nam and Lead 2008, Brar et al. 2010, Weinberg et al. 2011, ISO/TS 80004-1:2015; Gigault et al. 2016, 2018), while environmental NPLs have highly polydisperse particle sizes (Gigault et al. 2016, 2018; Lambert and Wagner 2016; Ter Halle et al. 2017) and consist of many plastics. Also, NPLs typically occur as colloids with heterogeneous aggregates of natural/anthropogenic materials (Hotze et al. 2010, Hüffer et al. 2017), and aggregate structures and surfaces depend on environmental conditions (e.g., pH, salinity, organic matter [Li et al. 2018b]). Their presence in polluted waters may pose risks, especially for NPLs, due to sorption of a variety of toxins that may subsequently transfer to organisms that ingest these particles, as discussed below (2.2.2).

2.2.2 Pollutant transfer

Another concern about N&MPs is their potential to serve as vectors for associated toxins (e.g., Teuten et al. 2007, 2009; Vethaak and Leslie 2016; Curren and Leong 2019), an issue not addressed by most toxicity studies. Transfer of particle-borne toxins in the planktonic food web may affect the fate of numerous toxic substances (Setälä et al. 2014). Ingested plastic pollution has the potential to release and concentrate associated environmental contaminants in tissues. Although sorption of pollutants by N&MPs could enhance their bioavailability, it also may reduce it. For example, in sediments the presence of MPs was reported to reduce transfer of hydrophobic organic compounds to sediment-ingesting organisms (Beckingham and Ghosh 2017). However, because sediment and suspended particles in aquatic environments are mainly naturally occurring organic and inorganic matter, pollutant transfer to aquatic organisms by dietary intake is expected to be dominated by these components rather than N&MPs (Beckingham and Ghosh 2017). The hypothesis that MP ingestion leads to bioaccumulation of POPs has played a major role in studies over the past decade. While some investigators believe

that MPs increase uptake, others do not, and both groups have data to support their positions (Besseling et al. 2019). According to a study by Besseling et al. (2019), N&MPs could significantly increase chemical exposure in some ‘hotspot’ locations, when they are a relatively large fraction of an organism’s diet and the pollutant concentration gradient favors transfer to the organism.

Because small MPs and NPIs can accumulate environmental pollutants, depending on the particle type and specific environment, their presence in polluted waters (e.g., wastewater) could result in substantial sequestering of pollutants, with potential for subsequent transfer to a variety of species. Transfer by NPIs may be more substantial than that from MPs due to their high affinity for POPs (Besseling et al. 2019), and their much higher surface area for sorption of organic compounds, metals, and/or pathogens (Eriksson and Burton 2003; Murray and Cowie 2011; Boerger et al. 2010; Rochman et al. 2013b, 2013c, 2015a; Eerkes-Medrano et al. 2015; Li et al. 2018a; Curren and Leong 2019; Li et al. 2019). Further, their small size allows them to reach other tissues more readily (Koelmans et al. 2015). They can enter cells by endocytosis, penetrate tissues, translocate from the digestive to the circulatory system, and cross the blood-brain barrier (Browne et al. 2008, Besseling et al. 2019). However, to assess the overall risks of exposure to N&MPs, it is important to consider their chemical transfer risks relative to other uptake routes. In most ecologically-relevant environments, chemical uptake from N&MPs is negligible relative to other sources (air, food, water) (Koelmans et al. 2016, Beekingham and Ghosh 2017, Horton et al. 2017a, Rehse et al. 2018). It also is important to distinguish the chemical risks from particle effects (EPA, 2016, Koelmans 2017, Besseling et al. 2017). Nevertheless, in ‘hotspot’ locations, or if N&MPs concentrations increase significantly, increased bioaccumulation is anticipated (Chen et al., 2018; Diepens and Koelmans 2018, Besseling et al. 2019).

2.3 Dietary intake in humans

Quantitative exposure assessments are essential to understanding the potential risks of N&MPs. Although N&MPs are pervasive in the environment, and dietary and airborne exposures are known exposure routes for humans, very little is currently known about their daily intake, biodistribution, and persistence in physiological environments. The extent of contamination of the food supply is uncertain, but a variety of MPs and plastic-related contaminants have been found in seafood (Van Cauwenberghe and Janssen 2014, Li et al. 2015, 2016), honey and sugar (Liebezeit and Liebezeit 2013), table salts (Yang et al. 2015, Karami et al. 2017), beer (Liebezeit and Liebezeit 2014), bottled water (Mintenig et al. 2014, Orb Media 2017, Schymanski et al. 2018, Oßmann et al. 2018), and drinking water and its freshwater (Koelmans et al. 2019) and groundwater sources (Mintenig et al. 2019). Textiles and packaging can be sources of N&MPs/additives that contaminate food and beverages. Plastic parts in water delivery systems also may contribute MPs (EPA 2017, Mintenig et al. 2019), though this route is uncertain. Given the widespread contamination, it is not surprising that MPs were found in all human stool samples collected in a preliminary study of eight international participants (Parker 2018), attributed to bottled water consumed as part of the study. The source of PET particles in stool samples may have been plastic bottles and food packaging, but it also could have been airborne fibers (from clothing, carpets, or other sources) that settled onto food. For example, Catarino et al. (2018) reported that the risk of plastic consumption to humans was greater from exposure to airborne fibers than seafood (muscles).

Cox et al. (2019) recently evaluated human consumption of MPs, based on commonly consumed foods and drinking water in the ‘American’ diet and the potential for ingestion of inhaled MPs. Their evaluation included 402 data points from 26 studies, representing over 3,600 samples. Considering approximately 15% of Americans’ caloric intake, an annual consumption of 39,000–52,000 MPs was estimated, depending on the rate of human consumption. Estimates rose to 74,000 and 121,000 when inhalation was included in the

estimate. A further increase was estimated for individuals that met the recommended water intake by drinking only bottled water, which added 90,000 MPs annually relative to 4,000 MPs for tap water only. Though these estimates are reportedly subject to large variation, the authors indicated that they are likely underestimated due to methodological and data limitations.

3. Urban watersheds: sources and transport of plastics

Land-based sources were estimated to account for up to 80% of the annual plastic pollution released to oceans (12.2 Mt/year), with primary MPs (0.5–1.4 Mt/year) accounting for about 9% of the land-based input (5.4–15.2 Mt/year) (Eunomia 2016). Approximately 94% of the current stock (total) of marine plastic pollution is at the seabed (70 kg/km² on average), while about 1% is at the surface (18 kg/km²), with an average global surface concentration just under 1 kg/km². Surface concentrations are higher at specific mid-ocean points, the highest being found in the North Pacific Gyre (18 kg/km²). Beach contamination is much higher, with an estimated global average of 2,000 kg/km² (Eunomia 2016).

Land-based sources of N&MPs, especially urban watersheds, also contaminate lakes and rivers globally. The atmosphere and soil are important sources in freshwaters, but relatively little is known about the mass fluxes between these compartments, including the extent to which flooding impacts transport. Studies of freshwater and terrestrial systems in urban/industrialized areas are especially relevant because of their proximity to plastic sources and initial transport media. Major MP sources in urban environments include microfibers from synthetic textiles and tire wear. Wastewater treatment plants (WWTPs) can effectively remove MPs in influents (e.g., 87-99 % [Rezania et al. 2018]), but they release N&MPs to aquatic and terrestrial ecosystems due to the high discharge volumes, and through application of contaminated sludge on agricultural land (e.g., Bayo et al. 2016; Horton et al. 2017a; Rillig et

al. 2017a, 2017b). The sources, transport, and environmental levels of N&MPs in urban watersheds are discussed below.

3.1 Microplastic sources and transport

Microplastic particles can be released directly to the environment, and the initial transport medium depends on the source. Major sources identified on a global scale are shown in Figure 3-1. These include synthetic textiles (35%), tire wear (28%), city dust (24%), road markings/dust (7%), marine coatings (3.7%), microbeads (2%), and plastic pellets (0.3%) (Boucher and Friot 2017). The category ‘city dust’ is a composite of common sources in urban environments, including MPs generated through abrasion, weathering, and pouring. Examples include MPs from abrasion of synthetic soles (footwear), plastic utensils, and infrastructure (e.g., household dust, artificial turf, building coatings); abrasive blasting; and pouring powders.

Figure 3-1. Sources of microplastic pollutants (Boucher and Friot 2017).

The many sources of MPs in urban watersheds contaminate household sewage, solid waste, storm water, wastewater and sewage sludge, agricultural runoff (e.g., from sludge-

treated soils), industrial effluents, road drainage ditches, and landfill leachates (Hoellein et al. 2014; Bayo et al. 2016; Boucher and Friot 2017; EPA 2017; Horton et al. 2017a, 2017b). Plastic pollution (aquatic and soil) also occurs through accidental spills (e.g., transportation accidents, leakages) and by intentional dumping. Spills of MP pellets (nurdles) at industrial facilities can enter drains and waterways, and eventually marine environments (Entwistle 2018). The relative contribution of tire wear particles to the total amount of plastics reaching oceans was estimated at 5–10%. Tire wear also contributes to air pollution globally: 3–7% of the particulate matter (PM_{2.5}) in air was attributed to tire wear. As with other MPs, debris from tires (Kole et al. 2017) can enter the food chain, but air pollution is likely the main exposure route.

Synthetic textile fibers are a major contributor to secondary MPs in aquatic and terrestrial systems (Browne et al. 2011; Dris et al. 2015a, 2015b, 2016, 2017; Dubaish and Liebezeit 2013; Napper and Thompson 2016; Hartline et al. 2016; Boucher and Friot 2017; Carney-Almroth et al. 2017; Hernandez et al. 2017a; Salvador Cesa et al. 2017). These microfibers originate from wastewater, especially in highly populated/urbanized areas; diverse non-point sources; and other aquatic and terrestrial systems. Commercial and domestic laundry are major sources (Browne et al. 2011, Hartline et al. 2016, Salvador Cesa et al. 2017, Boucher and Friot 2017). Items made of PP&A, spandex and nylon shed as many as 700,000 MP fibers/kg of laundry (Napper and Thompson 2016) from households and industrial facilities. Smaller amounts (100–300 fibers/L) in washing machine effluents have been reported (Browne et al. 2011). Hernandez et al. (2017a) reported that use of detergent had the greatest impact on fiber release of the variables tested: 0.025 and 0.1 mg fibers/g textile, without and with detergent, respectively. Neither the fabric type nor wash conditions affected the overall fiber length distribution, generally 100–800 µm. In another study, PES fleece had the highest fiber loss relative to acrylic and nylon fabrics (Carney-Almroth et al. 2017). Based on their sources and relatively small size, aqueous transport of microfibers mainly occurs through wastewater

effluents and sludge applications, as with primary MPs (Carr et al. 2017, Horton et al. 2017a). Fiber dispersion and transport in air, with subsequent deposition on land and water also occur (Dris et al. 2015a, 2015b, 2016, 2017; Gasperi et al. 2018; Allen et al. 2019).

Directly released MPs (primary and secondary), including microfibers, tire debris, pellets and microbeads in wastewater, may undergo further degradation in the environment. Information on how rapidly MPs are formed through breakdown of plastic products is limited. Data for several plastics were reported in a recent review (Fotopoulou & Karapanagioti 2017). Biodegradation of some polymers has been reported, but the extent to which microbes degrade N&MPs in different environmental matrices is unknown (SAPEA 2019). However, microfiber pollution from synthetic textiles may pose a greater problem than degradation of larger plastic items at sea (Boucher and Friot 2017, Henry et al. 2019). According to an IUCN report, between 15% and 31% of all plastics released to oceans (estimated at 4.8–12.7 Mt/year) originate as MPs released directly from households and industrial products, with 35% due to washing of synthetic textiles (Jambeck et al. 2015, Boucher and Friot 2017). Europe and Central Asia alone had annual MP releases into oceans equivalent to the addition of 54 light (e.g., 5 g) grocery plastic bags/person/year. In Europe and North America, regions with adequate waste management, direct release of MPs equaled or exceeded contributions of secondary MPs from mismanaged waste (Boucher and Friot 2017).

Rivers can transport plastic debris long distances, eventually reaching the oceans. Air transport (e.g., fibers from clothes dryers and dust dispersion) also occurs, contaminating land and water bodies as atmospheric fallout (Cai et al. 2017; Dris et al. 2015a, 2016, 2017; Gasperi et al. 2018; Allen et al. 2019). Deposition may be driven by wind and precipitation events (Dris et al. 2017, Allen et al. 2019). Data on MP aerosols are currently limited, but several studies have been reported. Dris et al. (2017) investigated fiber contaminants in indoor and outdoor air, finding concentrations of 1.0–60.0 and 0.3–1.5 fibers/m³, respectively. Indoor deposition

rates ranged from 1,586–11,130 fibers/day/m² surface (190–670 fibers/mg settled dust). Most (67%) were natural fibers (mainly cellulose); the remainder (33%) were synthetic, with PP being dominant.

Studies of atmospheric fallout in two major cities also were reported. Dris et al. (2015a) found 29–280 particles/m²-day in total atmospheric fallout (wet and dry deposition) collected on a rooftop in a dense urban area near (11 km from) the center of Paris (France). Most (> 90%) of the MPs were fibers: approximately 50% were > 1000 μ m in length, and the remainder were equally distributed in two classes: 100–500 and 501–1000- μ m. Cai et al. (2017) reported fiber and non-fiber MPs ranging from 175–313 particles/m²-day in atmospheric fallout in Dongguan (China). Fiber lengths were mainly in the 200–700 μ m range. Diverse shapes (e.g., fiber, foam, fragment, film) were found but fibers were dominant. Three polymers types were identified (PE, PP, and PS), and SEM images revealed adhered particles and evidence of particle degradation (e.g., grooves, pits, fractures).

In contrast to cities, where microfibers were dominant, fragments were the primary shape (followed by films and fibers) in a remote mountain catchment (French Pyrenees) (Allen et al. 2019). Contamination was attributed to aerosol deposition (wet and dry) after long-range air transport from populated areas. The average daily MP deposition was 365 items/m² catchment surface, with daily counts (items/m²) of 249 fragments, 73 films, and 44 fibers. Considering only fibers, daily deposition was 36 ($\pm$ 18) and 28 ($\pm$ 13)/m². The predominant polymer was PS (as fragments), closely followed by PE, both used in many single-use plastic items and packaging materials (Allen et al. 2019). The dominance of these two polymers in a remote area may relate to the relatively low densities of PS foam and PE particles (Allen et al. 2019). The relatively high abundance of PS also may relate to its high susceptibility to mechanical and UV degradation, especially expanded PS (Song et al. 2017).

3.2 Freshwater systems

Most (> 96%) studies of N&MPs have focused on the marine environment (Lambert and Wagner 2018), but studies of freshwater systems are increasing. Abundances of MPs in some freshwaters were reportedly comparable to those in marine waters (Peng G. et al. 2017) but results have been highly variable (Klein et al. 2018, Rezaei et al. 2018), from as few as 10^{-2} pieces/m³ (10^{-5} /L) to as many as 100 million/m³ (10^5 /L) (Koelmans et al. 2019). As discussed, variability likely relates to differences in monitoring methods, location (e.g., proximity to urban areas/WWTPs), and natural conditions (e.g., river catchment features, weather) (Eerkes-Medrano et al. 2015, Klein et al. 2018).

Freshwater systems can be sources (e.g., contaminated rivers) and sinks (lakes, sewage sludge) of N&MPs. Particle types and their relative proportions and properties may differ from those for marine waters, depending on matrix, weathering, and other conditions (Klein et al., 2018). For example, MPs in sewage sludge may be larger and contaminated by organic material, while those in clean waters may be relatively small and difficult to see without a microscope (Orb Media 2017, Li et al. 2018c). Lakes and rivers near highly populated and urbanized areas can have higher MP abundances than those in rural areas (Eriksen et al. 2013, Ballent et al. 2016, Di and Wang 2018). Plastic debris has been found in a range of freshwater systems: e.g., 1 MP per 8 gallons of Great Lakes tributary water (Baldwin et al. 2016), an average of 1,285 MPs/ft² of Saint Lawrence River (Canada) sediment (Castañeda et al. 2014), 112,000 MPs/mile² of Great Lakes (US) water (Eriksen et al. 2013), and MPs in 12% of freshwater fish (Sanchez et al. 2014).

Studies of MP contamination in freshwater systems and drinking water have found high variability in the detected concentrations (e.g., Horton et al. 2017a, Li et al. 2018c, Pivokonsky et al. 2018, Koelmans et al. 2019, Novotna et al. 2019, Triebkorn et al. 2019). Li et al. (2018c) reported surface water concentrations from 10^{-5} – 10^5 pieces/m³ and sediment levels of 4–40

$\times 10^4$ pieces/ m^3 (40–400 pieces/L) for freshwater systems. Koelmans et al. (2019) reported MP abundances from 10^{-2} – 10^8 MPs/ m^3 in a review of 50 freshwater and drinking water studies. Novotna et al. (2019) reviewed available information on MPs in drinking water sources and potable water. Results of the different studies varied significantly, from zero or few (< 10) to > 4000 MPs/L (Wang W. et al. 2017, Wang W. et al. 2018, Pivokonsky et al. 2018, Su et al. 2018, Bordós et al. 2019, Luo et al. 2019, Mintenig et al. 2019). Studies of freshwaters near urban/populated areas potentially impacted by residential, industrial, and WWTP effluents are summarized in Table 3-1. For comparison, several studies of drinking water and relatively clean water sources are included. Studies of freshwater sediments are reported in Table 3-2. These two tables (3-1 and 3-2) include studies published in 2017 and later. Earlier studies on freshwaters and sediments are reported as supplemental information (Tables S3-1 and S3-2, respectively).

Few studies of water supplies to drinking water treatment plants (DWTPs) have been reported. Pivokonsky et al. (2018) investigated MPs in raw and treated water at three DWTPs with different water sources. Average concentrations ranged from $1,473 \pm 34$ to $3,605 \pm 497$ MPs/L raw water and 338 ± 76 to 628 ± 28 particles/L in treated water. Their study was reportedly one of very few that determined MPs down to $1 \mu m$. Particles $< 10 \mu m$ accounted for up to 95% of the MPs in both raw and treated water. Fragments were dominant at two plants, while fibers and fragments were most abundant at the third plant. Twelve materials were identified, but the majority ($>70\%$) of MPs were PET, PP, and PE. Mintenig et al. (2019) investigated a drinking water system with a purified groundwater source. To assess possible

Table 3-1. Studies of Microplastics in Freshwater Systems: Freshwaters

Study	Sample type(s)	Collection and particle cut size	Sample preparation	Analysis	MP abundances and types		Comments
					reported units	converted ^a units and/or MP types	
Bordós et al. 2019 Hungary Carpathian basin	Surface water of fish ponds, rivers and reservoirs	1500 L pumped through connected filters, 2 mm–100 µm.	Density separation (1.2 g/mL NaCl) in MPSS unit (Imhof et al. 2012). WPO (30% H ₂ O ₂ , 1 h, 80 °C, 450 rpm). Filtered (0.2-µm Al ₂ O ₃ filter)	Visual (microscope) µ-ATR-FTIR. Suspect MPs auto analyzed.	3.52–32.05 MPs/m ³ (92% of samples). Mean = 13.79 ±9.26	0.003–0.03 MP/L Mean = 0.01 PP, PE dominant	Fish ponds may be MP deposition area.
Di et al. 2019 China Danjiangkou Reservoir (DJKR)	Surface water at 20 sites (drinking water reservoir). Sediments (Table 3-2)	See Di and Wang (2018). Pumped and sieved. Smallest size 48 µm.	Digested in 30% H ₂ O ₂ (24 h). Filtered (0.45-µm glass-fiber filter). Dried filter in Petri dish.	Visual (microscope) µ-Raman (140 suspect MPs) and SEM. Confirmed 118/140	Mean (DJKR) = 2594 ± 3875 par/m ³ Range: 467–15,017	Mean = 2.6 par/L (0.5–15) PP (45%), PS (35%), PE (20%). Fibers dominant.	Blue dominant (17–85%), then clear. Most MPs < 2 mm.
Dikareva and Simon 2019 New Zealand (Auckland) Small streams	Water, sediments (Table 3-2). Streams spanned urbanization gradient	Plankton net (83 mm diameter, 63 µm mesh) 190–4,520 L	Rinsed filtered (63 µm) material into beaker, covered with foil, dried (60 °C). Digested (75 °C) in 30% H ₂ O ₂ and 0.05 M Fe (II), H ₂ SO ₄ solution, with repeat additions of H ₂ O ₂ . NaCl (1.2 g/mL) density separation. Supernatant filtered (1.2-µm glass-fiber filter). Filter placed in petri dish to dry (60 °C) before analysis.	Visual (microscope) 3,309 suspect MPs. ATR-FTIR on 50. Library search: 10% of suspect MPs were non-MPs (counts adjusted). Mean blank = 16 MPs (correction made).	17–303 items/m ³ Some polymers (e.g., polyamide) may be underestimated due to degradation (digest > 70 °C).	Up to 0.30 par/L Fragments (39%), fibers (34%), and small particles (63–500 µm) dominant. Films also found. 70% of particles < 500 µm were fragments.	High variability across streams. Local factors likely more important than catchment-scale. No relation between abundance and population or combined stormwater overflows. Polymers varied and often not predictable based on size, shape, or color.
Hitchcock and Mitrovic 2019 Australia Estuaries	Water from 3 estuaries with different levels of human impact.	Plankton nets (45 and 37-µm). Flow meter. Stored in 250-mL PE containers in 50% ethanol.	Sieved (20 µm). NaCl density separation per Claessens et al. (2013). Digested 12 h in 30% KOH:NaClO solution. Stained with Nile Red.	Fluorescence microscopy Raman on particle subset of photo bleached samples	Means (MP/m ³) 98, 246, and 1,032	Means (MP/L) 0.098, 0.246, and 1.032 Fragments (< 200 µm) dominant	MPs higher in areas with more human impact (number of townships and WWTPs, and heavy industry). Procedural blanks < 3% of mean MP counts (3.2 particles/blank).
Liu et al. 2019 Denmark 7 retention ponds	Water from retention ponds receiving urban and highway stormwater: 3 with residential catchments, 2 with industrial, 1 with commercial, and 1 with highway. Sampled Sept. to Oct. 2017	Pumped samples through filter device with steel mesh (10-µm, 100 mm diameter). Hose placed in steel cage (2 cm mesh) to exclude large debris. Sampled until filter clogged (201 to 454 L). Filters from 3 sampling rounds at each pond were pooled as one sample (752–1139 L)	Soaked filters in SDS to dissociate MPs from solids. Added 50% H ₂ O ₂ to digest (2 days). Removed solids in ultrasonic bath. Collected in SDS (200 mL) and digested enzymatically (3 days, 50 °C), then by Fenton's reagent and 0.01 M NaOH. Filtered (10 µm) and extracted solids by ZnCl ₂ (1.7 g/mL) density separation (twice). Filtered (10 µm), added 50% ethanol, and applied suspension to 13-mm Zn selenide transmission window (with surface restricted by compression cell). Applied and dried in 100-µL increments to obtain proper loading for µ-FTIR.	µ-FTIR-FPA imaging (128x128 MCT FPA). 10-mm diameter window scanned in transmission mode. Image maps analyzed using MPhunter software (auto MP detection for µ-FTIR image datasets). Size fraction: 10–2000 µm	490–22,894 items/m ³ or an estimated 85–1143 µg/m ³ .	0.49–22.8 items/L Dominant polymers: PP, PVC, PES, PE, and PS. Ponds serving highway and residential areas had the lowest levels, while ponds serving areas with industry and commerce had the highest.	PVC tended to be in largest size fraction. Smallest MPs were mixes of less common polymers. Residential ponds tended to have the largest MPs and comparable levels, sizes, and composition. Study shows that land-based sources of MPs are significant, and retention ponds act as pollution hotspots that play a role in transport from land to aquatic systems.

Study	Sample type(s)	Collection and particle cut size	Sample preparation	Analysis	MP abundances and types		Comments
					reported units	converted ^a units and/or MP types	
Luo et al. 2019 China Yangtze Delta	Surface water (n=3) at 43 sites. Urban creeks and rivers, estuary, coastal.	5 L with pail. Used air lift pump from boat to collect estuary/coast samples	Nylon filter (20 µm). Washed into bottles containing KOH solution (10% w/v). Incubated 24-48 h (65 °C, 80 rpm). Filtered with same filter. Stored in Petri dish.	Visual (microscope) µ-FTIR (32% of suspect MPs). 20 µm smallest size detected	1.8–2.4 par/L in freshwaters. 0.9 par/L in estuary and coastal waters.	Fibers were 88% of MPs in city creeks, and 81% and 85% of those in 2 rivers.	No blank correction (blanks 2.6% of sample MPs). MPs levels higher in freshwater.
Mintenig et al. 2019 Germany DWTP	Groundwater, inlet, outlet, household water. 9 raw (8 m ³) and 15 drinking water (32 m ³) samples.	Filtered (3-µm) tap water at 10 L/min and DWTP water at 5 L/min. Filtered up to 1 m ³ raw and 2.5 m ³ drinking water.	Removed CaCO ₃ and iron in dilute HCl. Rinsed with H ₂ O-ethanol and filtered (3-µm). WPO (35% H ₂ O ₂). Sample enriched on Al ₂ O ₃ filter (0.2-µm) and dried. Density separation of raw water in ZnCl ₂ (1.6 g/mL) to remove iron oxide.	µ-FTIR-FPA. Analyzed entire filter. 20-µm lower limit CaFI windows held filter.	0 to 7 MPs/m ³ 14 samples had no MPs. Results blank corrected (n = 4)	0–0.007 MP/L	Filter unit lids contributed styrene acrylonitrile and PP. Large volumes collected (low MPs anticipated).
Triebkorn et al., 2019 Germany Elbe River	River water	µ-sieve cascade (5, 20, 100 µm) coupled to submersible pump at 0.5-m depth	Filtered 250-450 L. Extracted in ultrasonic bath, treated with ozone, centrifuged. Density separation in Na polytungstate solution. Filtered on PTFE filter.	Visual (microscope). µ-Raman to 4 µm. Laser counter for total particles, 1-200 µm	Range (par/m ³): 10 ⁵ – 9 x 10 ⁵ Total particles = 10 ¹¹ /m ³	100–900 par/L PE (96.5%), PS (1.8%), PP (0.7%), PA (0.5%)	MP loads: 4-30 kg/d. ≤ 10 of the 10 ⁶ total particles were plastic.
Wiggin and Holland 2019 United States Urban estuary in Long Beach, CA	Water from Los Angeles and San Gabriel rivers, and Long Beach Harbor	Triplicate 20-L grab samples (33 total) using pump and 4-L amber glass jars.	4 stainless sieves (500, 124, 63, and 20 µm). Filtered water with 3-µm PC filter (required 3-12 filters). Sieved material rinsed into filter (3-µm PC) units and digested in 15% H ₂ O ₂ (50 °C, 16 h). Rinsed filter units with 20% IPA. Dried filters (covered) Validated WPO protocol.	Visual (microscope) 40x-100x. Subset of filters in each size class stained with Nile Red and recounted under fluorescent microscope using FITC filter cube.	Means, MP/m ³ Without staining - Harbor: 8,130 - LA River: 13,622 - San Gabriel: 4,161 With staining (respectively) 13,627; 641,292; 63,359	Means, MP/L: Without staining - Harbor: 8 - LA River: 14 - San Gabriel: 4 With staining (respectively) 14, 641, 63	White and clear non-fibers not counted (too similar to background). Small fragments and beads could not be distinguished. Procedural blanks and recovery checks included. Counts corrected for controls. Fiber estimates likely low due to inability to stain.
Yuan et al. 2019 China Poyang Lake (largest freshwater lake in China)	Surface water, sediments, wild crucians 21 sampling sites. Sampled: 11/2017	2, 20-L water (0–1-m depth) with steel sampler. Sieved (50-µm). Residue rinsed into bottle.	Water and sediment processed as described previously (Di and Wang 2018). 30% H ₂ O ₂ overnight. Diluted digest and filtered (0.45-µm) onto gridded filter. Air dried, covered.	Visual (microscope) µ-Raman. Cleaned sample with alcohol. Used Ag slide to reduce fluorescence.	Range (par/L): 5–34 Highest at mid-lake Most < 0.5 mm.	Fibers and colored MPs dominant. PE and PP major types. No correlation between water and sediment.	Domestic sewage is a possible MP source. Human and topographic factors may affect distribution.
Di and Wang 2018 China Three Gorges Reservoir Region	Surface water and sediments (Table 3-2) at 29 sites along Yangtze River. Aug. 2016	2, 25-L samples pumped at 1-m depth. Sieved (48-µm), rinsed residue into 50-mL jar. Stored in 5% formalin (4 °C).	30% H ₂ O ₂ for 12 h. Filtered with gridded filter (0.45-µm glass-fiber filter). Dried (50 °C) in Petri dish. Kept covered.	Visually (microscope) µ-Raman (174 suspect MPs), SEM	Water (par/m ³): Mean = 4703 ±2816 Range = 1597-12,611 79.8% < 1 mm PS Total types: 38.5% PS, 29.4% PP, and 21% PE	Water (par/L): Mean = 4.7 Range = 1.6–12.6 Fibers 28.6% to 90.5% PP and PE dominant (PS main type in sediments).	MPs higher in urban water than rural. Clear (21-82%), followed by white, and blue. No correlations between sampling sites MP sorption of organics.

Study	Sample type(s)	Collection and particle cut size	Sample preparation	Analysis	MP abundances and types		Comments
					reported units	converted ^a units and/or MP types	
Gray et al. 2018 United States Charleston, South Carolina	Surface μ -layer water. 6 sites in each estuary: Charleston Harbor and Winyah Bay.	4-L collected with 2-mm steel mesh and drained into jars. Each dip gave ~ 75 mL.	Filtered with nested sieves (500, 150, and 63 μ m). Processed solids as described for sediments.	Visual (microscope). Subset of particles (> 500 μ m) in sediments by ATR-FTIR.	Means (par/L): Harbor = 6.6 ± 1.3 Bay = 30.8 ± 12.1	No correction for blanks or FTIR results.	Levels comparable to estuaries worldwide. But harbor had many black MPs attributed to tire wear.
Kay et al. 2018 England (North) Rivers near 6 WWTPs	Water upstream and downstream of WWTPs in river catchments with different features	Net (300- μ m) on frame (250 x 230 mm). Held frame against riverbed, facing upstream. 15-min sample.	Filtered by 6 stainless, stacked sieves (5.6 mm to 250 μ m). Discarded material on top sieve. Transferred MPs to petri dish for analysis.	Visually (microscope). MPs classified as pellets/beads, fibers, and fragments/flakes	Concentrations (not reported) generally higher downstream. In 4 cases, higher upstream.	Mean up-to-downstream concentration ratios > 1 . For 19/28 cases, ratios were 1 to 3.	Effluents contributed MPs to rivers. Prevalence of fibers indicates textiles. Source apportionment work needed.
Lin et al. 2018 China Pearl River, lower course along Guangzhou City	River water and sediments (Table 3-2) at 14 sites WWTP samples (Table 3-3).	60-L surface (top 50 cm) with 5-L pumped sampler (Seaward WS-5). Filtered on-site with steel sieve (20 μ m). Fixed residues in formaldehyde.	Transferred residue to 1-L flask. Added 200 mL 30% H_2O_2 , covered with foil and digested 24 h in incubator shaker (65 $^{\circ}$ C, 80 rpm). Followed by 24-h NaCl density separation ($d = 1.2$ g/mL). Filtered (5- μ m) and covered with petri dish.	Visual (optical microscope). μ -FTIR with MCT detector. Subset of suspect MPs. Size focus: 0.02-5 mm	Surface water (items/m ³): 379–7924 items/m ³	0.379–7.924 MPs/L PE and PP accounted for 64.3%	Fibers dominant. Noted number, size, shape (fibers, fragments, films, pellets) and color.
Pivokonsky et al., 2018 Czech Republic, Prague DWTP supplied by groundwater	Raw and treated drinking water Sampled: 11/2017 – 01/2018	3, 1-L samples over 24 h. Total of 27 L each of raw and treated water for SEM analysis. And 9 L each for FTIR, Raman and elemental analyses.	WPO (H_2O_2). Vacuum filtered onto successive PTFE filters (5 and 0.2 μ m) for SEM. Filtered onto Al_2O_3 filters for qualitative analyses. Dried filters dried in oven (30 $^{\circ}$ C for 30 min). Stored in Petri dishes in desiccator until analysis.	Qualitative analyses (on 25% of Al_2O_3 filter) by μ -FTIR (> 10 - μ m) and μ -Raman (1-10 μ m). Number, size, shape by SEM (3 pieces of PTFE filter). Elements in some particles by SEM-EDS.	Means (par/L): Raw: 1473 ± 34 to 3605 ± 49 Treated: 338 ± 76 to 628 ± 28 Up to > 4000 8–83% of particles not MPs.	Fragments dominant at 2 plants; fibers and fragments most common at one. PET, PP, and PE most common (accounted for $> 70\%$ of MPs).	One of few studies to determine MPs to 1 μ m. MPs < 10 μ m most common (up to 95%). Higher in raw water than treated.
Su et al. 2018 China Yangtze River	Water, sediment (Table 3-2), and Asian Clams in lakes, rivers, and estuaries	5-L water samples ($n = 3$) with bucket dipped (0–12 cm deep) from boat.	Vacuum filtered water through net (20 μ m). Washed residue into flask with 100 ml 30% H_2O_2 . Digested using oscillation incubator (65 $^{\circ}$ C, 80 rpm, ≤ 72 h). Filtered and stored filter in Petri dish.	Visual (microscope) μ -ATR-FTIR on 150/1303 particles	0.5–3.1 par/L	Fibers dominant. MP sizes ranged from 0.021–4.83 mm. Most were 0.25–1 mm.	Clear and blue particles most common. Clear dominant. 0.4–5.0 par/clam (μ -fibers 60–100%)

Study	Sample type(s)	Collection and particle cut size	Sample preparation	Analysis	MP abundances and types		Comments
					reported units	converted ^a units and/or MP types	
W. Wang et al. 2018 China Hong and Dongting lakes	Surface water. Inland freshwater systems. 20 sites at Hong Lake and 30 at Dongting. Sampled in Sept. 2017	20 L bulk water (0–20 cm depth) collected with pump at each site. Sieved (50- μ m). Rinsed residue into bottle. Preserved in 4% formalin solution.	30% H ₂ O ₂ (48 h, room temperature). Filtered with glass-fiber filter (0.45- μ m). Placed filter in Petri dish for visual examination.	Visual (microscope) SEM, μ -Raman of 50 suspect MPs from each lake.	par/m ³ • Hong Lake: Mean = 2867 $\pm$ 989 Range = 1250–4650 • Dongting Lake Range = 900–2800 • Mean granules: Hong 685 $\pm$ 375 Dongting 385 $\pm$ 221	par/L: • Hong Lake: Mean = 2.9 $\pm$ 1.0 Range = 1.2 – 4.6 • Dongting Lake: Range = <1 – 2.8 71–78% of MPs colored. Most < 1 mm; >20% <330 μ m. PE and PP dominant.	Fibers 42–92% of MPs, many transparent. Possible sources: fishing nets, sewage, runoff, air. Granules irregular or spherical, and colored. Possible sources: domestic effluents and waste from residents. Films (14–16% of MPs) appeared irregular and weathered.
Ravit et al. 2017 United States Raritan and Passaic rivers, NJ	River water, adsorbed compounds, toxicity Sampled: 2016 (May–Aug.).	Manta trawl (333 μ m net). Triplicates (n = 45 each location). Rinsed residue into jar. Added IPA to 1 sample. Other 2 shipped on ice.	Digested 1 of each replicate by Fenton reaction (1:1 0.05 M iron sulfate + 30% H ₂ O ₂). Rinsed any large organic debris and discarded. Recovery verified with blue microbeads (330 μ m) spiked samples.	Visual (microscope). Pyr-GC-MS on individual particles. HSSPME-GC-ITMS to identify organics on MPs and in water.	28,000 to >3 million particles km ⁻²	Not reported	Watersheds are among most developed (residential and industrial) in state; (Raritan largest).
Leslie et al. 2017 Netherlands Amsterdam canals Rhine and Meuse Rivers (Germany and Netherlands)	Canal water. Riverine suspended particulate matter (SPM). WWTPs (Table 3-3), biota, sediments (Table 3-2). Sampled 2012–2013	2-L bulk water in glass bottles, at 6 sites in Amsterdam canals.	Density separation in saturated NaCl (1.2 g/mL). Filtered subsample on glass-fiber filter (0.7 μ m) for visual inspection. Al ₂ O ₃ filter (0.2 μ m) used for μ -FTIR analysis of sediment and biota.	Visual (microscopy). μ -FTIR on particles in sediment and biota (6% of total counted overall)	Amsterdam canal water (par/L): Mean = 100 ($\pm$ 49) Range = 48–187 River SPM (par/kg, dry wt.) (SD) Meuse = 1400 (520) Rhine (P2) = 4900 (540) Rhine (P3) = 1700 (390)	Fibers, spheres, and foils. Fibers dominant. Two size categories: >300 and < 300 μ m 10 μ m smallest size detected	MPs present in all urban surface water samples. Canal levels similar to effluent from local WWTPs.
Miller et al. 2017 United States Hudson River	River water	Top 8–18 cm surface water, with bucket. Top 1 L decanted into jars.	Vacuum filtered onto gridded filters (47-mm, 0.47 μ m). Stored filters in metal dishes.	Visual (microscopy 45x). Counted fibers with lengths $\geq$ 100 μ m Counts background corrected. μ -FTIR	Mean microfibrs (MPs/L): 1.24 $\pm$ 0.14, with lengths 0.33 to 3.59 mm Mean = 0.98 anthropogenic fibers/L (0.625 to 2.45 fibers/L)	No significant change in fiber abundance from river source to sea μ -FTIR indicated half of fibers were plastic and half non-plastic but anthropogenic.	MP fibers: blue dominant, then black, clear, red, and others. Fibers: 43% cotton, 22% PET, 22% fluoropolymer, 7% PP, 7% nitrocellulose/clay Estimate: 34.4% of Hudson River watershed adds 300 million anthropogenic fibers to Atlantic Ocean/day. No relation between counts and population or WWTP location.

Study	Sample type(s)	Collection and particle cut size	Sample preparation	Analysis	MP abundances and types		Comments
					reported units	converted ^a units and/or MP types	
Anderson et al. 2017 Canada Lake Winnipeg	Water	Manta trawl, 333- μ m net. Collected material preserved in 70% ethanol.	30% H ₂ O ₂ 250- μ m sieve separation substrate	SEM-EDS	Mean: 1.93×10^5 par/km ² (max: 7.48×10^5)	Converted units not available.	23% of particles were non-plastic
W. Wang et al. 2017 China (Wuhan) Lakes/rivers	Surface waters of urban lakes and rivers. Residents and industries along shore.	20-L (0.2 m depth) pumped through 50- μ m sieve. Rinsed residue into 50-mL jar. Preserved in 5% formalin at 4 °C	30% H ₂ O ₂ (24 h, in dark, room temperature). Added distilled water and filtered by glass-fiber filter (0.45 μ m). Placed filter in Petri dish for visual (microscope) examination.	Visual (microscope), particle subset by SEM, FTIR 44 suspect MPs analyzed.	$1,660 \pm 639$ to $8,925 \pm 1591$ par/m ³	1.7-8.9 par/L PET and PP dominant >80% of MPs <2mm Fibers dominant.	Wuhan is largest city in central China. Anthropogenic factors greatly affected MP abundances. Colored particles 50.4% to 86.9%; 24.7% were transparent.

^aValues converted from particles km⁻² to particles L⁻¹ are from Horton et al. 2017 except where indicated. Sample volumes were based on sampling area and depth. ^b2011 values. ^cUrban/industrial and suburban/residential. Abbreviations: CaF₂ = calcium fluoride, FITC = fluorescein isothiocyanate, FPA = focal plane array (detector), HCl = hydrochloric acid, H₂O₂ = hydrogen peroxide, HSSPME-GC-ITMS = headspace solid phase micro extraction with GC and ion trap MS, IPA = isopropyl alcohol, MCT = mercury-cadmium-telluride, μ -ATR-FTIR = Fourier transform infrared spectroscopy (FTIR) coupled with an attenuated total reflectance microscope objective (μ -ATR), NaCl = sodium chloride, par = particles, PTFE = polytetrafluoroethylene, Pyr-GC-MS = Pyrolysis gas chromatography with mass spectrometry, SDS = sodium dodecyl sulfate, SEM-EDS = scanning electron microscopy with energy dispersive X-ray spectroscopy, WPO = wet peroxide oxidation, WWTP = wastewater treatment plant.

Table 3-2. Studies of Microplastics in Freshwater Systems: Sediments.

Study	Sample type	Collection	Sample preparation	Analysis	MP abundances reported/converted ^a	Types/sizes/comments
Bordós et al. 2019 Hungary Carpathian basin	Sediments and water (Table 3-1)	2-3 kg with Van Veen Grab and spade. Shipped cooled.	Weighed amount was added to MPSS for NaCl (1.2 g/mL) density separation. Stirred 4 h and processed by water method (Table 3-1). 30% H ₂ O ₂ .	μ-FTIR. Particles selected visually and auto analyzed in ATR mode.	Mean: 0.81 ± 0.37 par/kg Range: 0.46–1.62	PP and PS dominant (identified by library match). Fish ponds may act as MP sink.
Di et al. 2019 China Danjiangkou Reservoir (DJKR)	Sediment at 20 locations	See Di and Wang (2018)	2-step density separation, in NaCl and NaI (Di and Wang 2018). 30% H ₂ O ₂ . 0.45 μm smallest mesh size	Visual (microscope). μ-Raman of 142 suspect MPs, and subset by SEM.	Mean (DJKR) = 24 ± 9 par/kg sediment Range: 15-40 par/kg (wet wt.)	Colors: transparent dominant (12.5–100% for 13 samples), followed by blue. Fibers (25%-100%) in 19 samples, fragments (14.3%-75%) in 13, pellets in just 4. No Styrofoam.
Dikareva et al. 2019 New Zealand (Auckland) Streams	Sediments and surface water (Table 3-1) in small streams spanning urbanization gradient	Scooped 10-20 samples (5-cm depth) into 150 mL container. Mixed in bucket, sieved (5 mm) into 1 L container, and placed in zip-lock bag for transport to lab.	2-step extraction (Claessens et al. 2013). Placed 1-L sediment in elutriation device; added water. Sieved (63 μm) overflow water from device. Transferred filtered solids to centrifuge tube for density separation (NaI, 1.6 g/mL). Centrifuged, filtered (1.2 μm glass-fiber filter) top liquid, dried filter. Repeated density separation on remaining sample. Filtered onto same (dried) filter.	See Table 3-1. Visual (microscope) and physical properties. ATR-FTIR	9–80 items/kg (dry wt.) Fragments (79%) and fibers (20%) dominant. 88% of fragments < 500 μm. Foam and films rare; pellets absent. Smallest fraction (63–500 μm) dominant.	See Table 3-1. Counts blank corrected (Mean procedural blank = 15 particles, <i>n</i> = 6). Polymers varied and often not predictable based on size, shape, and color.
Yuan et al. 2019 China Poyang Lake.	Sediments, water (Table 3-1), crucians 21 sites, 11/2017	Collected 2, 500-g surface (0.25 m ²) samples from boat with Van Veen grab. Placed 1 kg in foil bag.	Sediments dried (48 h at 50 °C). MPs extracted by density separation. 5 size classes: < 0.1 mm, 0.1–0.5 mm, 0.5–1 mm, 1–5 mm, and > 5 mm.	Visual (microscope) μ-Raman on 100 par (on silver slide to reduce fluorescence). Library search.	Sediments (par/kg dry wt.): 54–506	Highest MP levels in northern area. Largest freshwater lake in China.
Di and Wang 2018 China Three Gorges Reservoir	29 sites, sediment and water (Table 3-1)	2, 1-L surface samples (0.25 m ² area) per site, by Van Veen Grab. Placed in jar. 0.45 mm smallest mesh size	Stored at 4 °C. 2-step density separation (saturated NaCl then 60% NaI solution). Suspension digested in 30% H ₂ O ₂ . Next steps same as water (Table 3-1).	μ-Raman (see Table 3-1)	Mean (par/kg wet wt.) = 82 ± 60 Range: 25-300 PS dominant.	87% recovery of spiked PE microbeads. Fibers: 33.9–100% of MPs. Higher MP levels in countryside. High-density MPs more likely to deposit in sediment. Most transparent, then blue.
Gray et al. 2018 United States (South Carolina) Estuaries: Charleston Harbor, Winyah Bay	Intertidal sediments (<i>n</i> = 9–12)	Removed top 2 cm with trowel and weighed in steel buckets.	Density separation (800 g NaCl in 4 L seawater). Sieving (500, 150, 63 μm). Digestion in 30% H ₂ O ₂ (1 week). Filtered (38 μm).	Visual (microscope) ATR-FTIR (80 particles) and SEM imaging.	Means (par/m ²): - Charleston Harbor = 413.8 ± 76.7 - Winyah Bay = 221.0 ± 25.6, respectively (high = 441, low = 51)	Fragments dominant (76.2% in harbor and 77.5% in bay). Fragments mainly black (73%). White blue, clear, other colored MPs also present. 18.9% of par in harbor were foam (98% white). First study in Southeastern US estuaries. Variation due to currents, wind, and sources.
Imhof et al. 2018 Italy Lake Garda	Sediments at 5 beaches. Three, 20-m 'transect' lines at each.	20-m transect (parallel to water), with 10 core samples from each (5-cm depth, 10-cm diameter)	Extraction in MPSS (Imhof et al. 2012) with ZnCl ₂ (<i>d</i> = 1.6–1.7 g/mL) (Imhof et al. 2016). Filtered on glass-fiber filters.	Visual (microscope) μ-Raman down to 1 μm (Imhof et al. 2016)	3508 ± 8855 par/m ²	Sampling approach reduced number of samples and improved representativeness (combines quadrat and core sampling approaches).

Study	Sample type	Collection	Sample preparation	Analysis	MP abundances reported/converted ^a	Types/sizes/comments
Lin et al. 2018 China Pearl River, lower course along Guangzhou City	River sediments and water (Table 3-1) at 14 sites. WWTP samples (Table 3-3)	2 kg sediment (top 5 cm) by Van Veen grab sampler. Shipped in foil bags (storage at -20 °C).	Dried, crushed, removed large items. Multiple density separations (200-g:1 L sat. NaCl). Filtered (2-µm) supernatants, digested in 10% KOH (65°C, 80 rpm, 24 h), added NaCl solution, filtered (5-µm).	Visual (microscope) µ-FTIR (MCT) Spectral library match (quality > 80%).	Sediments (items/kg dry wt): 80–9,597	Fibers dominant. PE and PP accounted for 73.8% MP abundances varied and may be affected by multiple factors.
Peng et al. 2018 China Shanghai	Sediment	Shovel to collect upper 5 cm of 0.5 x 0.5 m area (n = 3). 500 g each.	Density separation (NaCl). (Masura et al. 2015). No wet sieving or WPO.	Visual (microscope) µ-FTIR, ATR Library match (>70% criterion)	802 ± 594 items/kg sediment (dry wt.) 57% PP, 17% PES, 11% rayon 5.7% cotton + viscose	Spheres dominant (88.98%) in river sediments, followed by fibers (7.55%) and fragments (3.47%). Most (90%) were white spheres. Other MPs (blue, transparent, white, red) relatively minor. Tidal flat sediments had mostly fibers. 62.15% of particles 100–500 µm.
Su et al. 2018 China Yangtze River	Sediment, water (Table 3-1), and Asian clams in lakes, rivers, and estuaries	Top 10 cm of sediment by Peterson sampler (n =3). See Su et al. 2016.	Dried sediment. Used 300 g for NaCl (1.2 g/mL) density separation. Supernatant treated same as water samples (H ₂ O ₂ digestion).	Visual (microscope) µ-ATR-FTIR	15–160 par/kg (dry wt.): sediment	Sizes = 0.021–4.83 mm. Most 0.25–1 mm. Fibers dominant. Clear and blue particles most common; clear dominant. Sediment MPs more similar to those in clams than water. Asian Clam could serve as bioindicator of MPs in freshwater systems, especially sediments.
T. Wang et al. 2018 China Yellow Sea wind farm	Sediment and water (Table 3-1) Sept. 2016	Collected 3-kg sample with a bottom grab. Stored in Al foil bag.	Extracted by 2-step process. Fluidization in saturated NaCl. Second extraction in saturated NaI solution. Filtered on cellulose nitrate filter (1.0 µm) and dried.	Visual (microscope 80 x). µ-FTIR on 20% of particles. Library search.	Sediment: 2.58 ±1.14 items/g (dry wt.) PET dominant, followed by cellophane and PE	Black, and transparent particles. Most 0.05–5mm. Fibers dominant in sediment (68.7%) and water (75.3%). Cellulose fiber and calcium carbonate pellets major interferents.
Xiong et al. 2018 China Qinghai Lake	Lakeshore sediment (and others) and water.	Collected top 2 cm of 20 cm x 20 cm area with shovel. 3 replicates.	Sieved (2 mm mesh size). Transferred particles (on sieve) for analysis. Water passing sieve was treated by density separation in potassium formate (density = 1.54 g/mL). Floating particles digested in 30% H ₂ O ₂ (60 °C). Filtered (1.2 µm) and transferred particles for analysis.	Visual (microscope) 3 classes: 0.112 -0.5 mm, 0.5-1 mm, 1-5 mm. µ-Raman on all suspect MPs if counts < 100, or 10-15% if > 100.	50-1292 MP/m ² sediment Higher abundances in tourist areas.	PE and PP dominant. Sediments dominated by fibers and sheets. Raman analyses were on particles > 200 µm as smaller particles could not be manually transferred. Wore cotton lab coat and nitrile gloves
Abidli et al. 2017 Northern Tunisia Bizerte Lagoon	Sediment	Removed top 2-3 cm of a 0.25 m x 0.25 m area with stainless spatula.	Dried (50 °C, 48 h). High-density NaCl (140 g/L) for density separation. Filtered supernatant (7-10 µm).	Visual (microscope) Fibers dominant: 21-91%, followed by fragments	3,000-18,000 items/kg sediment (dry wt.) High MP pollution	Mean sizes: fiber = 1.39 ±0.27 mm, fragment = 0.51 ±0.19 mm, No pellets. Fibers clear, followed by white, blue, red, green, black. Fragments blue, green, black, and red. MP sources: cities, industry, and fishing.
Horton et al. 2017b UK River Thames Basin (4 sites)	Benthic sediment Sites had range of sewage input. 28 Aug.–3 Sept. 2014	4 samples per site. 10-cm depth collected with stainless scoop. Filled 1-L Kilner jar. Focus was large MPs (1–4 mm)	Wet sieved into 2 fractions: 1-2 mm and 2.4 mm and dried (80°C). Used 3 steps to check processing requirements. Visually removed suspect MPs under microscope. Separated particles in remaining material by floatation (1.7-1.8 kg/L ZnCl ₂). Rinsed and filtered settled material (1.2 µm glass-fiber filter). Inspected under microscope for any suspect MPs that did not float.	Visual (microscope) µ-Raman on 20% of particles. BioRad KnowItAll® Informatics System – Raman ID Expert software	33.2 ±16.1 par./100 g sediment (or 332/kg dry wt.) Ave. abundance (par/kg): 185–660, depending on site MPs at all sites, most 1–4 mm	96% fragments in highest-load sample. Site was downstream of storm drain. Fragments (mostly red and yellow) from thermoplastic road paint. Fibers dominant at other sites (from sewage). Site with high sewage input had highest number. PP, PES, and polyarylsulphone most common polymers. ‘Unidentified’ polymer was largest category, inferred by a synthetic dye.

Study	Sample type	Collection	Sample preparation	Analysis	MP abundances reported/converted ^a	Types/sizes/comments
Leslie et al. 2017 Netherlands Canals and coastal areas	Sediment Amsterdam canals, 12 sites on Dutch North Sea coast, and 3 in Walden Sea	Van Veen grab 2, 1-L samples combined	All sample homogenized before taking sub-sample for analysis. 20 g sediment added to flask with saturated NaCl solution (1.2 g/mL).	Visual (microscope) μ -FTIR (transmission mode) on 6% of suspect MPs. Library search.	par/kg sediment (dry wt.): Canal: mean = 2,071 ($\pm$ 4146), range: < 68–10,500 Coast: 100–3,600	Results indicate partial MP settling in freshwater and that seabed is a sink. Canal sediment particle sizes: 10–300 μ m: 0–75%, 300 μ m–5 mm: 19–100% MPs present in benthic macroinvertebrates (muscles, oysters): 10–100 MP/g (dry wt.)
Matsuguma et al. 2017 Japan, Thailand, Malaysia, S. Africa	Sediment cores (as trends in plastic pollution)	Gravity corer (8 or 11 cm diam. x 50 or 100 cm length acrylic pipe) 315 μ m to 5 mm	10 g freeze-dried sediment. 1 week 30% H ₂ O ₂ to remove biofilms. Density separations in NaI (d = 1.6 g/mL). Tweezers used to transfer suspect MPs for analysis.	ATR-FTIR. Carbonyl and vinyl indices used to avoid counting biopolymers. Library hit > 60%	100–1,900 par/kg sediment (dry wt.)	White (57%), brown (17%) and black (14%) dominant. Main types: PE, PP, PS, PET, PVC, acrylics, polyamides. 79.8% < 1mm (0.315–1 mm). Increase in MPs towards sediment surface indicates increasing MPs over time.
Sruthy and Ramasamy 2017 India Vembanad Lake	Sediment	Van Veen grab (25 cm ²)	Wet samples sieved (< 5 mm), dried, and sieved again (< 5 mm). WPO (30% H ₂ O ₂). Saturated NaCl (d = 1.3 g/mL) density separation of WPO mixture. Filtered supernatant on glass-fiber filter and dried.	Visual (microscope) μ -Raman, KnowItAll® (BioRad) Raman library search.	96–496 par/m ² Mean 252.80 $\pm$ 25.76 par/m ² Main types: PE, PS, PP	Transparent and white particles dominant. Film and foam dominant shapes. LDPE dominant Biofilms may make plastics negatively buoyant. May be one of the reasons for PE in the sediments (usually in water column).
Volertsen and Hansen 2017 Denmark Soils, WWTPs, sludge	10 farmland soils: 5 treated with sludge, and 5 untreated WWTP water and sludge (See Table 3-3).	Cores of about 300 mL	50 g soil sub-sample treated and MPs concentrated in 5 mL ethanol.	Aliquot of ethanol suspension transferred to slide for μ -FTIR-FPA analysis. Size range: 20-500 μ m. (See Table 3-3)	Mean concentrations (dry wt.): - Sludge-treated soils: Mean = 82,000 MP/kg (6.2 mg/kg), Median = 71,000 MP/kg (5.8 mg/kg) - Non-treated soils: Mean = 236,000 MP/kg (51 mg/kg), Median = 145,000 MP/kg (12 mg/kg)	Polymer types by mass (13 MPs identified): - Sludge-treated soils: 56% PP, 39% PE, 5% nylon. - Non-treated soils: 89% PE, 10% nylon, 1% PP. MP concentrations in soils were considered low (about 10 mg/kg, comparable to heavy metals in Danish soils). Soils without sludge had more MPs (13 in treated, 24 in untreated). But results had high uncertainty due low MP numbers.
Wang J, et al. 2017 China Beijiang River	Benthic sediment MPs and associated heavy metals	Collected 3, 20 x 20 cm area (2 cm deep) with shovel. Transferred to foil bag as 1 sample.	Dried samples (50 °C) $\geq$ 48 h. Density separation (sat. NaCl). Cleaned floating particles in ultrasonic bath. Let stand overnight. Filtered supernatant with 47-mm glass-fiber filter (1 μ m).	Visual (microscope) SEM-EDS, μ -FTIR (reflectance mode), ICP-MS	Abundances (items/kg dry wt.): 178 $\pm$ 69 to 554 $\pm$ 107	Brown and blue particles, and PE and PP dominant. FTIR and EDS indicated weathering. Most associated heavy metals were inherent load. Copolymers found at all sites.

^aValues initially reported as particles m⁻² were converted to particles kg⁻¹ by Horton et al. 2017. Abbreviations: DWTP = drinking water treatment plant, FPA = Focal plane array (detector), H₂O₂ = hydrogen peroxide, ICP-MS inductively coupled plasma with mass spectrometry, MBR = membrane bioreactor, MCT = mercury-cadmium-telluride (detector), μ -ATR-FTIR = Fourier transform infrared spectroscopy coupled an attenuated total reflectance microscope objective (μ -ATR), NaCl = sodium chloride, par = particles, SEM-EDS = scanning electron microscopy with energy dispersive X-ray spectroscopy, WPO = wet peroxide oxidation, WWTP = wastewater treatment plant, ZnCl₂ = zinc chloride.

MP sources, samples were collected at different locations, from the groundwater supply to household taps. Fourier transform infrared (FTIR) microscopy coupled with a focal plane array (FPA) detector (FTIR-FPA) was used for identification of MPs down to about 20 μm (Löder and Gerdt 2015, Mintenig et al. 2017). The authors reported concentrations from 0–7 MPs/m^3 , with an overall mean of 0.7 MPs/m^3 . No concentration differences were found for different treatment stages, and all detected MPs were fragments (50–150 μm), identified as PE, PA, PES, PVC or epoxy resin. Four of these polymers were used in the system: tanks in the DWTPs were coated with epoxy resin, pipes (in the plants/households) were often PVC or PE, and fittings were usually PA. The MPs were attributed to abrasion of plastic equipment used to purify or transport water (Mintenig et al. 2019).

The reported widespread occurrence of MPs in drinking water has raised concerns for human health, though the reliability of these findings has been questioned due to method limitations. Koelmans et al. (2019) proposed best practices to sample, extract, and detect MPs in water. They further proposed a set of quality criteria to assess the reliability (data quality) of a study and applied them to 50 studies of drinking water and its sources. Data quality for each study was assessed against the criteria and a total accumulated score (TAS) was calculated, with a maximum of 18. The average (minimum - maximum) scores for the different water types were: 13.7 (13–14) for bottled water, 11.5 (8–15) for treated tap water, 12.5 (11–14) for DWTP water, 7.9 (4–15) for surface water, and 7.3 (3–13) for wastewater. The authors noted that the average score likely reflects the relative ease of sample preparation (e.g., bottled and tap water required no digestion and were assigned the maximum for this criterion). They also noted that the average scores for DWTPs and tap water ($n = 2$ for each), and for bottled water ($n = 3$), are less rigorous because of the relatively few studies on these water types. Of the 50 studies evaluated, 46 (92%) lack reliability due to inadequate quality assurance. On average, the studies were assigned a TAS of 8.4, about half the maximum. The average is comparable to

that for studies of MP ingestion by biota (Hermesen et al., 2018). In addition to reporting MP concentrations and quality scores, the authors summarized polymer types and particle shapes. In order of decreasing prevalence, the following polymers were reported for the 50 studies: PE ~ PP > PS > PVC > PET. The abundances reportedly reflect the global plastic demand, and the tendency of PVC and PET particles to settle due to their higher densities. Fragments, fibers, film, foam and pellets were the most frequently reported shapes.

Luo et al. (2019) reported MP abundances in city creeks, rivers, an estuary, and coastal waters (East China Sea) in the Yangtze Delta region. Abundances in freshwater bodies (1.8-2.4 items/L) were higher than those in estuarine and coastal waters (0.9 items/L). Abundances in two rivers tended to be higher downstream, at sites closer to the city center. The authors suggested transport from pollution sources to sinks throughout the river networks. Fibers were highest in city creeks (88%), followed by two rivers (85% and 81%), an estuary (66%), and the sea (37%), with mainly PES in creeks and rivers. Results suggest that the levels and types of MPs vary across the different water bodies, and that small, freshwater bodies are more polluted than estuarine and coastal waters.

Wastewater treatment plants are recognized sources of N&MPs that might affect freshwater quality (e.g., Sun et al. 2019). Kay et al. (2018) investigated MPs in freshwaters upstream and downstream of 6 WWTPs in river catchments across northern England over a 6-week period. All plants were associated with increased MPs in rivers, as reported elsewhere (McCormick et al. 2014, Morritt et al. 2014, and Estahbanati and Fahrenfeld 2016). The potential MP sources were diverse and varied within and across catchments, and no patterns were found in the types of MPs detected. The identified sources included atmospheric deposition (fibers), sludge applied to soils, and secondary MPs. Fibers, fragments, and flakes were dominant, consistent with Dris et al. (2015a, 2015b) and Ballent et al. (2016). The prevalence of fibers indicates textile sources, while fragments and flakes suggest secondary

MPs. Beads and pellets were dominant only at one site, upstream, as reported by Mani et al. (2015). The temporal variability was small at some sites and high at others. The concentrations were not reported, but the mean ratio of up-to-downstream MPs was > 1 , and between 1 and 3 for 19 of 28 paired samples. On four occasions, MPs were higher upstream, possibly due to concurrent increases upstream and decreases downstream. Further study of MP sources in river catchments was identified as a research priority for improved waste management to reduce MPs in freshwater and marine environments.

Ravit et al. (2017) reported MP surface densities, types, and sizes for 15 locations at two New Jersey (US) rivers in heavily urbanized areas: the Raritan and Passaic rivers. Many point sources discharge into the rivers within a 5-mile radius of the sampling sites, including companies that produced personal care products, companies with 'plastic' in their names, and WWTPs. Surface water MPs ranged from 28,000 to > 3 million particles/km². The most common forms in order of abundance were: "hard fragment" (38%), foam, line (fiber, filament), film (from bags/wrappers), and pellet (nurdles or microbeads). A hard fragment was identified as PP, a pellet as PE, and black foam as PE and either PVC or a PE-vinyl acetate copolymer. By size, 71% of the MPs ranged from 1 to > 4.5 mm. Analyses of MP and water fractions found similar compounds, indicating transfer between phases and bioavailability to aquatic biota.

Estahbanati and Fahrenfeld (2016) investigated the impact of WWTPs on MP pollution in a recipient river, the Raritan (New Jersey). The river basin covers 2,850 km² and provides water for drinking, irrigation, agriculture, recreation, and industry. The primary land use of the main stem is urban and suburban (51.3%), while the branches have predominantly agricultural and forest lands (61.3%). More than 10 municipal WWTPs discharged into the river, five of which are major (> 1 MGD). Water samples were collected upstream and downstream of four major plants. Recovered MPs were counted in three, quantitative size categories (500–2000

μm, 250–500 μm, 125–250 μm) and one semi-quantitative category (63–125 μm). Particles were classified under a light microscope as primary or secondary MPs, based on morphology. Concentrations in the 125–250 and 250–500-μm fractions were significantly higher downstream: 72 ± 60 downstream and 24 ± 11 MPs/m³ upstream. The smaller size classes were in high relative abundance across sites. The primary MPs increased significantly downstream, but the secondary MPs were dominant (66–88%) in the quantitative size categories. A moderate correlation between MP concentration and downstream distance was observed.

McCormick et al. (2016) examined MP pollution in surface waters of nine streams in the Chicago, Illinois (US) metropolitan area (Table S3-1). The streams received wastewater from ten facilities with effluent flowrates of 2.16–225 million gallons per day (MGD) (0.095–9.86 m³/s) and different treatments. Microplastic particles were counted and classified as fiber, film, fragment, pellet, or foam, as described by Eriksen et al. (2013). Fibers included filament/line-shaped plastic pieces. Films were very thin relative to their surface area and typically had irregular shapes, while pellets were more regular and rounded. Foam particles had sponge-like textures and included PS. Fragments had jagged edges and appeared to be broken from larger pieces of plastic. The average MP flux was about ~ 1.3 million particles/day, but results were highly variable between sites, ranging from 15,000 to 4.7 million particles/day. Pellets, fibers, and fragments were dominant. The main polymers were PP, PE, and PS; film and foam MPs were uncommon. Except for two rivers, MP concentrations were significantly higher downstream. Assuming similar concentrations across seasons, the estimated discharges represent 488 million MPs per river annually, with a minimum of 5.6 million and maximum of 1.7 billion. Further work was recommended for “more robust calculations of annual flux.”

Dris et al. (2015a) found MPs (100–5000 μm) in freshwater samples collected in Paris, France, near city WWTPs (Table S3-1). Sampling by manta trawl and a plankton net gave

different results for the types and amounts of MPs collected. Concentrations in surface waters of the Seine River ranged from 3–108 particles/m³ (0.003–0.108 particles/L), with a mean of 30 particles/m³ (0.03 fibers/L) for samples collected with an 80-mm mesh plankton net, which gave a predominance of fibers relative to a 330-mm mesh manta trawl. Greater variety in the shapes and types of MPs was found with the manta trawl, but at much lower concentrations: 0.28–0.47 particles/m³ ($2.8\text{--}4.7 \times 10^{-4}$ particles/L; mean = 3.5×10^{-4} particles/L). For comparison, raw wastewater from a WWTP contained high levels of fibers, $260\text{--}320 \times 10^3$ particles/m³ (260–320 MPs/L), while the treated effluent contained $14\text{--}50 \times 10^3$ particles/m³ (14–50 MPs/L) (89% reduction) (Table S3-3). The authors suggested that a combined sampling approach may provide more accurate assessments of MP distributions. No upstream-downstream patterns were observed in river concentrations. Levels were higher in April (0.014–0.108 particles/L) than in May (0.003 to 0.036 particles/L), except at one site. Differences were attributed to local variability, a water flow decrease (20 %), and difficulties in visual observations of MPs due to large amounts of natural debris in the May samples, attributed to weather (e.g., higher rainfall) and possibly other factors such as river traffic. The presence of MPs, mostly (>90%) fibers, in atmospheric fallout also was reported, indicating an atmospheric deposition of 29–280 particles/m²-day.

Yonkos et al. (2014) studied four estuarine rivers in the U.S. Chesapeake Bay, finding high variability in MP types, both within and between sites (Table S3-1). The watersheds at these sites had different land uses and a wide range of populations. Surface water samples were collected monthly using a manta net trawl (based on an operational definition of MP sizes: 0.3 to 5.0 mm) during a six-month period in 2011. Particles were counted as: fibers, thin sheets, hard colored fragments, preproduction pellets, and extruded polystyrene (e.g., styrofoam). Microplastics were found in 59 of 60 samples, at surface concentrations from < 1.0 to > 560 g/km². Small fragments (0.3–2.0 mm) and flexible sheets were most abundant, followed by

synthetic fibers, extruded PS, and larger fragments (2.0–5.0 mm). Preproduction pellets were common only in one river (Patapsco). Particles analyzed by optical microscopy coupled with Raman spectroscopy (μ -Raman) all had peaks indicating PE. Several had peaks attributed to other constituents: two had a color and peaks consistent with cobalt phthalocyanine (a dye), and a black particle had two broad peaks indicative of black carbon (possibly burnt plastic). Positive correlation was found between MP concentration and population density, and the extent of urban/suburban development. In three of four sites, the highest MP levels occurred after major rain events.

Studies of urban watersheds, especially those with high population densities, highlight the role of WWTPs as potential sources of environmental N&MPs. The potential contributions of WWTPs are discussed in the next Section (3.3).

3.3 Wastewater treatment plants

Many types of plastic debris in urban watersheds reach WWTP influents. Depending on the plant design, primary, secondary, and tertiary treatments purify influent by physical, chemical, and biological means. A schematic illustrating this series of treatments is shown in Figure 3-2. Incoming floating/suspended solids are removed by screening, sedimentation, flotation, coagulation-flocculation, and filtration in treatment ponds or settling tanks, which capture substantial amounts of solid particles (Stephenson and Stuetz 2009). The capture of common MPs is thought to relate to their relatively low densities, permitting floatation and removal in the grease layer (Murphy et al. 2016) by skimmers (Carr et al. 2016) in the primary treatment. Another possible mechanism is surface fouling, which can cause MPs to sink in a settling tank or associate with flocculants (Carr et al. 2016).

Figure 3-2. Illustration of serial treatment stages of a tertiary wastewater treatment plant.

Though just a small fraction of MPs in influent is released in the final effluent, WWTPs can be significant sources of MPs (Browne et al. 2011, McCormick et al. 2014, Lares et al. 2018). Multiple literature reviews on WWTPs have been published (e.g., Kang et al. 2018, Lares et al. 2018, Prata 2018, Raju et al. 2018, Enfrin et al. 2019a, Sun et al. 2019, Gatidou et al. 2019). Studies of WWTPs published since 2017 are summarized in Table 3-3, while earlier studies are reported in the supplemental material (Table S3-3). Several studies are highlighted below. Details on the sample collection, preparation, and analysis methods are discussed in Section 4.

3.3.1 WWTPs: selected studies

Many studies of WWTPs have focused on N&MPs in final effluent, while others have examined the removal efficiencies of different treatment processes (Carr et al. 2016, Murphy et al. 2016, Mintenig et al. 2017, Talvitie et al. 2017a, Gies et al. 2018, Lares et al. 2018, Conley et al. 2019, Yang et al. 2019). For example, Lares et al. (2018) examined a conventional activated sludge process and an advanced, pilot-scale membrane bioreactor (MBR). The overall removal efficiency was 98.3% (89.8% for fibers and 99.1% for non-fiber MPs). Most MPs were removed before the CAS process, and 99.0% were removed before aeration. The MBR

was slightly more efficient: 99.4%. The MBR permeate contained 0.4MP/L while the final effluent of the CAS process was 1.0 MP/L. Lares et al. (2018) also reviewed studies at primary, secondary, and tertiary facilities, representing 15 studies and 52 WWTPs globally (by Browne et al. 2011; Magnusson and Norén 2014; Talvitie et al. 2015, 2017b; Mason et al. 2016; Murphy et al. 2016; Carr et al. 2016; Ziajahromi et al. 2017; Michielssen et al. 2016; Dyachenko et al. 2017; Mintenig et al. 2017; Leslie et al. 2017; Lares et al. 2018). Mean concentrations in final effluents ranged from 0 MP/L (Carr et al. 2016) to 81 MP/L (Leslie et al. 2017). Differences in sample collection methods; lower particle size limit, typically 20–300 μm (Lares et al. 2018); MP types and concentrations; treatment processes (Mahon et al. 2017); and sample preparation and analysis methods (e.g., Underwood et al. 2017) contributed to the observed variability, as did the different periods over which the studies were conducted. Most were completed over relatively short periods (one day to 1.5 months). A need for long-term studies to assess temporal variation has been emphasized (Magnusson and Norén 2014, Murphy et al. 2016, Ziajahromi et al. 2017).

In a one-year study, (Conley et al. 2019) determined MP loads and removal efficiencies of three WWTPs with different treatment sizes, operations and service compositions (Table 3-3). The plants discharged into the Charleston Harbor, South Carolina (US). Overall, MP concentrations at each plant varied within a factor of 2.5 in influent and 4.8 in effluent, with no seasonal trends in concentrations or efficiencies. Mean concentrations in influents ranged from 126-147 MP/L, while those for final effluents ranged from about 4-18 MP/L. Microfibers were most common (60–70%). The largest plant, which employed primary clarification, had the highest efficiency ($97.6 \pm 1.2\%$), while the two smaller facilities averaged $85.2 \pm 6.0\%$ and $85.5 \pm 9.1\%$, suggesting that retrofitting secondary plants with primary

Table 3-3. Studies of Microplastics in Wastewater Treatment Plants

Study	Sample and plant/study details	Sample collection	Sample ^a preparation	Analysis ^b	Main findings ^c	Comments
Bayo et al. 2020 Spain Cartagena Urban WWTP AS process with 1° treatment and 2 parallel AS reactors PEq = 210,000 (35,000 m ³ /d)	Sampled 4 stages: grit/grease removal (GGR), 1° clarifier (PCL), activated sludge reactor (BRT), and 2° clarifier effluent (EFF). Determined MP and 'microlitter' (ML) particles. Survey between Sept. 2016 and April 2018.	128 grab samples. Volume depended on treatment stage. Total L: GGR = 60.1, PCL = 59.3, BRT = 103.4, EFF = 143.0. Precise volumes for tests: GGR = 0.5-3.7, PCL = 0.5-3.6, BRT = 1.4-3.7, and EFE = 2.7-17.0 L	Processed 352.6 L of wastewater. EEF sample was directly filtered. All others treated by NaCl density (d) separation (final d = 1.08 g/mL) in 1:3 salt solution to wastewater. Sample filters placed in Petri dishes to dry (100 °C) overnight. Clothes of natural fabric were worn.	Trinocular microscope. Transferred particle subset for FTIR using diamond anvil compression (DAC) cell. Low background but samples blank corrected.	Influent maximum: 13.04 MP/L MPs 46.6% of total ML. Overall MP and ML removals: 90.3% and 90.1%, respectively. Mean concentrations: - MPs/L: 3.20 (±0.67), 2.59 (±0.85), 2.13 (±0.38), 0.31 (±0.06) for GGR, PCL, BRT, and EFF, respectively. - ML/L: 12.43 (±2.70), 9.73 (±3.04), 3.21 (±0.50), and 1.23 (±0.15), respectively Fibers and fragments dominant in effluent, most 400–600 µm. 17 polymers identified. LDPE most prevalent (52.4%) as films (27.7%). Attributed mainly to nearby greenhouses and plastic bags.	Biodegradable polymers likely degrade in first treatment stages. Non-plastic fraction > MP fraction, except BRT (66.2% MPs). Influents with high suspended solids (SS) had fewer and larger MPs, possibly due to aggregation with SS. PS and PET have surface energies > 25 mN/m and could associate with organic matter.
Conley et al. 2019 United States Charleston, South Carolina 3 WWTPs with CAS processes: Plum Island (PI), Rifle Range (RR), Center Street (CS)	Influent and effluent. PI treated residential, commercial, and industrial wastes. RR and CS treated mainly residential. Sampled June, Oct. 2016, Jan., April, July 2017. Further RR samples in June, July 2017.	Influent: downstream of headworks and upstream of sludge return. Effluent: post-disinfection (before discharge). Duplicate influent and effluent samples.	Filtered (43-µm) and transferred solids to Petri dishes. Processed by sonication, 30% H ₂ O ₂ and 1M HCl. Filtered as 3 size fractions and treated filters with final sonication and rinse. Transferred solids to clean dish or gridded cellulose membrane.	Visual (optical microscope). 3 filter fractions: >418 µm, 178-418 µm, 60-178 µm. Used gridded filter or grid under Petri dish. µ-ATR-FTIR (Ge crystal) on select MPs.	Means (par/L): PI, RR, CS, respectively (n = 5 or 8): - Influent: 147 ±62, 126 ±45, 146 ±57 - Effluent: 3.7 ±1.5, 17.6 ±6.1, 17.2 ±10.4 - 60-70% microfibers Average removals (%) of total, fiber, and non-fiber MPs, respectively: - PI = 97.6 ±1.2, 97.2 ±1.0, and 98.4 ±1.3 - RR = 85.2 ±6.0, 80.2 ± 8.0, and 95.4 ± 2.4 - CS = 85.5 ±9.1, 83.7 ± 8.2, and 88.8 ±9.6 Colors: translucent or white (60%), black (22%), blue/green (13%), red (5%).	No seasonal trends in MP levels or efficiency. High potential for MP sorption of toxins in wastewater. High numbers may pose risks. Average daily influent MPs per capita: PI = 83,500 ± 29,200 CS = 53,500 ± 28,400 RR = 49,600 ± 15,400
Lv et al. 2019 China Wuxi City WWTP with parallel treatment systems	Influent, effluent pots, mixed liquors and excess sludge of OD and A ² /O-MBR Parallel treatments: oxidation ditch (OD) and MBR Collected February 2018	5 steel sieves (diameter 20 cm): 500, 250, 125, 62.5 and 25 µm in sequence. Gravity feed through sampler until sieve clogged (at least 1 L), or to a total volume of 200 L	Digested 3 h in Fenton's reagent (30% H ₂ O ₂ + Fe (II) catalyst, 20 mmol/L) and dried (60 °C). Density separation in NaI solution (1.49 g/mL). Centrifuged (3500 rpm, 5 min). Filtered supernatant (25 µm), rinsed, transferred solids to petri dish.	Visual (microscope) ATR-FTIR, and confirmation by library search.	Concentrations (MPs/L) Influent: 0.28±0.02 Effluent: OD = 0.13±0.01, MBR = 0.05±0.01 Overall removal (by number): 53.6% OD and 82.1% MBR (systems A and B, respectively). And 99.5% and 97% by mass (respectively) Rotary grit chamber (OD system): 1.32±0.1 Aerated grit chamber (MBR system): 0.22±0.03 MLSS of membrane tank (MBR system): 1.6±0.2 OD system sludge (settling tank): 0.7±0.1 Influent: PET (47%), PS (20%), PE (18%), and PP (15%). Fragments (65%) and fibers (21%) dominant, most PET. Limited films (12%) and foams (2%). No microbeads.	Dominant MP sizes in influent: > 500 µm (40%) and 62.5–125 µm (29%). MPs accumulated in sludge. MBR has higher removal efficiency than OD system. Results suggest source control (e.g., eliminating MP fibers from laundry effluents) and proper treatment units could significantly reduce MPs in WWTPs. No contaminants in negative controls.

Study	Sample and plant/study details	Sample collection	Sample ^a preparation	Analysis ^b	Main findings ^c	Comments
Magni et al. 2019 Northern Italy Tertiary WWTP PEq = 1.2 million	Inlet, after the settler, effluent, and sludge Sampled 3 days over 1 week, during dry weather, in spring.	30 L wastewater collected in steel bucket. Sieved on-site (5 mm, 2 mm, and 63 µm). Sludge in 50-mL beaker.	Density separation (NaCl, 1.2 g/ml). Stirred overnight (4 °C). Supernatants filtered (8-µm) and solids rinsed. Samples partially digested (3 days in 15% H ₂ O ₂).	Visual (optical microscope) µ-FTIR	Mean wastewater concentrations (par/L): - Influent = 2.5 ±0.3, After settler: 0.9 ±0.1 - Effluent: 0.4 ±0.1, - Sludge: 113 ±57 MPs/g sludge (dry wt.) 84% removal efficiency Effluent mainly PES (35%) and polyamide (17%)	Potential discharge of 160 million MPs/day. Sludge traps about 1.1 billion MPs/ton (30 tons daily) Sludge could be an important MP source in agroecosystems.
Wolff et al. 2019 Germany WWTP, mechanical and biological treatments. Service population: 98,500	Effluent from secondary clarifier Surveyed 11/2017 to 01/2018. Wet and dry days	7 grab samples. Sampler was a pump, 10-µm cartridge filter and housing (all ss), and silicone hose. Filtered 40–200 L.	Oxidation (H ₂ O ₂ [50%] and NaClO) and density separation (ZnCl ₂ , 1.9 g/cm ³). Filtered DI water and n-hexane used to rinse small MPs adhered to surfaces.	MPs ≥ 10 µm analyzed on sub-area of silicon substrate by µ-Raman (785 nm). Counts based on total deposit area.	Non-fiber means (par/m ³) Wet days: 5,900, Dry days: 3,000, Overall: 3,500 Most 30 to < 100 µm. Mainly PET, PP, PE, PS. Fiber means (fibers/ m ³) Wet days: 1,500, Dry days: 730, Overall: 1,100 Fiber lengths: 100–1000 µm. Most were PET.	Effluent: about 0,000 m ³ /d. Dry weather samples tended to have fewer MPs than wet weather. Hexane rinse removed small, adhered particles (< 20 µm) that could not be removed with water.
Yang et al. 2019 China (Beijing) Advanced tertiary STP (Gaobeidian). Serves population of approximately 2.4 million.	Influent, effluents, and primary and secondary sludge. Largest water reclamation plant in China. Treats mainly domestic effluents. Sampled between April and June 2018.	30-L samples in glass bottles. Stored at 4 °C.	Sieved (5 mm, 50 µm). Rinsed into water, filtered (10 µm), sonicated filters in water, dried, digested (60 °C) by WPO (Fenton's reagent). ZnCl ₂ density separation (then HCl added to remove ZnCl ₂). Transferred to separatory funnel, drained solids, filtered solution (10-µm PTFE filter).	Visual (optical microscope). Subset of particles removed for µ-FTIR analysis and count correction.	Mean wastewater (MPs/L): Influent: 12.03 ± 1.29 Effluent: 0.59 ± 0.22; > 95% MP removal Sludge (MPs/kg dry wt.): 22.7 ±12.1 x 10 ³ (sludge range: 1.6–56.4 x 10 ³) Identified 18 polymers (10 colors), mainly PET (42%), PES (19%), and PP (13%). Microfibers dominant (86%) and attributed to domestic washing. Average size of 1,111 ± 863 µm. Average non-fiber MP was 681 ± 529 µm (most near 300 µm).	Treated mean of 10 ⁶ m ³ /day of wastewater. Estimated daily release of 0.59 ± 0.22 x 10 ⁹ MPs. 50-µm filter used because smaller size clogged. And MPs <50 µm could not be manually transferred for µ-FTIR. Inspected (visually) solids from separatory funnel. No MPs found.
Gies et al. 2018 Canada (Vancouver) Secondary WWTP Plant treats combined wastewater and stormwater. PEq = 1.3 million	Influent, primary and secondary effluents and sludges. Collected in 2016: 9/16, 9/29, 10/28 (water), and 9/14, 9/27, 10/11 (sludge).	1 L influent in jars. Filtered (0.63 µm) 30 L effluent and rinsed solids into jars. 250-mL jars for sludge. 17 wastewater and 12 sludge samples.	All samples spiked with MPs. Wastewater allowed to settle 24 h. Extracted water layer with canola oil, filtered (1 µm) oil layer, and digested solids (30% H ₂ O ₂). Same process for sludge (after H ₂ O added).	Visual (optical microscope). ATR-FTIR (> 3 mm). µ-ATR-FTIR (< 3 mm). 37 suspect MPs (4.7% of total). Dark and white fibers not counted due to high blanks. MP estimates are conservative.	Wastewater means (MPs/L): - Influent: 31.1 ±6.7 - After primary clarification: 2.6 ±1.4 - After secondary treatment: 0.5 ±0.2 Sludge (MPs/g): - Primary: 14.9 ± 6.3, Secondary: 4.4 ± 2.8 97–99% removal (92% by primary treatment). Fibers (mostly cellulose) dominant (65.6%) MPs in wastewater, then fragments (28.1%) and pellets (5.4%). PES main MP fiber (4 red, 1 blue, 1 green). Counts blank corrected.	Treats > 180 billion L/year Influent MP loads (FTIR-corrected) of 1.76 trillion/year. Estimates: 1.28 ±0.54 and 0.36 ±0.22 trillion MPs trapped in primary and secondary sludge, respectively. Estimated discharge: 0.03 ±0.01 trillion MPs/year. Of the 4.8% (37/770) of suspect MPs analyzed by FTIR, only 32.4% were plastic.
Simon et al. 2018 Denmark 10 major WWTPs: primary and secondary.	Inlet and outlet. All plants used AS; 1 had RSF. 9 plants managed mainly wastewater from households, and one mainly (75%) from industry.	Autosamplers for influent (24 h). 1-L sent to lab. Effluent collected in bottles and filtered (10-µm) on-site until 3 filters clogged (4.1–81.5 L)	Influent (+ SDS) sieved (500 µm). 200 mL digested (enzyme, WPO). Effluent solids removed from filters by ultrasound and processed similarly.	Focal Plane Array FTIR (FPA-FTIR) Reported MP concentrations based on number and mass. Colors not reported.	Median (range) (MP/L): - Influent = 7,216 (2,223–10,044) - Effluent = 54 (19–447) Removal efficiency = 99.3% (98.3% by mass). Identified 176 MPs in raw wastewater and 222 in treated: 393 non-fiber and 5 PES fibers. Main polymer types (by number): - Raw wastewater: 27% acrylate, 14% PES, 13% PE-PP, 12% PP, and 10% PE - Treated: 27% PE, 25% PES, 14% PE-PP, 12% PP, and 12% acrylate	Estimated 3 t/year of MPs (10–500 µm) released in effluent, or 0.56 g MP/(capita year), for Danish WWTPs. <0.5% of total MP emissions from Denmark. Sludge may be significant source. 188 t/year of MPs removed from wastewater in Denmark and presumed trapped in sludge.

Study	Sample and plant/study details	Sample collection	Sample ^a preparation	Analysis ^b	Main findings ^c	Comments
Lares et al. 2018 Finland Municipal WWTP CAS process. Also operated pilot-scale MBR	Influent, effluent, and sludge. Lake water near discharge site. Sampled every 2 weeks, between 10/2017 and 01/2018	Collected water in 10-L bucket. Sieved (5 mm and 250 µm) water. Solids on 250-µm sieve rinsed into jars. Sludge (150-200 mL) collected in bucket or 0.25-L cup was poured into flasks.	Water processed by method of Masura et al. (2015), but no density separation. WPO (Fenton's reagent) of dried samples. Dried influent also treated with cellulase (cellulose fiber removal). Samples filtered onto gridded membrane filters and dried.	Visual (microscope). µ-FTIR and µ-Raman 5200 MPs classified as 54 groups. Subset of each group examined visually and by µ-FTIR/µ-Raman.	Means (MPs/L water and MP/g sludge [dry wt.]): Influent = 57.6 (±12.4) Digested sludge = 170.9 (±28.7) CAS effluent = 1.0 (±0.4) MBR sludge: 27.3 (±4.7) MBR permeate = 0.4 Lake water (near discharge) = 0.3 (±0.1) 98.3% overall removal. Most MPs removed before CAS process; 99.0% before aeration. MBR slightly more efficient (99.4%). PES most common fiber (79% of total MPs). PE most abundant non-fiber (11%)	Treats 10,000 m ³ /day (3.65 x 10 ⁶ m ³ /year). Discharge of 10 ⁷ MPs/day 1.3% and 1.4% of all particles and fibers were examined by FTIR and Raman. Automatic composite sampling may be a better approach (Talvitie et al., 2017a). Single monitoring event not generally representative.
Li et al. 2018d China 28 WWTPs in 11 provinces of China	79 dewatered sewage sludge samples Collected in 2014 and 2015.	Samples stored at 20 °C. Dried at 105 °C (24 h) to determine total solids. Heated at 600 °C (1 h) to determine volatile solids.	Homogenized sample. Performed NaCl (1.2 g/mL) density separation on 20-g sub-samples (n = 3). Sieved (37-µm) water layer, rinsed solids with distilled H ₂ O, and digested (30% H ₂ O ₂) sequentially. Poured digest into distilled H ₂ O, filtered, rinsed, dried.	Visual (microscope). SEM on selected samples (~10). Transferred suspect MPs (about 10%) for µ-FTIR. Used polymer, additives and synthetic textile libraries.	Concentrations (MPs/kg sludge [dry wt.]) - Range = 1.6–56.4 x 10 ³ - Average (n = 79) = 22.7 ± 12.1 x 10 ³ Average MP percentages by shape and color (79 samples): - Shape: Fiber (62.5%), shaft (14.9%), film (14.0%), flake (7.3%), sphere (1.3%) - Color: white (59.6%), red (9.0%), green (2.3%), black (17.6%), orange (3.3%), blue (1.7%), other (6.5%)	Estimated average release (in China) of sludge-based MPs of 1.56 x 10 ¹⁴ MPs/year. Total sludge production in 2015 estimated at 40 million tons (moisture 80%). Predicted to increase to > 60 million tons in 2020.
Lin et al. 2018 China (Guangzhou) 3 large WWTPs Capacities: W1 = 0.55, W2 = 0.20, and W3 = 1.20 million tons/day	Influent and effluent. Plant discharges to Pearl River. River water (Table 3-1) and sediment (Table 3-2) also collected A ² /O main process	Collected influent (15 L) after coarse screening and effluent (15 L) after sterilization.	Filtered immediately in lab (Leslie et al. 2017). Digested (30% H ₂ O ₂ , 65°C, 80 rpm, 24 h). Density separation 24 h in NaCl (d = 1.2 g/mL). Membrane filtered (5 µm).	Visual (optical microscope) Classified fibers, fragments, films, and pellets. µ-FTIR with MCT detector on subset of suspect MPs.	Concentrations (MPs/L) at W1, W2, and W3: - Influent: 4.2, 0.5, and 1.4; - Effluent: 2.7, 0.3, and 0.6 Removals: 35.7%, 40.0%, and 57.1% Main shapes (in influent): foams (57%) and fibers (43%) in W1; fibers (60%), fragments, and pellets (20% each) in W2; fragments (57%) and fibers (43%) in W3. Fibers dominant (67-100%) in all effluents. Fragments 33% in W3.	A ² /O process included coarse screening, upgrade pumping, fine screening, sedimentation, AS, second sedimentation, sterilization. Blanks (lab, field) had little (<2%) background (< 2 cotton fibers). Polymer confirmed by library match quality > 80%
Gündoğdu et al. 2018 Turkey (Adana) 2 Secondary WWTPs	Influent and effluent Seyhan and Yüreğir plants supply 1 and 0.5 million people. 6 days in August 2017	5 L, 24-h composite samples daily. (automatic collection)	Sieved (55-µm) and digested solids by WPO (30% H ₂ O ₂ and 0.05 M Fe(II)). Density separation in NaI (1.8 g/mL) using centrifuge (5 min, 3500 rpm). Filtered (55-µm) supernatant and rinsed. Stored in Petri dish.	Visual (microscope) µ-Raman	Means ±SD (MPs/L), Seyhan and Yüreğir plants, respectively: Influent: 26.6 ±3.2 and 23.4 ±4.1 Effluent: 7.0 ±0.8 and 4.1 ±0.3 73% -79% removal rates 7 polymers identified. PES most common.	Influent: 10 ⁶ –6.5 x 10 ⁶ particles/day. Effluent: 220,000–1.5 x 10 ⁶ particles/day

Study	Sample and plant/study details	Sample collection	Sample ^a preparation	Analysis ^b	Main findings ^c	Comments
Dyachenko et al. 2017 United States Secondary WWTP Serves about 680,000 people. Discharge to San Francisco Bay	Effluent collected after secondary treatment, prior to dechlorination and discharge to SF Bay.	Collected effluent 24 h as 2-h composite samples. Collected on 4, stacked sieves: 5, 1, 0.355, and 0.125 mm. 5-mm sieve prevented clogs (its content discarded). Max. of 1 gallon/min.	Rinsed sieves (1, 0.355, and 0.125 mm) into 0.25 L jars with DI water. Stored at 4 °C. Extracted by adapted NOAA protocol. Digested by catalytic WPO (0.05 M FeSO ₄ and 30% H ₂ O ₂ , 70 °C, 30 min). Added H ₂ O ₂ as needed. Membrane filtered.	Visual (microscope). Suspect MPs (355 µm sieve size) transferred to IR transmitting substrate for µ-FTIR.	Significant differences between 2-h (peak flow) and 24-h composite effluent samples: - Maximum (5 monthly 24-h samples) = 0.09 MPs/gallon (0.02 MP/L) - 2-h sample = 0.64 MPs/gal (0.17 MP/L). Mostly fragments. Pellets or microbeads < 10%. Cellulose fibers major interference, followed by undigested fatty acids (in 2° WW). 87% recovery of PS beads (200 µm) in spikes.	Discharge (based on 24-h sample): 5 x 10 ⁶ MPs/day Discharges about 57 million gallons wastewater daily (216,000 m ³ /day, or 7.88 x 10 ⁷ m ³ /y) Cellulose fibers confirmed by Raman. Authors emphasized challenges in quantifying MPs in wastewater (no standard methods).
Kalciková et al. 2017 Slovenia Ljubljana, Lab-scale WWTP	Single stage, mechanical and biological treatment. (sequencing batch reactor [SBR])	Tested 5 body and facial scrubs with 4.82 and 0.74 g of microbeads per 100 mL, respectively.	None specified. PE in 4 of 5 products. Mostly white particles (2 scrubs also had small amounts of red and blue, and 1 had brown, likely husks and shell powders)	IR, SEM Mean microbead size = 37–96 µm by number (by laser diffraction analysis).	Estimated means: 21 par/m ³ discharged. 112,500,000 MPs released daily 52% efficiency for sludge. Overall of 77% (based on estimated efficiency of primary treatment)	Smaller particles (up to 60–70 µm) removed in sludge. Authors suggest high affinity of PE for negatively charged AS flocks due to positive surface charge.
Leslie et al. 2017 Netherlands 7 municipal WWTPs: designated R1–R7 in paper. R7 was testing an MBR (no longer in use).	Influent and effluents at 4 plants (R3, R4, R5, R7). Effluents and sludge at 3 (R1, R2, R7). Effluent at R6. River suspended particulate matter, canals (Table 3-1), sediments (Table 3-2)	2-L of influent, effluent, and sludge collected in jars. Stored in dark (4 °C). Sampled 2012–2013	NaCl (1.2 g/mL) density separations (all samples). Used 100-g aliquots water, and 20-g sludge and sediment subsamples. H ₂ O fractions filtered with 0.7-µm glass-fiber filters. Sediment (and biota) filtered onto Al ₂ O ₃ filters (0.2 µm).	Visual (microscope). Counts, shape, and size (10–300 µm, 300 µm–5 mm) µ-FTIR on subset of particles (6% of total) on Al ₂ O ₃ filter.	Mean concentrations: - Influent means: 68–238 MP/L (range: 20–910) - Effluent means: 51–81 MP/L (range: 9–142) - Sludge mean (1 plant): 660 (±410) MP/kg (wet wt.) (range: 370–950) % of MPs in 10–300-µm fraction: effluent: 49% ±27, influent: 68% ±20, and sludge: 62% ±8 Mean retention (in sludge): 72% (SD 61%). (variability not representative of a given plant). Fibers dominant; spheres and foils also found. Spheres and colored fibers confirmed MPs.	Discharge (MPs/day): 7.48 x 10 ⁸ –4.32 x 10 ¹⁰ Efflux: 3.37 x 10 ⁶ –2.63 x 10 ⁸ m ³ /year Procedural blanks had (0–3 fibers). Samples blank corrected. Could not obtain spectra of 75% of thin colorless fibers (10% of total MPs) or identify black fibers (37.4%).
Mahon et al. 2017 Ireland 7 WWTPs with PEqs from 6.500 to 2.4 million.	Sludge from plants using anaerobic digestion (AD), thermal drying (TD), or lime stabilization (LS) Plants received wastewater from industry, stormwater, and domestic sources	3 replicates (30 g) of treated sludge from each plant. Stored at -20 °C. Dry matter contents from 24% (AD) to 87% (TD). Wore cotton lab coats and nitrile gloves. Covered samples. Cleaned work surfaces with alcohol LS samples (10 g) too oily to extract. Filtered directly onto glass-fiber filter.	TD pellets soaked in water 1 week, placed in water bath (30 °C, 24 h), then on shaker (≥12 h). Sieved (250 µm), then filtered (212, 63, and 45 µm) a portion of the washed-through fraction to check for MPs. AD and LS sludges soaked in water and sieved (250, 212, and 45 µm). Extracted AD and TD samples in elutriation column. Filtered (250 µm) extract and rinsed filtered material into separatory funnel for ZnCl ₂ (1 M) density separation. Drained settled matter and filtered remainder onto glass-fiber filter (1.2-µm).	Visual (microscope). 4 size categories (µm): 250–400, 400–600, 600–1000, 1000–4000 About 10% of suspect MPs per filter analyzed by µ-ATR-FTIR. 20 samples of LS and TD sludges, and pristine MPs analyzed by SEM.	Average concentrations: 4,196–15,385 MPs/kg sludge (dry wt.) Recovery for elutriation column based on spiked sediments with known MPs: 90–94% for HDPE and 80% for PVC. SEM images showed signs of melting/blistering of TD MPs, and shredding/flaking of LS MPs. Higher numbers of smaller MPs in LS samples may be due to shear during processing. Lower MP abundances in AD samples suggests this process reduces MPs. Authors reported bias towards larger particles as they were cut into 2 pieces for FTIR and SEM. AD samples were dark and heavy with cellulosic material. TD samples had entrained cellulosic material that was difficult to separate by elutriation/density separation.	Visual classification as described by Hidalgo-Ruz et al. 2012. Study highlights potential for treatment process to affect MPs, and possible impact of sludge and biosolids use on terrestrial and aquatic ecosystems. It may have implications for regulations on sludge/biosolids. Oily LS samples may be due to breakdown of cellulosic matter by alkaline hydrolysis. MP degradation by microorganisms in AD systems should be investigated as a potential remediation method.

Study	Sample and plant/study details	Sample collection	Sample ^a preparation	Analysis ^b	Main findings ^c	Comments
Mintenig et al. 2017 Germany 12 municipal WWTPs in Lower Saxony PEq = 7.0×10^3 – 2.1×10^5	Effluents, sludge Primary, secondary and tertiary (4 plants) treatments Sampled between April 22 and 29, 2014.	Collected effluent (390–1,000 L) with custom mobile pumping device with 10- μ m stainless steel cartridge filter and flowmeter. Filtered treated water onto 0.2 μ m Al ₂ O ₃ filter. Collected 500 g (wet wt.) sludge with shovel.	Samples stored at 4°C - Sludge: alkaline treatment, neutralization (HCl), followed by density separation (NaCl). - Water: multi-step digestion (enzymatic-oxidative). Samples sieved (500 μ m). Retained matter stored in petri dish for ATR-FTIR. Fraction < 500 μ m further processed, filtered on Al ₂ O ₃ filters and stored in petri dish.	ATR-FTIR (> 500 μ m) FPA-based transmission μ -FTIR (< 500 μ m down to about 20) Included negative controls	Effluents (MPs/L): 0–0.05 (> 500- μ m) 0.01–9 (< 500 μ m) PE most common MP, in both size classes, and in water and sludge. Synthetic fibers ranged from 0.09–1/L and mainly PES (74%), followed by polyamide (PA) (17%) and PP (9%). Most (61%) ‘transparent.’ Sewage sludge (variable): 10^3 – 2.4×10^4 MPs/kg (dry wt.). No MPs in fraction >500 μ m. MP <500 μ m in all sewage samples.	Estimated annual discharges: 9–400 $\times 10^7$ MP/plant. MPs/day: 4.19×10^4 to 1.24×10^7 Annual efflux: 1.9×10^5 to 1.3×10^7 m ³ /year Produces 72-3000 t sludge per year. > 50% of sludge from 46 plants in region used for energy. 1.24 – 5.67×10^9 MPs/year in sludge. Fibers not counted as background not assessed.
Talvitie et al. 2017a Finland Advanced tertiary WWTP PEq (800,000). Largest WWTP in Finland.	Influent, after pretreatment and after AS process, plant effluent, excess sludge, reject water, and dried sludge AS process had aeration tanks and secondary clarifiers. Sampled during a 1-week period (10/2015).	Grab samples (vol. depended on water quality), and automated 24-h composite and sequential (n=3) samples. Except influent, samples filtered onto filters (300, 100, and 20 μ m) in custom filtering device (Talvitie et al. 2015). Collected influent, excess sludge, and reject water in a beaker. Dry sludge hand collected.	Subsamples of wet sludge, reject water, and dry sludge mixed with 1 L tap water. Filtered with device used for wastewater. All filters stored in Petri dishes until analysis.	Visual (microscope) Counted natural and synthetic microliter and color noted. Imaging FTIR (FTIRi). Custom textile fiber library. Transferred 752 particles from 3 effluent filters (for each day) to ZnSe windows for analysis.	Mean microliter concentrations (par/L): Grab samples: Influent: 380 ($\pm$ 52.2)–686.7 ($\pm$ 155.0). Effluent: 0.7 ($\pm$ 0.6)–3.5 ($\pm$ 1.3) Composite (24-h) samples: Influent: 390–900 Effluent = 1.4–2.8 Blank: 0.4–0.8 Sludge and reject water: Excess + raw sludge: 76.3 ($\pm$ 4.3) par/g; 6,3611 ($\pm$ 3544) par/L Dry sludge: 188 ($\pm$ 26) par/g Reject water: 12.9 ($\pm$ 0.3) par/g; 12,867 ($\pm$ 275) par/L > 99% retention after secondary treatment. 66% of fibers were natural. Clear PE fragments in effluent were similar to those in scrubs, but most clear particles were hard crystals that could not be identified.	Average discharge: 270,000 m ³ /day Average MP releases: 1.7×10^6 to 1.4×10^8 MPs/day to Gulf of Finland, Baltic Sea. Most (> 97%) microliter removed by pre-treatment; further removal in sludge. Produced 60,000 tonnes dried sludge annually. Huge amounts of microliter released in sludge. Identified only 18% of MPs/microliter in wastewater. Weak spectral matches due to interfering peaks (1000-2500 cm ⁻¹) of biofilms. SEM-EDS could assist analysis (e.g., confirm inorganics).

Study	Sample and plant/study details	Sample collection	Sample ^a preparation	Analysis ^b	Main findings ^c	Comments
Talvitie et al. 2017b Helsinki, Finland 4 municipal WWTPs with advanced final treatments $PEq = 5 \times 10^4 - 8.0 \times 10^5$ CAS + BAF + DF* (Efflux = 100) CAS + disinfection or MBR* (Efflux = 5) CAS + DAF (Efflux = 7) CAS + RSF (Efflux = 32) Efflux: million m ³ /year *pilot	TriPLICATE samples before and after treatments. Primary, secondary, tertiary (BAF, DF, MBR, DAF, RSF) MBR treated primary effluent. RSF, DAF, and DF treated secondary. Sampled after CAS unit at 1 plant to compare with MBR. Sampled April 2014–August 2015	RSF and DAF wastewater pumped into custom filtering device (300, 100 and 20-μm). Collected DF and MBR samples using taps designed for sampling into device. Volume varied with water quality and filter (0.5–1000 L). Also collected automated 24-h composite samples (4.0–27.4 L).	No further processing	Visual (microscope) Imaging FTIR (FTIRi) of representative particles (>20 μm) Commercial polymer and additives spectral library, and user-made textile fiber library.	Influent and effluent (MP/L), respectively, and removal (%): Grab samples (means, $n=3$) MBR = 6.9 and 0.005 (99.9%) RSF = 0.7 and 0.02 (97.1%) DAF = 2.0 and 0.1 (95.0%) DF10 = 0.5 and 0.3 (10-μm filter) (40%), DF20 = 2.0 and 0.03 (20-μm filter) (98.5%) Composite (24-h) samples (smaller volumes) RSF = 0.4 and <0.04 (>90%) DAF = 2.3 and 1.2 (48%) MBR = 3.2 and 0.2 (94%) CAS = 3.2 and 0.5 (84%) PES dominate (mean = 60%), then PE (14%), polyacrylates (7%), PVC (5%), PS (4%), and PP (3%). Most PES MPs were textile fibers, and PE MPs were mainly microbeads.	Discharge = $1.75 \times 10^6 - 8.22 \times 10^7$ MPs/day Efflux = $8.03 \times 10^2 - 8.82 \times 10^7$ m ³ /y (Sun et al. 2019) Advanced final-stage treatment can substantially reduce MPs; needed to remove small MPs (<100 μm). Small fraction should be included to assess releases. 13 polymers identified: PES, PE, PP, PS, PU, PVC, PA, acrylamide, polyacrylate, alkyd resin, polyphenylene oxides, ethylene vinyl acetates.
Volertsen and Hansen 2017 Denmark 10 WWTPs	Influent and effluent (20 samples for each of 3 sampling events). Anaerobic digested sludge from 5 plants. 10 farmland soils: 5 treated with sludge and 5 untreated (See Table 3-2).	Collected 1-L raw influent as flow proportional 24-h samples using autosamplers at plant. Effluent collected in bottles. Filtered on-site with 3 steel filters (10-μm) until filters clogged (4.1–81.5 L). Collected about 1 kg sludge (at dewatering unit after sludge digestion).	Pre-filtered 1 L with 500 μm sieve. 1 mL SDS (150 g/L) added before wet sieving. Treated 200 mL of filtrate (by hydrolysis: 48 h, 40 C, cellulase, and oxidation: 50% H ₂ O ₂ + catalyst), then concentrated MPs in 5 mL ethanol. Effluent filters treated similarly to influent, and MPs concentrated 5 mL ethanol. 0.1 g sludge (dry matter) suspended in 50 mL water and processed similarly to influent. MPs concentrated in 5 mL ethanol. Analyzed particle 20–500 μm.	μ-FTIR-FPA imaging. 0.02–0.3 mL of ethanol suspension applied to slide (Kevley MirrIR) for reflectance mode and to ZnSe transparent slide for transmission (700 x 200 μm area) Reflectance on particles ≥ 80 μm, and transmission on smaller particles. Fast scan to reject non-MPs (MPs absorb in 2820–2970 cm ⁻¹ range). Unrejected particles analyzed.	Large variability between plants: - Influent: mean = 127,000 MP/L (8.0 mg/L), range = 13,000–442,000 MP/L (0.22–29.6 mg/L), median = 86,000 MP/L (5.9 mg/L) - Effluent: mean = 5,800 MP/L (0.034 mg/L), median = 6,400 MP/L (0.016 mg/L). 92.6% retention by number, 99.7% by mass. Median size in effluent (42.5 μm) 20% smaller than in influent (50 μm). Particle Types (% by number): - Influent (181 particles) mainly polyamide/nylon (76.8%) and PE (13.3%). 6.6% Zn stearate, 2.2% PP, and 0.6% each PVC and PP-PE copolymer. - Effluents (150 particles) similar: nylon (77.3%) and PE (16.7%), 4% Zn stearate, 2% PP. Digested wastewater sludge: - MPs about 2% of total dry matter (25–30%). - Mean = 169,000 MP/g (4.5 mg/g). - Median = 158,000 MP/g (6.5 mg/g). - Identified MPs (29): 65.5% PE, 31.0% nylon, and 3.4% PP by number (79.2% PE, 20.3% nylon, and 0.5% PP by mass).	Cannot identify MPs with Zn stearate coating (used on household plastics and others), but coating indicates MP. Authors concluded MP mass is better for assessing removal because it is conserved, but number important in assessing impact. Lassen et al. (2015) estimated total Danish MP emissions (1 μm–5 mm) to aquatic environment of 600 to 3,100 tons/year. Relative to authors' estimate of MPs in effluents (11 tons/year in 20–500 μm range) they concluded that wastewater from Danish WWTPs is a minor fraction of total MP emissions. Mass concentrations based on MP size (estimated volume), polymer density, and scaling to total sample.

Study	Sample and plant/study details	Sample collection	Sample ^a preparation	Analysis ^b	Main findings ^c	Comments
Ziajahromi et al. 2017 Australia Sydney 3 major WWTPs: 1 primary, 1 secondary, and 1 tertiary with reverse osmosis (RO) treatment. PEq = 0.15–1.2 x 10 ⁶	Wastewater at different treatment stages Population and plant capacity (ML/day): Primary = 1.2 million and 308; Secondary = 67,130 and 0.48; Tertiary = 150,870 and 0.28 Sampled over 3 days (10/2015)	Filtered 3–200 L with stacked-sieves (500, 190, 100, and 25-µm), depending on water type. Removed 25-µm screen if blocked (e.g., clogged after 8 L of primary effluent). Tertiary and RO samples were 200 L. Sampled 1-h at max. of 10 L/min. Stored screens in foil-wrapped petri dishes.	Samples concentrated to 100 mL in 90 °C oven. Digested (30% H ₂ O ₂) and treated by density separation in NaI (1.49 g/mL). Centrifuged (15-mL tubes, 5 min, 3500xg). Filtered (25 µm) buoyant particles and rinsed. Stained with Rose Bengal to reduce false positives.	Visual (microscope). ATR-FTIR on particle subset.	Mean effluent concentrations (MPs/L): Primary = 1.54, Secondary = 0.48, Tertiary = 0.28, RO = 0.21 > 90% removal of MPs in primary effluent. 100% removal of particles >190 µm PET fibers and irregular PE particles most common. Attributed to clothing and personal care products. No MPs in controls. Main (75% of suspect) MPs in 1 ^o effluent of tertiary plant: PE (42%), PET (36%), PS (15%), and PP (8%). Most were white and blue irregular particles, followed by black fibers (100–190 µm) PET fibers 65% and 88% of MPs in tertiary and RO samples, respectively. 75% of suspect MPs in tertiary and 60% in RO samples confirmed.	Treated 13–308 x 10 ⁶ L/day Discharge = 3.6–460 x 10 ⁶ MP/day Some differences in plastic composition along treatment train may relate to different sample volumes. Despite rigorous protocol, 22–90% of suspect MPs were non-plastic (e.g., castor oil, stearic acid/stearate. Authors stressed need for careful analyses and long-term monitoring. Recoveries of PS MPs (250–500 µm) in spikes were 92% (25-µm screen) and 99% (500-µm screen).

A²/O = anaerobic, anoxic, oxic (aerobic), AS = activated sludge, BAF = biologically active filter, CAS = conventional activated sludge, DAF = dissolved air flotation, DF = discfilter, FPA = Focal plane array (detector), HCl = hydrochloric acid, H₂O₂ = hydrogen peroxide, MBR = membrane bioreactor, MCT = mercury-cadmium-telluride, µ-ATR-FTIR = Fourier transform infrared spectroscopy coupled with an attenuated total reflectance microscope objective (µ-ATR), NaCl = Sodium chloride, par = particles, PEq = population equivalent, PTFE = polytetrafluoroethylene, RSF = rapid sand filtration (filters), SDS = sodium dodecyl sulfate, SEM = scanning electron microscopy, WPO = wet peroxide oxidation, WWTP = wastewater treatment plant.

clarifiers could improve removal. Source modeling indicated that MP fiber loads in influent were consistent with service area populations laundering textiles. The estimated total discharge from all three plants was 500 million to 1 billion MPs per day, equating to an annual discharge of 0.34-0.68 g MP per capita in treated wastewater. This release may account for <0.1% of the annual plastic input to this metropolitan area's surface waters on a mass basis, considering mismanaged plastic waste. However, as discussed, N&MPs in wastewater may pose unique toxicological risks because of their potential for efficient sorption of toxins and biological uptake.

Yang et al. (2019) reported mean concentrations in influent and final effluent of 12.03 ± 1.29 and 0.59 ± 0.22 MPs/L, respectively, for samples collected at an advanced sewage treatment plant (STP) in Beijing (Table 3-3). The plant treats mainly domestic effluents using: an aerated grit chamber, a primary sedimentation tank, A²/O (anaerobic, anoxic, and aerobic) treatment followed by secondary sedimentation, and a final series of advanced processes (denitrification, ultra-filtration, ozonation and UV). Primary aerated grit treatment removed $58.84 \pm 8.05\%$ of the MPs. Subsequently, $54.47 \pm 14.73\%$ and $71.67 \pm 11.58\%$ of the MPs were removed after the A²/O and final treatments, respectively. An overall reduction > 95% was found. The authors indicated that further improvement would be difficult as advanced technology was already being used. For example, post-filtration units can lower fiber concentrations (Mintenig et al. 2017), and this technology (ultra-filtration) was used after secondary sedimentation. Microfibers were dominant (85.92%) relative to non-fiber MPs, in both influents and effluents, and > 60% were removed by the aerated grit chamber. The authors suggested that an aerated grit chamber treatment might be an effective alternative for reducing microfiber loads. Of the 18 polymers identified, PET, PES and PP accounted for > 70% of the detected MPs. The PET and PES particles were mainly fibers and thought to originate from domestic washing. In contrast to some studies, PE accounted for just 1.64% of the total MPs

and was found in only one of three sample sets. According to the authors, the small PE fraction relative to those found for studies in Europe (average = 14%) (Talvitie et al. 2017a) and the US (> 90%) (Carr et al. 2016) may relate to differences in MP use between China and Europe/US. Some (about 2%) non-fiber MPs contained both PE and PP, and MPs (1.30%) identified as synthetic rubber were found in effluents, possibly from tire wear. Most MPs were black (37%), transparent (34%), or blue (12%), indicating different sources. Particles < 50 µm could not be isolated by the extraction method because high levels of suspended particulate matter quickly clogged the filters. Also, for confirmatory analysis by µ-FTIR, the collected MPs were isolated by hand, under a microscope, and MPs < 50 µm could not be transferred for analysis. The authors noted that a previous study (Erni-Cassola et al. 2017) found higher MP concentrations when filters with a smaller particle capture size were used and recommended future work on identification and quantification of these smaller MPs.

As mentioned, direct comparisons of studies may not be valid due to the many procedural differences. Nevertheless, all studies of WWTPs have found significant reductions of MPs. Primary treatment alone removed an average of 65% (50–78%) of MPs in influents. Secondary treatment increased removal to 97.7% (96–99%), while a final tertiary treatment averaged 94% removal (range = 90–99.9%) (Burns and Boxall 2018). Overall, typical removal efficiencies range from 96%–99%. For example: 99% removal after mechanical, chemical and biological treatments (15,000 particles/L in influent and 8 in effluent) (Magnusson and Norén 2014); 96% after secondary treatment (467 textile particles/L in influent and 16/L in effluent) (HELCOM 2014); and 98% after tertiary treatment (430 particles/L and 180 textile fibers/L in influent, and 4.9 and 8.6, respectively, in effluent), with estimated daily discharges of 2.3×10^9 particles and 1.3×10^9 fibers, based on a flow rate of 270 million L/day (Talvitie et al. 2015). Carr et al. (2016) reported 99.9% removal for tertiary U.S. WWTPs, and a total daily discharge of 9.3×10^5 MPs. Murphy et al. (2016) reported 98.4% removal for a secondary plant in Scotland.

Volertsen and Hansen (2017) reported releases of 0.3% of influent MPs (i.e., 99.7% removal) in Danish WWTPs, with effluent MPs being mostly fibers and fragments.

Though treatment technologies were not designed for MP removal, WWTP retentions of 96%–99.9% represent high capture efficiency. However, MPs are constantly discharged. Thus, even small levels in effluents can result in substantial MP releases to aquatic environments due to the high discharge volumes (Ziajahromi et al. 2017, Conley et al. 2019). Nevertheless, the relative contribution of effluents may be small (Burns et al. 2018, Conley et al. 2019). Volertsen and Hansen (2017) estimated that WWTP effluents contribute only 3% of the total MP load to the environment. And based on fate modeling, recipient rivers were expected to retain the most common MP sizes (Besseling et al. 2017). If so, freshwater sediments are the most relevant compartment to consider with respect to discharges to rivers (Nizzetto 2016b, Burns et al. 2018). However, if most (>95%) MPs are trapped in sludge (Magnusson and Norén 2014; Bayo et al. 2016, 2020; Michielssen et al. 2016; Talvitie et al. 2015, 2017a; Yang et al. 2019), sludge may be a more relevant route of environmental releases from WWTPs (Zubris and Richards 2005, Nizzetto et al. 2016a, Rillig et al. 2017a, Talvitie et al. 2017a, Alimi et al. 2018). Estimates of the MP contents of sludge from 28 WWTPs across 11 provinces in China ranged from $1.60\text{--}56.4 \times 10^3$ MPs/kg sludge (dry wt.), with an average ($n = 79$) of $22.7 \pm 12.1 \times 10^3$ MPs/kg sludge (dry wt.) (Li et al. 2018d). For China, the average environmental release of sludge-based MPs was estimated at 1.56×10^{14} particles per year (Li et al. 2018d). According to an estimate for Europe and North America, the annual amount of MPs transferred from WWTPs to agricultural soils as biosolids exceeded the total burden in oceans (Nizzetto et al. 2016a). Organic fertilizers from biowaste composting and digestion also may be an important source of environmental MPs: 20–24 and 14–895 MPs/kg material were found in biowaste composts and digests, respectively (Weithmann et al. 2018).

4. Methods review: sample collection, preparation and analysis

A variety of methods have been used to collect, process, classify (type, size, number), and identify N&MP particles (e.g., Burns et al. 2018, Renner et al. 2018, Prata et al. 2019b, Strungaru et al. 2019, Sun et al. 2019). Most studies (42%) have relied solely on visual observations for particle identification, by the unaided eye and/or under a dissecting microscope. Of those published in 2016–2017, 43% were based on visual observation only (Burns et al. 2018). Visual identification techniques are relatively simple, fast and low cost, but they are limited to a lower particle size of about 500 μm (Löder and Gerdts 2015). Further, they are subject to positive bias due to misidentification of naturally occurring materials such as cotton and other cellulose fibers, coal/coal fly ash (Eriksen et al. 2013), quartz and calcium carbonate (Ballent et al. 2016), and organic compounds (Ziajahromi et al. 2017). Studies that identified MPs based on visual inspection, without confirmation by an analytical technique, likely overestimated concentrations (Lusher et al. 2017), especially of synthetic fibers, which can be difficult to distinguish from natural fibers (Fischer et al. 2016). Burns et al. (2018) reported error rates from 33% to 70% for 10 studies (Hidalgo-Ruz et al. 2012, Dekiff et al. 2014, Lenz et al. 2015, Lusher et al. 2015, Ballent et al. 2016, Clunies-Ross et al. 2016, Fischer et al. 2016, Horton et al. 2017a, Imhof et al. 2017, Kanhai et al. 2017).

High variability in the total MP counts for different studies has been observed. In part, variability likely relates to the different sampling locations. However, as discussed, it also relates to the many different methods used. Standardized, accurate monitoring methods are key to tracking the sources of N&MPs in the environment. Method requirements depend on the particle properties, sample matrix, and sampling site. Given the pervasiveness of microfibers (e.g., from clothing, air, and settled dust), precautions to avoid MP fiber contamination during sample collection and handling are important (Foekema et al. 2013, Talvitie et al. 2015, Dris et al. 2016, Conley et al. 2019). Examples include wearing cotton clothing, rinsing equipment

with filtered (e.g., 0.8- μm membrane) DI water, processing samples in a laminar flow hood, and procedural blanks (i.e., samples processed by the same procedure used for environmental samples). Pre-rinsed filters should be checked (under a microscope) for contamination and stored covered (e.g., in Petri dishes) until use (Talvitie et al. 2015). An overview of the various methods that have been used for collection, preparation, and analysis of environmental samples impacted by urban/industrial activities is provided below (Sections 4.1–4.3). The discussion applies mainly to MPs as reliable methods for NPIs in environmental samples are lacking (Lehner et al. 2019). Method limitations and potential approaches are discussed further in Section 5. Additional details on methods can be found in recent reviews (e.g., Burns et al. 2018, Renner et al. 2018, Prata et al. 2019b, Strungaru et al. 2019, Sun et al. 2019, Zarfl 2019).

4.1 Sample collection

Sample collection methods based on neuston, manta, and plankton nets have been widely applied to marine waters, with typical mesh sizes of 300 μm or 333 μm . These methods have been adapted to some freshwater studies, but they are not optimal for sampling inland freshwaters (e.g., Bordós et al. 2019), and they can introduce errors for particles in the lower μm size range due to sample loss. Moreover, they are not suitable for studies of important N&MP sources in urban watersheds, such as WWTPs. As discussed, a variety of methods have been used to sample N&MPs in wastewater, but methods have not been standardized (e.g., Ziajahromi et al. 2017). Given the anticipated low concentrations (e.g., 1 particles/L in influents of tertiary WWTPs in the U.S. [Carr et al. 2016]), large water volumes must be sampled. Tables 3-3 and S3-3 summarize sampling methods used in studies of WWTPs.

Most methods for wastewater collection have involved some type of filtration technique. For example, Talvitie et al. (2015) used a custom sampling device to capture different MP size fractions. An electric pump pumped (1.0 ml/min) water from a wastewater stream into the

sampler. The sampler consisted of three, clear plastic tubes (60-mm diameter) and screw-on connectors (with o-rings) to join the tubes. Up to three filters (80-mm diameter), cut from plankton nets with different mesh sizes (corresponding to 200-, 100- and 20- μm filters), could be used in the device. Filters were inserted at the tube connections and the tubes were screwed tightly together. Wastewater was pumped (1 L/min) into the top of the device and through the filters, largest to smallest mesh opening. The filtered volume depended on the filters used and sampling location, from 0.3 L for influent to 285 L for effluent. Reference samples of seawater were collected with the same device, with a petrol driven pump. One thousand liters were filtered through the 200- μm and 100- μm filters. Volumes through the 20- μm filter varied from 20–30 L.

Ziajahromi et al. (2017) reported a stacked-filter sampler with removable, 12-cm diameter, stainless steel mesh screens (500, 190, 100 and 25 μm). It allowed continuous sampling of large volumes of wastewater. The stacked-sieve (largest to smallest size) unit was placed inside a PVC cover that directed water through the sampler to give size-classified particulate fractions. A flow meter at the head of the device measured sample volume. Sampler efficiency was determined using tap water spiked with MPs (60 μm to > 500 μm), based on duplicate spikes and four size fractions: 60-125, 125-250, 250-500 and > 500 μm . Recovered MPs were weighed and the efficiency was calculated based on the masses of added and recovered particles. Average efficiency was calculated for each size fraction. Recoveries of PS MPs (Table 3-3) ranged from 92% for the 25- μm mesh screen to 99% for the 500- μm screen. A negative control found no PVC particle contaminants from the sampling device.

4.2 Sample extraction/preparation

Environmental samples usually require pretreatment to isolate plastic particles prior to analysis. A two-step procedure is often used to separate plastics from other solids (e.g., wood,

sand) and remove any associated organic matter (e.g., biofilms). Isolation of MPs is typically accomplished by density separation, while (bio)chemical treatments are used to digest organic matter. Saturated salt solutions have been commonly used to isolate plastic particles based on density. A saturated sodium chloride (NaCl) solution (1.202 g/cm^3) has been used most often because it is inexpensive and non-toxic (Van Cauwenberghe et al 2015, Hanvey et al. 2017, Renner et al. 2018, Prata et al. 2019b). Repeat extractions can improve recovery of N&MPs (Nuelle et al. 2014, Crawford and Quinn 2017). For example, using a saturated NaCl solution to extract PE MPs from sediment, efficiencies of 61%, 83% and 93% were reported for the first, second, and third extractions, respectively (Crawford and Quinn 2017). A variety of sample pre-treatments to remove non-plastic organic matter also have been used, including protocols based on H_2O_2 , acids and bases (HNO_3 , HCl , NaOH , KOH) and enzymes (Renner et al. 2018, Prata et al. 2019b). A key criterion is removal of non-plastic components without degradation/loss of N&MPs (Bergmann 2015, Renner et al. 2018). Harsh treatments such as digestion with strong acids and bases, and ultrasonic baths, can degrade MPs (Renner et al. 2018). A wet peroxide oxidation procedure (e.g., 30% H_2O_2 with 0.05 M Fe(II)) at about 75°C) can reportedly remove organic matter with little or no impact on MPs (e.g., Eriksen et al. 2013, Tagg et al. 2015).

Although a saturated NaCl solution is efficient for isolation of lower-density plastics, including PE ($0.917\text{-}0.965 \text{ g cm}^{-3}$), PP ($0.85\text{-}0.94 \text{ g cm}^{-3}$) and PS ($1.04\text{-}1.1 \text{ g cm}^{-3}$) (Crawford and Quinn 2017, Zobkov and Esiukova 2017), higher-density plastics such as PET ($1.4\text{-}1.6 \text{ g cm}^{-3}$) and PVC ($1.3\text{-}1.7 \text{ g cm}^{-3}$) have poor extraction efficiencies. Higher-density salt solutions such as sodium iodide (NaI , 1.8 g cm^{-3}), zinc chloride (ZnCl_2 , $1.5\text{-}1.7 \text{ g cm}^{-3}$), and sodium polytungstate (SPT, 1.4 g cm^{-3}) can significantly improve extraction of higher-density plastic particles, but these reagents are more expensive, and some are environmentally hazardous. To maximize efficiency and minimize pollution, multiple extractions and recycling heavy salt

solutions were recommended (Crawford and Quinn 2017, Dris et al. 2018). A small-scale, portable method to separate MPs from sediments by density separation was described by Coppock et al. (2017). The Sediment-Microplastic Isolation (SMI) unit in combination with ZnCl solution (1.5 g/cm^3) reportedly extracted MPs from sediments in one step, with a mean efficiency of about 96%.

Fuller et al. (2016) developed a method for MPs in complex samples (e.g., municipal waste, soil), based on pressurized fluid extraction (PFE). Benefits of PFE include fast, simple, and low-cost extractions, with potential for automation. Extracted residues can be analyzed by a variety of techniques (e.g., FTIR, Raman), but mixtures produce complex spectra, and particle sizes and shapes cannot be determined as they are altered by the extraction. Gies et al. (2018) applied an oil extraction procedure (OEP) (Crichton et al. 2017) to wastewater matrices that reportedly improved sludge extraction relative to previously used methods. An objective was to avoid using sieves, unlike the method of Mahon et al. (2017), to prevent potential air contamination during processing and the loss of fibers, which can penetrate sieves based on diameter. Sample volumes of 1 L could be processed without sieving, and with a lower particle cutoff of $1 \text{ }\mu\text{m}$. Further, no harsh reagents/high temperatures were required, as used previously (Carr et al. 2016, Mintenig et al. 2017). A sample mass of 5 g gave good precision. A method described by Talvitie et al. (2017a) also excluded chemical treatment, but the sample volumes were smaller (0.2–1 g) than those processed by OEP. Depending on the type of sludge and treatment process, MP levels as low as 4 MP/g and as high as 187 MP/g (Lares et al. 2018, Mahon et al. 2017, Murphy et al. 2016, Talvitie et al. 2017a) have been reported. Thus, sample volumes may need to be adjusted to ensure extraction of an adequate number of MPs. Based on the sludge masses examined (2.5, 5, and 10 g), the authors concluded that a 5-g sample should give sufficient accuracy even at relatively low concentrations.

Of the various digestion methods described in the literature, H_2O_2 was used most often, reportedly with negligible effects on N&MPs (Renner et al. 2018). Strong acids, bases, or enzyme solutions also have been used to remove organic contaminants (Renner et al. 2018). Dehaut et al. (2016) recommended KOH for sample digestion. However, peroxide treatment was not tested because the objective was to evaluate digestion methods for seafood tissue. Of the methods and plastics (15 common types) tested, a KOH method was reportedly superior. Plastic integrity was evaluated by MP inspection, weighing, pyrolysis gas chromatography coupled with mass spectrometry (pyr-GC-MS), and Raman spectroscopy. Five of the six protocols tested showed plastic degradation and/or insufficient tissue digestion. A KOH method (10% KOH solution, 24 h, 60 °C) gave efficient digestion with no significant polymer degradation, except cellulose acetate. It was reported to be the best compromise for extraction and identification of MPs in biological samples and recommended for environmental and seafood quality studies. However, in a more recent study, Fenton's reagent was reported to be superior to H_2O_2 , NaOH, and KOH (Tagg et al. 2017, Hurley et al. 2018). In conjunction with density separation, its use was recommended for organic-rich, complex environmental samples (Hurley et al. 2018). Temperature, pH, and the ratio of peroxide to ferrous ions have been reported as key factors in maximizing removal of organic matter by Fenton's reagent (Simon et al. 2018). For example, Simon et al. (2018) adjusted (with NaOH) the pH to about 3 to avoid formation of complex species of ferric or ferrous ions, which lowers the amount of free iron(II), slowing the reaction at pH values below 2 and above 4 (Simon et al. 2018). In addition, the reactor was kept in an ice bath maintained at 15 to 30 °C. Temperature control is essential because the oxidation is exothermic and the mixture can boil violently, and because a yellow substance (possibly a ferric compound) tended to precipitate below ~15 °C (Simon et al. 2018).

A novel method using 96% ethanol (EtOH) was reported for isolating N&MPs from vegetal-rich samples (e.g., beach sand) (Herrera et al. 2018). Biomaterials such algal and plant

components have lower densities than most N&MPs and float on the EtOH surface. Only very low-density particles such as EPS and XPS remained at the surface. After separation, the supernatant was removed and the remaining sample was filtered to recover the plastics. Six plastics (PP, PE, PVC, PUR, PET [fiber], and PS) were tested using five digestion protocols: HCl (3%), NaOH (40%), NaOH (4% in 0.5% surfactant), KOH (10%), and H₂O₂ (30% with 0.05 M iron [Fe(II)] catalyst). The EtOH density separation removed biological matter more efficiently than the digestion methods tested. The method was reported to be simple, safe, and inexpensive, and it was suggested as a standard protocol for separation of N&MPs in environmental samples (Herrera et al. 2018). No degradation was reported for the tested materials, but they were larger (mm scale) plastic items (pieces or pellets). Other types of plastics, and possibly small particles of those tested, could potentially be damaged by ethanol extraction. However, further testing can address this issue.

Density separation and digestion protocols for water and sediment/sludge samples have been described in numerous publications (e.g., Song et al. 2015a; Tagg et al. 2015; Bayo et al. 2016, 2020; Dehaut et al. 2016; Crichton et al. 2017; Karami et al. 2017; Wagner et al. 2017; Renner et al. 2018; Prata et al. 2019b; Sun et al. 2019). For example, two recent reviews reported frequencies of use for different N&MP collection, separation, digestion, and identification methods (Renner et al. 2018, Prata et al. 2019b). Renner et al. (2018) reported that a 30% H₂O₂ solution was the most commonly used (41%) digestion method, and NaCl was the most commonly used (53%) salt for density separations, based on 67 (density separation) and 53 (digestion) published papers. Based on 20 articles, Prata et al. (2019b) also reported H₂O₂ (30%) and NaCl as the most commonly used reagents for digestion and density separation (respectively): 30% H₂O₂ was used for digestion of 25% and 35% of the water and sediment samples (60% used no digestion procedure), while NaCl was used for 65% and 75% of the density separations of water and sediment samples. Regardless of the method applied, additives

can significantly impact polymer density and particle separation (Crawford and Quinn 2017). For example, Wang et al. (2014) reported significant decreases in polymer density due to addition of wetting agents.

4.3 Analysis

In some cases, MPs can be identified by visual inspection under a microscope, based on their physical features. For example, MP fibers have been described as having a consistent diameter, and 3-dimensional bending (Norén 2007), while cellulose fibers are typically ribbon-like (Dyachenko et al. 2017, Murphy et al. 2016). However, Lares et al. (2018) also found PES fibers with a flat, cotton-like appearance. Because visual inspection under an optical microscope is unreliable, a variety of confirmatory methods have been used to identify and characterize N&MPs in environmental samples, the complexity of which depends on particle size and sample matrix (Burns et al. 2018, Mai et al. 2018, Silva et al. 2018).

Spectroscopic and thermal degradation methods have been applied for MP identification. For example, ATR-FTIR (Löder and Gerdt 2015) has commonly been used to identify larger ($> 500 \mu\text{m}$), manually sorted MPs, but pyr-GC-MS also has been applied. Käßler et al. (2018) compared pyr-GC-MS and μ -ATR-FTIR and found these to be complementary techniques. A rapid method to determine MPs directly in soil by hyperspectral imaging (wavelengths: 400–1000 nm) was described by Shan et al. (2018). This imaging technique reportedly has potential for MPs from 0.5–5 mm, giving size, shape, surface characteristics, and composition, and it also has been applied to water samples (Serranti et al. 2018). A portable instrument for fast, direct measurement of MPs in soils was described, based on vis-NIR reflectance spectra (Corradini et al. 2019). It was tested with spiked (LDPE, PET, and PVC MPs) soils and reported as a promising method for pollution hotspots ($>15 \text{ g MPs/kg soil}$). Further testing and development of a spectral library was recommended, and a sample preparation step likely

would be required for general application. For microscopic particle determination, the coupling of a microscope with FTIR or Raman spectroscopy (i.e., μ -FTIR or μ -Raman) provides both the chemical identity and size of individual particles. Unsorted samples also can be analyzed using thermal degradation methods, which provide MP mass but not size or number. Methods based on pyrolysis/thermal decomposition GC-MS (pyr/TD GC-MS) (e.g., Dümichen et al. 2017, Ravit et al. 2017), thermal extraction-desorption GC-MS (TED-GC-MS) of size-fractionated water samples (Bannick et al. 2019), and μ -FTIR or μ -Raman have been applied. The FTIR and μ -Raman methods have the advantages of being non-destructive, selective, and sensitive, and they were reported as the most commonly used (58% of MP studies) (Burns et al. 2018, Mai et al. 2018). Both methods can distinguish MPs from natural particles and identify polymer types. Micro-FTIR and μ -Raman analyses can lower the particle size detection limit to about 10 and 1 μ m (or lower), respectively (Löder and Gerdt 2015, Song et al. 2015b, Duis and Coors 2016). In 64% of the studies, confirmation was performed on < 50% of the particles sampled. A confirmatory technique was used on > 50% of the sampled particles in 13% of the studies, while 23% confirmed 100% of the suspected MPs (Burns et al. 2018). A confirmatory technique is required for MP identification. Previously, a “hot point-test” was used (e.g., Hermesen et al. 2018), wherein suspect particles are considered MPs if the particle deforms and/or forms a dark sticky mark when touched with a hot needle. This test may be useful for visual sorting, but it does not provide polymer identification and is unsuitable for smaller particles.

Methods based on selective staining of MPs with fluorescent dyes have been reported (Maes et al. 2017, Hitchcock et al. 2019, Wiggin and Holland 2019). Maes et al. (2017) described a rapid screening method based on Nile Red (NR), followed by density separation and filtration. Fluorescence was detected under an orange filter and MPs were counted by image analysis. Microplastics extracted from sediments could be observed down to a few μ m.

Also, because NR is solvatochromic (i.e., changes color with solvent polarity), MPs can reportedly be classified into polar (nylon and PET) and nonpolar (PE, PP and PS) groups. Individual polymers cannot be identified, but the small amount of dye used does not interfere with FTIR or Raman analyses. Thus, the method could be combined with these techniques for confirmatory analysis.

Wiggin and Holland (2019) reported a low-cost, time-efficient, reproducible NR method with improved detection of smaller (3–500 μm) MPs relative to visual (microscope) examination of unstained samples. Water samples (Los Angeles River, California) were found to contain 3,622 MPs/ m^3 by unstained visual examination, while 641,292 MP/ m^3 were found by the NR protocol (Table 3-1). The authors concluded that more extensive work is needed on smaller MPs using harmonized testing and reporting standards. Ziajahromi et al. (2017) also applied selective staining to minimize interference of non-MPs, but Rose Bengal was used. Samples (WWTP effluents) were stained after density separation and MPs were identified by FTIR. However, despite thorough sample processing, 22–90% of the suspected MPs were non-plastic particles.

Different FTIR techniques have been applied to MP analysis, including μ -FTIR (e.g., Vianello et al. 2013, Magni et al. 2019), ATR-FTIR (e.g., Cheung et al. 2016), and μ -FTIR imaging based on FPA detection (Löder and Gerdt 2015, Primpke et al. 2017, Simon et al. 2018). In a comparison between reflectance μ -FTIR and ATR-FTIR, both techniques gave acceptable results in identifying PE MPs, but ATR-FTIR produced better spectra for particles with irregular shapes (Harrison et al. 2012). However, relatively large particles ($> 500 \mu\text{m}$) were required for ATR-FTIR (Löder and Gerdt 2015), while the lower size limits for μ -FTIR and μ -Raman were reported to be about 20 μm and sub- μm , respectively. Methods based on μ -FTIR (and ATR) and μ -Raman have typically relied on an initial sorting (categorization) of particles under an optical microscope. After visual sorting, a subset of particles in each group

is then examined spectroscopically to identify the polymer type. For example, a binocular microscope was used to examine particles in sieved sediments, grouped into four categories (fibers, granules, films, and spherules). Polymer types in a representative subset of each group were then identified by μ -FTIR, by comparison of acquired spectra to those in a reference library (e.g., Ng and Obbard 2006, Claessens et al. 2011). A resolution of 8 cm^{-1} was reported to be optimal for μ -FTIR, considering both data quality and analysis time (Löder and Gerdtz 2015). At this resolution, μ -FTIR allowed MP identification and counting directly on dried filter paper (Song et al. 2015b). It also permitted identification of MPs in bottom sediment from a lagoon (Vianello et al. 2013). However, when polymer identities are based only on a small subset of particles from the groups, there may be considerable uncertainty in the distribution of polymers (Hermsen et al. 2018).

Relative to μ -FTIR, the main advantage of μ -Raman is higher resolution, especially important in identifying very small ($< 20\text{ }\mu\text{m}$) MPs (Käppler et al. 2016, Elert et al. 2017, Ivleva et al. 2017, Araujo et al. 2018) not detectable by IR techniques. The number of publications including Raman analysis is sharply increasing, and a semi-automated method was described (Frère et al. 2016, 2017), but FTIR techniques have been more widely applied to MP analysis. Araujo et al. (2018) found 71 original articles in a literature search (2018) on MP identification by Raman spectroscopy. Of these, 86% applied μ -Raman methods (traditional Raman was used in early studies). Also, 6 book chapters and 21 reviews addressed Raman analysis of MPs, with a chapter (by Ribeiro-Claro et al. 2017) being dedicated to the technique. In part, drawbacks such as long measurement time and spectral interference due to fluorescence of some sample constituents (e.g., biofilms) have slowed progress on Raman methods. However, some common limitations have relatively simple solutions that can both expedite analyses and improve MP identification. For example, signal quality can be enhanced with improved, commercially available detectors and spectrum processing methods. Automated particle

selection can expedite Raman mapping, and comprehensive reference libraries can improve spectral matching. Advanced applications such as real-time Raman detection and imaging of MPs also may be possible with less conventional techniques (Araujo et al. 2018). The prospects and challenges of Raman and FTIR imaging techniques for analysis of MPs in environmental samples were reported in a recent review (Xu et al. 2019). A new, fully automated, robust μ -FTIR method also was reported that accurately (> 98%) identified MPs (Renner et al. 2019). The approach detects and numerically describes vibrational bands in an absorbance spectrum by curve fitting, giving a highly characteristic list of peaks for improved library searches. It is based on a published MP identification algorithm (*μ IDENT*), extended to μ FTIR data and focused on relevant broad, overlapped, or complex vibrational bands (Renner et al. 2019).

Levels of NPIs in environmental and biological matrices are virtually unknown due to the difficulty in their measurement. Methods applied to engineered nanoparticles (e.g., UV–vis spectrometry, electron microscopy, field flow fractionation (FFF), and dynamic light scattering [DLS]) for hydrodynamic diameter) have been suggested as appropriate for NPIs, but a combination of these is required for NPI identification (e.g., Correia et al. 2018). Further development of methods based on FTIR (Huth et al. 2012) and Raman (Chernenko et al. 2013, Oßmann et al. 2017) spectroscopies, and atomic force microscopy coupled with IR spectroscopy (AFM-IR) (Dazzi et al. 2012) also has been suggested (Lehner et al. 2019). Promising methods for NPIs and MPs are discussed below (Section 5).

The techniques discussed are useful in classifying the types of N&MP pollution but cannot necessarily identify their origin. To this end, methods based on stable carbon isotope ratio (Berto et al. 2017, Berto et al. 2019) and radiotracers (Lancôt et al. 2018) have been reported. However, the latter technique is technically challenging, requires specialized facilities, and has limited availability (Lancôt et al. 2018), while stable carbon isotope ratio mass spectrometry (IRMS) is relatively simple and widely available. In IRMS, carbon isotopic

ratios ($^{13}\text{C}/^{12}\text{C}$) are expressed by convention, relative to the ratio for a standard (Pee Dee Belemnite), as the $\delta^{13}\text{C}$ value, and this isotopic measurement can distinguish some polymer types. It has been applied to studies of the origin of organic matter in the environment and shows promise for tracking MP fate and transport (Berto et al. 2017, Berto et al. 2019). Findings of our studies of the utility of IRMS for characterization of macro/MP debris are presented in Section 6.

5. Current status and research needs

The environmental impact of N&MPs has attracted increasing scientific, public, and regulatory interest over the past decade. Microplastics have been found in surface waters, sediments, and WWTPs globally, with fibers and fragments being dominant. Growing evidence of environmental N&MPs and their uptake by a wide range of organisms (e.g., Eerkes-Medrano et al. 2015, Lusher 2015, Curren and Leong 2019) has drawn attention to these emerging, persistent contaminants. Concerns about ecotoxicity and other adverse effects have prompted numerous studies, especially of aquatic systems. As discussed, findings of concern include: environmental releases by WWTPs, adverse effects on aquatic organisms, bioaccumulation and amplification in the food chain, sorption/release of toxic organic compounds and metals, biofilms with toxic bacteria, ingestion and translocation, and the presence of MPs in human stool. Efforts to ban products such as microbeads were a positive step, but major releases of plastic items continue, including microbeads in unregulated products. Moreover, although microbeads have been a focus of attention, synthetic fibers may be a much larger issue (Boucher and Friot 2017, Ziajahromi et al. 2017, Henry et al. 2019). A better understanding of the actual risks is needed to establish effective waste management policies and a sound regulatory framework for plastic pollutants. Several important knowledge gaps are discussed below (5.1–5.4).

5.1 Potential risks

The environmental impact of N&MPs is uncertain and the human health risks are unknown. Further study is needed to assess their ecotoxicological and human health risks, especially of NPIs (Andrady 2011, Hidalgo-Ruiz et al. 2012, Galgani et al. 2015, Vethaak and Leslie 2016, Peng J. et al. 2017, Smith et al. 2018, de Sá et al. 2018, Wang F. et al. 2018, Curren and Leong 2019, Lehner et al. 2019, Triebkorn et al. 2019, Wang et al. 2019), but Burns et al. (2018) reported a general perception among investigators that N&MPs adversely affect marine life and may pose risks to humans (Cole et al. 2011, Wright et al. 2013, Ivar do Sul et al. 2014, Van Cauwenberghe et al. 2015, Eerkes-Medrano et al. 2015, Galloway 2015, Koelmans et al. 2015, Lassen et al. 2015, Lusher 2015, Oberbeckmann et al. 2015, Duis and Coors 2016, Auta et al. 2017, Anbumani and Kakkar 2018). Several investigators expressed concerns about the quality of some studies (e.g., Song et al. 2014, Phuong et al. 2016, Lusher et al. 2017), though little attention has been given to the findings in a risk context (Koelmans et al. 2017). Burns et al. (2018) examined evidence of the following apparent assertions: WWTPs do not adequately remove MPs, MPs contaminate waters and sediments, MPs are taken up by organisms and have adverse effects, and MPs are vectors for entry of POPs into organisms and the food chain. They concluded that the evidence of MPs serving as vectors for bioaccumulation of organic compounds was weak, and that available data suggest that MPs are not harming the environment, because levels are orders of magnitude below those associated with adverse endpoints in organisms. However, as recognized by others, the authors acknowledged that the relevance of toxicity assays is suspect due a mismatch between the concentrations and types of materials tested and those in the environment (e.g., differences in particle types, sizes, surface contaminants, and concentrations).

Several major knowledge gaps must be addressed to assess the potential environmental and human health risks of N&MPs. There is a critical need to understand the mechanism(s) of action and ecotoxicological effects of environmentally relevant N&MPs (de Sá et al. 2018). Though N&MPs are a negligible fraction of plastic waste by mass, their potential for efficient sorption of toxins and biological uptake due to small size may present unique toxicological risks, especially for discharges from WWTPs (Conley et al. 2019). In particular, NPIs have much higher surface area for chemical sorption, and relative to larger particles, their uptake by organisms, tissues, and cells is more likely. However, high concentrations of natural particles with similar size ranges have been reported. Triebskorn et al. (2019) found that < 10 in 10^6 particles in river surface water were plastics. These particles also burden ecosystems and their consideration may be relevant to understanding the relative ecotoxicological risks of N&MPs.

The possible impacts of N&MPs on aquatic organisms and humans depend on their environmental fates. In the case of nanomaterials, aggregation and deposition greatly affect availability in aquatic environments. Because different nanomaterials can have widely different surface chemistry (e.g., adsorbed species, extent of ionization) and colloidal stability, and their properties depend on environmental conditions (e.g., pH, ionic strength), it is not possible to generalize the behavior of these materials (e.g., Wiesner et al. 2009). Similarly, N&MPs undergo transformations in the environment, making their fate and impacts difficult to predict. However, trophic transfer of N&MPs through the food web has been observed (e.g., Farrell and Nelson 2013, Setälä et al. 2014), and inhalation/ingestion are human exposure routes (Wright and Kelly 2017, Cox et al. 2019, Toussaint et al. 2019).

Reported contamination of drinking water and food and beverage items has raised concerns about the possible health effects of N&MP ingestion, but uncertainty in the existing data has impeded reliable risk assessments. Based on 50 studies, Koelmans et al. (2019) concluded that MPs are present in water (and some foods) but found no evidence of harmful

effects. However, the authors reported inadequate overall data quality. Limitations (e.g., unreliable comparisons due to use of different methods, unrepresentative data) mainly relate to a lack of standardized methods and quality assurance measures (Hermsen et al. 2018). In the field of toxicology, the validity of studies is commonly assessed by consensus criteria (e.g., Klimisch score, CRED [Criteria for Reporting and Ecotoxicity Data]) (Hermsen et al. 2018). Such criteria have not yet been established for studying the impacts of plastic debris (Koelmans et al. 2017).

High-quality data on N&MP abundances in food, drinking water, and other beverages are needed to better assess the human health risks. Available evidence on N&MPs in a normal diet is scarce and unreliable. Toussaint et al. (2019) used an indirect approach to assess exposure. They reviewed peer-reviewed publications (since 2010) that document the presence of N&MPs in food products, and in animals (> 200 species) in the human food chain. Study methods and the quality criteria that were applied were considered. Overall, the authors concluded that precise data to accurately assess dietary exposure of humans to N&MPs cannot be produced until standardized methods are available. Meanwhile, based on an assessment of human consumption, Cox et al. (2019) reported that avoiding bottled water might significantly reduce MP exposure. They further recommended studies of MP contamination in food groups representing major sources of nutrition globally (grains, vegetables, beef, poultry). Also, where existing data on foods/drinks are suspect due to use of older (unreliable) methods, additional studies were recommended. These new data will provide better estimates of human consumption of plastic particles that ultimately will permit assessment of the potential ingestion risks (Cox et al. 2019).

5.2 Monitoring Methods

As emphasized in this review, a lack of standardized monitoring methods remains a critical knowledge gap and impediment to risk assessment and N&MP studies generally. Targeted particle sizes and analytical procedures have varied considerably, and commonly used visual methods are interference prone. Method limitations have limited the available data on the environmental concentrations and size distributions of plastic particles, especially NPIs and very small MPs (e.g., a few μm), including microfibers. In some cases, N&MP concentrations may be significantly underestimated. For example, Conkle et al. (2018) reviewed 50 aquatic surveys and reported that about 80% accounted only for MPs $\geq 300\ \mu\text{m}$. They also characterized 770 personal care products for microbeads, reporting concentrations of 1,649–31,266 MPs g^{-1} of product (1.9–71.9 mg g^{-1}). More than 95% were $< 300\ \mu\text{m}$ (minimum diameter). Studies employing mesh screens $>125\ \mu\text{m}$ also can underestimate N&MPs (Conley et al. 2019). Further, commonly applied analytical techniques have inadequate size resolution: e.g., about $20\ \mu\text{m}$ for $\mu\text{-FTIR}$. Size distributions in several recent studies appear to be dominated by relatively small particles (e.g., 81–92% $1\text{--}10\ \mu\text{m}$ [Pivokonsky et al. 2018], 96% $4\text{--}20\ \mu\text{m}$ [Triebkorn et al. 2019], 61% $10\text{--}300\ \mu\text{m}$ [Leslie et al. 2017], and 73% $50\text{--}500\ \mu\text{m}$ [Yuan et al. 2019]). However, other investigators (W. Wang et al. 2018, Di et al. 2019, Luo et al. 2019) reported a majority of MPs in larger size classes (Novotna et al. 2019). Improved monitoring methods for NPIs and small MPs are needed to resolve apparent discrepancies and collect accurate data as these particles may pose greater risks, especially if their environmental concentrations are much higher than currently realized.

To accurately identify and quantify N&MPs in environmental samples, multiple analytical techniques likely will be required, and characterization of the full range of plastic particles may not be possible using existing methods. Ideally, identities of all N&MPs in a sample should be confirmed (Tagg et al. 2015, Dyachenko et al. 2017). However, due to

method limitations and time constraints, most FTIR, Raman, and other confirmatory techniques have been applied to a relatively small subset of MPs, after manual sorting of particle types under a stereomicroscope. These techniques have trade-offs with respect to spatial/size resolution, sensitivity, and sample throughput. Further, the sorting process is biased (e.g., missed transparent/small particles) relative to direct spectroscopic identification of particles enriched on filters (Koelmans et al. 2019), and the recommended subsampling procedures differ for different particle size ranges and analysis methods (Mintenig et al. 2018). Identification of small MPs has typically been by vibrational spectroscopy, but FTIR point-mapping microscopes require very small apertures for this work, which degrades the signal-to-noise ratio. In addition, over a minute is required for a single particle. An FTIR array and Raman microscopes also require long analysis times. Faster, automated analysis and data processing methods that cover a larger sample area (e.g., automated FPA-FTIR) can improve data quality. Micro-Raman instruments with current generation detectors and fast spectral processing can extend analyses to the sub-micrometer range, not possible with current FTIR techniques. Automated particle selection, Raman mapping, and comprehensive reference libraries can expedite analyses and improve spectral matching. Advanced applications such as real-time Raman detection and imaging also may be possible (Araujo et al. 2018).

Although FTIR and Raman techniques can identify the types and possible sources of plastics, single particles are required, introducing a selection bias, and μ -FTIR has a lower size limit of about 20 μm . Further, these techniques are not available to some groups, and the manual methods are time intensive. Automated methods are expected to reduce bias and the analysis time, but they are not yet widely available and likely to be expensive. The relatively inexpensive, simple method reported by Wiggin and Holland (2019), based on NR staining and fluorescence, was capable of detecting MPs down to about 3 μm , though μ -fibers could not be

stained. Nevertheless, depending on the required performance criteria, this method may be fit to purpose for some studies.

Combination techniques such as scanning electron microscopy (SEM) with energy Dispersive X-Ray Spectroscopy (EDS or EDX), a chemical microanalysis technique, and Raman spectroscopy can provide detailed information on particle morphology and composition, along with color-coded maps for particle identification, including NPLs, and powerful techniques continue to be developed. For example, Gillibert et al. (2019) described a combination of ‘optical tweezers’ and Raman spectroscopy (Raman Tweezers, RTs). The RTs optically trap plastic particles, from tens of μm to as small as 90 nm, and confirm their composition. The technique also provides particle size and shape, and it can be used to investigate particles coated with biofilms and other organic materials. A commercially available Laser Direct Infrared (LDIR) Imaging system with a quantum cascade laser (QCL) was recently introduced for MP applications (Agilent Technologies 2019). It reportedly allows fast, automated analysis to characterize the chemical identity, size, shape, and total mass of relatively small MPs, down to 10 μm . A point detector and rapidly scanning optics allow MP identification within seconds. Combined with suitable protocols to collect and isolate N&MPs from environmental samples, emerging analytical techniques are expected to advance environmental studies of N&MPs, including studies of the mechanisms by which plastics degrade. In particular, methods to detect NPLs in environmental samples, including methods to assess their fate and effects in tissues and organisms, will greatly advance our knowledge of NPLs, a critical research gap. Microplastics and NPLs are typically referred to as group. However, methods to determine the occurrence, types, fate, and effects of NPLs are much less developed.

A major difficulty in monitoring N&MPs relates to the diversity of plastic types and particle sizes potentially present in environmental matrices. Further, particles can contain

fillers/additives, and they may be copolymers, which can alter density and confound spectral identification, as can environmental transformations (e.g., weathering, biofouling). Isolating these varied particles by a single digestion/extraction procedure, and identifying them by a single analytical procedure, are major challenges. Establishment of best practices to collect, extract, and detect N&MPs in different matrices, and criteria by which to evaluate data quality are needed (e.g., Koelmans et al. 2019, Hermsen et al. 2018). Digestions appropriate to the specific matrix components may improve recovery, while quality assurance/control (QA/QC) procedures can give an indication of particle recovery and minimize and account for sample contamination. Because this field is relatively new, it has lacked a QA/QC framework. Development of relevant N&MP reference materials, proficiency testing, appropriate controls and field blanks, and proper training will assist methods standardization and improve data quality.

Small plastic particles are a special challenge because they are more difficult to isolate and identify, particularly in complex matrices. Consequently, our understanding of the environmental prevalence of NPIs and small (e.g., low- and sub- μm) MPs is quite limited. As discussed, density separations are commonly used for particle isolation, wherein particles rise to the surface of the separation medium passively or with elutriation. However, these methods are not appropriate for very small particles. Flotation is hampered by low their buoyancy, and particles at the surface of the separation medium are difficult to process (Nguyen et al. 2019). Further, surface fouling can significantly affect the density of small particles. Adapting techniques used in other fields could improve particle separation, as suggested in a recent review (Nguyen et al. 2019). For example, density gradient separations based on centrifugation, common in biological applications, may offer improved recoveries of small particles. Other analytical separation techniques such as field-flow fractionation, gel electrophoresis, size exclusion chromatography, and ‘froth’ (foam) fractionation also may have promise. However,

method development will be required to improve efficiency and minimize particle losses in the separation medium (Imhof et al. 2012, Nguyen 2019).

5.3 Source apportionment

The relative contributions of N&MPs from terrestrial and freshwater sources are not well understood, slowing advancement of source management. Fahrenfeld et al. (2019) reviewed the evidence and methods for possible source apportionment of MPs in freshwater systems, including use of MP characteristics and mass balance techniques. The authors concluded that the data indicated potential for differentiating some sources within studies, based on differences in polymer types and shapes, but that clear cross-study patterns were lacking (Fahrenfeld et al. 2019). Major obstacles to source apportionment, and to N&MP studies generally, include challenges in identifying polymer types and surface contaminants; different classifications of particle shapes; lack of data for terrestrial sources; and poor understanding of fate, transport, and weathering processes for N&MPs and surface contaminants. Accurate data on particle composition and size are key to effective pollution prevention and mitigation.

5.4 WWTPs

5.4.1 Long-term studies

Most studies of WWTPs have relied solely on visual-sensory identification of MPs, with counts based on specific physical characteristics (e.g., Conley et al. 2019). Confirmation by μ -FTIR (and μ -Raman) is becoming more common; however, as mentioned, only a sub-sample of MPs is usually examined. Different sampling, isolation, and enumeration methods have been used. Various grab or composite methods have typically been used to collect MPs, different digestion/separation procedures (or none) have been used to isolate them, and different size fractions have been collected and classified. Grab sampling of the waste stream is useful to

capture samples during peak flows, and detect variability or over short time periods, but the sample may not be representative. Long-term studies of WWTPs and urban watersheds are needed to capture temporal variations, using improved sampling strategies (Leslie et al. 2017, Lares et al. 2018, Conley et al. 2019). Seasonal and weather-related (rainy or dry) variations occur, and diurnal variations in WWTP influents may exist. Automated composite sampling could provide more representative samples, but sampled volumes are generally low (10-20 L), causing false negatives, and potential contamination by background textile fibers may be significant (30%).

Additional research on wastewater treatment processes is needed to better understand the factors that affect N&MP retention. A comparison of three unit processes indicated that granular sand filtration and MBRs are more efficient in removing small anthropogenic litter (SAL or ‘microlitter’), though the final effluent had higher fiber content than effluent treated by activated sludge (Michielssen et al. 2016). Studies of WWTPs that treat water from combined sewers may be especially relevant as MPs in stormwater may differ from those in municipal wastewater, and because high MP levels were reported for combined systems in 17 US municipalities (Mason et al. 2016).

In addition to concerns about water and sludge contamination, N&MP loads in WWTPs could adversely impact their operational stability (Enfrin et al. 2019a). Because of their small size and diverse chemical properties, N&MPs enter different treatment processes to varying extents. Further, shear forces of processing could degrade MPs and generate undetectable N&MPs. Degradation of MPs by treatment processes reportedly increased the number of N&MPs in water by an order of magnitude (Enfrin et al. 2019b). Their impact on different treatment units is unclear, but membrane filtration processes could be affected (Enfrin et al. 2019a). Considering degradation and removal in WWTPs, the total N&MPs discharged could

be double the number in influent, with significantly increased particle surface area and porosity for sorption organic pollutants, metals, and pathogens (Enfrin et al. 2019a, Li et al. 2019).

Systematic studies of WWTPs using standardized methods could have important implications for source management. New water treatment processes and approaches to limit N&MPs in water and wastewater are needed, to maintain water quality and reduce risks to ecosystems (Enfrin et al. 2019a). In the case of microfibers from textiles, methods for trapping fibers at their sources (e.g., laundry) could greatly reduce levels in influents and accumulation in sludge. Development of new fabrics with reduced shedding also could provide significant reductions.

5.4.2 Sludge

The potential ecological risks of MP-contaminated sludge should be assessed prior to agricultural or other applications. For such assessments, improved methods are needed for processing sewage sludge and wastewater with high levels of suspended solids. Even after rigorous oxidation procedures, naturally occurring organic debris may remain in the samples (Dyachenko et al. 2017, Lares et al. 2018). Incomplete digestion has contributed potential bias due to natural fibers, especially in studies based solely on visual identification of MPs. An acid digestion step post oxidation (H_2O_2) was reported to reduce interferences of cotton and semi-synthetic textiles, but it also can reduce any acid-sensitive MPs (Conley et al. 2019), and any residual debris may confound results. No digestion or separation procedures were used to remove non-plastic SAL in some studies of WWTPs, rather this microlitter was designated as a category of interest (Talvitie et al. 2017a, Michielssen et al. 2016). Conley et al. (2019) reported thin fibers with white/translucent appearances as suspect MPs. Identification by μ -ATR-FTIR was not possible due to problems with the ATR crystal contact, especially with particles $< 500 \mu m$ (Mintenig et al. 2017). Similarly, Leslie et al. (2017) could not obtain

spectra for most (75%) of the thin transparent fibers in some samples. Staining procedures have been applied to assist fiber identification but improved spectral matching through use of a custom library could better expedite MP identification. As discussed, Talvitie et al. (2017a) obtained poor spectral matches for natural textile fibers with commercial libraries. After generating a custom library, using model fibers, all fibers were successfully identified. User-built libraries also could improve identification of other types of N&MPs. Improved FTIR, Raman, and/or other techniques to identify a wide variety of fibers, including plastic, natural, and semi-synthetic materials, especially for smaller particles, is a priority research need (e.g., Lares et al. 2018). Semi-synthetic fibers, cellulosic materials, and other microliter also may have risks (Remy et al. 2015). Selective measurement could help identify and mitigate sources, if appropriate, and allow assessment of the risks of different materials.

5.5 Conclusions

Concerns about the potential impacts of N&MPs have been a major focus of plastic pollution and continue to be debated. Microplastics have been detected in air, soils, freshwaters, drinking water, oceans, food and beverages, biota, and human stool. Regardless of whether current levels pose ecological and human health risks, plastic pollution is an enormous and escalating problem. The abundances of N&MPs in air, soil, water, and biota are likely to increase due to increasing plastic production and use, projected emissions, and continuous fragmentation of long-lived plastic reservoirs. The prevalence of plastics across ecosystems is increasing, and data indicate that N&MP contaminants will continue to be present in most food and beverages (Cox et al. 2019). Ultimately, the most effective way to reduce N&MP pollution is to reduce plastic production, use, and improper disposal. Multiple, sustained efforts, including improved waste management and development of new materials and technologies (e.g., recycling, waste-to-energy) will be required, evolving towards a circular economy, to

minimize waste and maximize resources, rather than reliance on linear processes and end-of-life cleanup (SAPEA 2019).

Further research is necessary to determine the potential environmental and human health impacts of N&MPs. Studies to identify the types, sources, and abundances of environmental N&MPs, and to investigate their transport, transformations, and fate; size distributions; associated toxins; and potential toxicity are critical research needs. Air, freshwaters, and soils are known sources/sinks of plastic pollution, especially in urban watersheds, but systematic studies of these important environmental compartments are lacking. Additional research priorities include studies of N&MPs in food and beverages, development of analytical reference materials to support method standardization, QA/QC protocols, interlaboratory comparisons, and environmentally relevant toxicity studies. To obtain reliable data, robust, standardized methods to collect, process, and analyze N&MPs in complex matrices are urgently needed, especially for soil, sediment, sludge, wastewater, and biological tissues. Accurate, automated, high-throughput methods that maximize recovery and minimize contamination will permit comparisons across studies and advance our understanding of the potential risks of N&MPs.

6. Experimental: IRMS, FTIR, and μ -Raman methods

Abstract

As detailed in previous chapters, in addition to direct releases of micro- (< 5 mm and ≥ 100 nm) and nano-sized (< 100 nm) plastic particles (MPs and NPIs), degradation of larger plastic debris due to environmental exposure and aging further contributes to environmental N&MPs. Relative to macro-sized plastics, these small particles are of special concern as they have been found to disrupt marine ecosystems globally. The presence of N&MP pollutants in

freshwater and terrestrial systems has also been reported. Because plastic is a persistent and growing environmental pollutant, an enormous reservoir of plastic pollution is expected to increase the environmental levels of N&MPs substantially. While plastic pollution has been widely studied in geographic regions globally, many questions remain about its origins. It has been assumed that marine contamination is mainly of terrestrial origin, but systematic studies of the sources are lacking. A lack of standardized methods to characterize and quantify N&MPs in environmental and biological matrices has slowed progress in understanding their sources, distribution, and impacts. Methods to study the sources, types, levels, and transformations of plastic litter (all sizes) are needed to establish their potential environmental and human health risks. To this end, isotope ratio mass spectrometry (IRMS), attenuated total reflectance Fourier transform infrared spectroscopy, and optical microscopy coupled with Raman spectroscopy were investigated as complementary techniques for characterization of common plastics. The impact of simulated weathering conditions (ultraviolet light [UV], heat) on the IRMS results also was examined. The IRMS method is based on stable carbon isotope ratios, expressed as $\delta^{13}\text{C}$ values. It has been applied previously to study the origins of organic matter in the environment. Our results show a significant difference between the $\delta^{13}\text{C}$ values for plant-derived and petroleum-based polymers. Differences also were found between plastic items composed of the same polymer, both for items from different countries and the same country. The $\delta^{13}\text{C}$ values for recycled materials were either comparable to nonrecycled or much higher. Increasing $\delta^{13}\text{C}$ values were observed after exposure to UV light. Results of the three techniques and their advantages and limitations are discussed.

6.1 Introduction

As discussed in previous chapters, the environmental occurrence and impact of nano and microplastics (N&MPs) has attracted increasing scientific, public, and regulatory interest over

the past decade. Numerous studies have been conducted, especially of aquatic systems. Findings of concern have included accumulation of N&MPs in sludge and discharge by WWTPs, contamination of freshwater and terrestrial systems, potential adverse effects on aquatic organisms, bioaccumulation and amplification in the food chain, sorption of toxins, ingestion and translocation, and the presence of MPs in human stool samples. Though the pervasiveness of plastic pollution has been clearly demonstrated, major research gaps must be addressed to better understand their potential environmental and human health risks. In particular, systematic studies of relevant environmental compartments are needed to determine the environmental sources, types, abundances, dispersion, and environmental transformations of N&MPs, especially of freshwater and terrestrial systems. Currently, environmental levels may be underestimated as most of the prior aquatic surveys (80% in a review of 50 studies) reportedly accounted only for MPs $\geq 300\ \mu\text{m}$ (Conkle et al. 2018). In large part, method limitations and a lack of standardized methods have slowed progress on N&MP research.

Spectroscopic techniques are increasingly being applied to MP analysis to improve the accuracy of particle identification. In particular, optical microscopy coupled with Fourier transform infrared (μ -FTIR) spectroscopy (e.g. Vianello et al. 2013, Magni et al. 2019) or Raman spectroscopy (μ -Raman) (e.g., Di et al. 2019, Triebkorn et al. 2019, Yuan et al. 2019), attenuated total reflectance FTIR (ATR-FTIR) (e.g., Cheung et al. 2016), and μ -FTIR imaging based on FPA (focal plane array) detection (Löder and Gerdts 2015, Primpke et al. 2017, Simon et al. 2018) have been applied. Both μ -FTIR and ATR-FTIR can identify MPs, but ATR-FTIR reportedly produced better spectra for particles (PE) with irregular shapes (Harrison et al. 2012). However, relatively large particles ($> 500\ \mu\text{m}$) are required for ATR-FTIR (Löder and Gerdts 2015), while μ -FTIR and μ -Raman lower size limits are about $20\ \mu\text{m}$ and in the sub- μm range, respectively. Combination techniques such as atomic force microscopy (AFM) coupled with Raman spectroscopy can provide detailed information on particle morphology,

and color-coded maps for particle identification, including NPLs. Other combination techniques (e.g., Raman and SEM) also are available, and powerful techniques continue to be developed. Further details on methods are provided in Section 4.

Although spectroscopic techniques are useful in classifying the types of N&MP pollution, they cannot necessarily identify their sources. Stable carbon isotope ratio mass spectrometry (IRMS) is a specialized technique that can provide information on the geographic, chemical, and biological sources of materials. This relatively simple technique has become routinely available and has been widely applied to trace the origins of organic matter in the environment (Muccio and Jackson 2009, Duarte et al. 2018). It reportedly showed promise for tracking plastic debris and changes in polymer structure due to aging/weathering processes (Berto et al. 2017, Berto et al. 2019). Tracing the sources of organic substances relies on their relative isotopic abundances. Namely, the isotope ratios of lighter elements common to organic substances (typically $^{13}\text{C}/^{12}\text{C}$, $^{18}\text{O}/^{16}\text{O}$, $\text{D}/^1\text{H}$, $^{15}\text{N}/^{14}\text{N}$, and $^{34}\text{S}/^{32}\text{S}$). Because isotope ratios depend on local kinetic and thermodynamic factors that affect isotope enrichment or depletion, isotope signatures can be used to distinguish materials by geographic origin that otherwise have the same composition (Muccio and Jackson 2009). This information may be difficult or impossible to obtain by other methods. Because many research fields require accurate, precise measurement of the isotopic ratios of light elements, and technological advances have made this type of analysis more routine, IRMS is increasingly being applied in a variety of fields including archaeology, biology, medicine, food authenticity, and forensics.

In this study, stable carbon IRMS was investigated as a complementary tool for monitoring environmental plastic debris. Isotopic signatures ($^{13}\text{C}/^{12}\text{C}$) for a variety of common plastics used in food packaging and other consumer items were determined and expressed as $\delta^{13}\text{C}$ values. To confirm polymer identities, samples also were analyzed by ATR-FTIR and μ -Raman spectroscopies. In addition, the impact of simulated weathering conditions (ultraviolet

light [UV], heat) on the $\delta^{13}\text{C}$ values was examined. Results for the three techniques and their advantages and limitations are discussed.

6.2 Methods

6.2.1 IRMS

Common mass spectrometers (e.g., single quadrupoles, ion traps, and time-of-flight [TOF]) are not adequate for measurement of the small differences in naturally-occurring, stable isotope abundances. For these measurements, a specialized instrument (e.g., multi-collector magnetic sector) commonly known as an isotope ratio mass spectrometer (IRMS) is required (Muccio and Jackson 2009). Several sample introduction methods are available for IRMS, depending on application. For powders and other bulk materials, flash combustion is the most commonly applied technique, using an elemental analyzer.

An elemental analyzer (EA) works by combusting a solid or liquid sample in the presence of oxygen at high temperature, and reducing the sample to its elemental forms: N_2 , H_2 , CO_2 , SO_2 etc. For this study, an NC 2500 Elemental Analyzer (Carlo Erba) with a Thermo Conflo III coupled to a DeltaPlus (Thermo Finnigan) stable isotope mass spectrometer (EA-IRMS) was used. The instrument components are shown in Figure 6-1 and a general scheme for the EA interface design is given in Figure 6-2. Samples were dropped by an autosampler carousel into an oxidizing furnace (chromium cobaltous oxides) at 1020 °C, where they are flash combusted under a stream of oxygen, generating CO_2 , NO_x (oxides of nitrogen), and H_2O , plus SO_2 if sulfur is present.

Figure 6-1. Elemental analyzer isotope ratio mass spectrometer (EA-IRMS), with autosampler tray and tin sample capsules (EPA, Cincinnati, Ohio).

Figure 6-2. Elemental analyzer interface for stable carbon isotope ratio mass spectrometer (IRMS)

Depending on the isotopes of interest, the combustion products may require treatment to minimize interferences. In the case of carbon (Figure 6-2), the most common application, combustion products are carried by helium flow into a reduction furnace (with elemental copper at 650 °C), which removes excess oxygen and reduces NO_x to N_2 gas. The gas stream

then passes through a water trap (typically magnesium perchlorate $[\text{Mg}(\text{ClO}_4)_2]$ to remove water prior to entry to a gas chromatograph (Porapak-GS column at 50 °C in this work) for separation of CO_2 and N_2 . Effluent from the EA enters the IRMS under continuous flow (controlled by Conflo III regulating device) for stable isotope measurement and determination of stable isotope ratios against ratios for secondary standards. A reference gas is used as an internal standard, introduced by the interface unit, which quickly switches between sample and reference. The internal standard for our instrument is ultrahigh purity (UHP) CO_2 calibrated with NIST standards.

To prevent bias and minimize error, the ratios of these isotopes are determined relative to an isotopic standard. The standards are, or can be related to, international standards (Muccio and Jackson 2009). Because the primary standards can be rare or depleted over time (e.g., the Pee Dee Belemnite [PDB] international standard for carbon is exhausted), several available secondary standards are used. The International Atomic Energy Agency (IAEA, Vienna, Austria) and NIST (National Institute of Standards and Technology, United States) have a variety of natural abundance standards. An L-glutamic acid standard (NIST 8573, USGS40), for carbon and nitrogen isotopes, also has been used. Established standards represent a stable material that is highly enriched in the minor (heavy) isotope. Because most samples are depleted in the heavy-isotope relative to the standard, they have negative delta values. Guidelines on working standards and establishment of universal isotopic referencing procedures are reported elsewhere (Werner and Brand 2001).

For optimal results, the signal intensities of the samples and standards should be similar, and external standards should be included with the sample set. In this study, 100–250- μg of the samples and standards (acetanilide and sulfanilamide) were used, but lesser amounts can be analyzed (e.g., 20–25 μg [Berto et al. 2017]). The samples were weighed in tin capsules and

the tops of the capsules were gently closed, forming a roughly spherical shape with no protruding edges. The capsules were then placed in an autosampler for analysis.

By convention, stable carbon isotopic ratios ($^{13}\text{C}/^{12}\text{C}$) are expressed relative to the ratio for an international standard (Pee Dee Belemnite [PDB] or Vienna PDB [VPDB]). Higher $\delta^{13}\text{C}$ values indicate enrichment of the sample in the rarer, heavier ^{13}C isotope. Results are reported as $\delta^{13}\text{C}$ values, in parts per thousand (‰) (per mil), calculated as follows:

$$\delta^{13}\text{C} (\text{‰}) = \left[\frac{^{13}\text{C}/^{12}\text{C}_{\text{sample}}}{^{13}\text{C}/^{12}\text{C}_{\text{VPDB}}} - 1 \right] \times 1000 \quad (1)$$

The raw values for the standards and samples are blank corrected (if necessary). If the standard deviation for the standards is $\leq 0.6 \text{ ‰}$, no drift correction is applied. The raw (or blank-corrected) values for the standards (dependent variable) are plotted against their expected values (known). A typical r^2 for the regression curve ranges from 0.95-0.99 (corrective action is taken if r^2 is < 0.95). A precise fit indicates complete combustion of the sample and proper operation of the EA unit. The linear regression curve is then applied to generate $\delta^{13}\text{C}$ results for the samples. Additional details can be found in Appendix B.

Our EA-IRMS instrument has a precision of 0.1‰ for carbon (and nitrogen), typical of continuous flow instruments. Instrumental (e.g., ion current stability) and other factors affect precision and accuracy. However, certain performance benchmarks can be expected. Analytical precision and accuracy of $\delta^{13}\text{C}$ results of a study by Duarte et al. (2018) were ± 0.13 and $\pm 0.69\text{‰}$, respectively.

6.2.2 ATR-FTIR

A desktop FTIR spectrometer (Bruker Vertex-80) located at the US EPA Center Hill Facility (Cincinnati, Ohio) was used for analysis of plastic items. The system (Figure 6-3) is

equipped with accessories for measurement of absorption/transmittance spectra of solid, liquid and gas phase samples including an ATR accessory for ATR-FTIR spectroscopic analysis of particles and other solids. Several instrument operating parameters (default) for the ATR-FTIR analysis are listed in Table 6-1.

Figure 6-3. FTIR spectrometer system with microscope (left) and ATR (right) accessories.

Table 6-1. ATR-FTIR scanning and system parameters.

Advanced Parameters	Settings
Resolution	4
Sample Scan Time	6 scans
Background Scan Time	6 scans
Save Data	From 4000 to 400 cm^{-1}
Result Spectrum	Absorbance
Optics Parameters	
Source Setting	MIR source (#1)
Beamsplitter	KBr
Optical Filter Setting	Open
Aperture Setting	8 mm
Accessory	A225/Q Platinum ATR, Multiple Crystals CRY Diamond
Sample/Background Measurement Channel	Sample Compartment
Detector Setting	RT-DLaTGS (#1)
Scanner Velocity	10 kHz
Sample Signal Gain	Automatic

A spectral library search (SEARCH package in Bruker OPUS software) was used for polymer identification. A peak based algorithm searched the library spectra database and reported top results in order of ‘hits.’ High quality matches (> 500) and characteristic spectral bands of the polymer were considered confirmatory.

6.2.3 Micro-Raman

As a technique complementary to FTIR, Raman analyses also were performed. A Renishaw inVia™ Raman microscope is equipped with a Leica DM2500 microscope (x5, x50 and x100 objectives) and multiple lasers (442, 457, 488, 514, 633, and 785 nm) was used for μ -Raman analysis. The instrument, shown below in Figure 6-4, is located at the University of Cincinnati (Cincinnati, Ohio), in the Department of Chemistry. Operating and system parameters are listed below in Table 6-2.

Figure 6-4. Renishaw inVia™ micro-Raman instrument.

Table 6-2. Micro-Raman instrument operating and system parameters.

Parameter	Settings
Grating Scan Type	Extended
Default values for Extended ^a	10s exposure, 1 accumulation, 100% power
Exposure Time	60s or less (typically >10s)
Accumulation ^b	1 at 10s (default)
Spectrum Range	100–3–0 cm ⁻¹
Power (%)	variable (1% safe for most samples)
Configuration (laser-grating)	
Laser	Grating
785 nm (300 mW)	1200 1/mm
633 nm ^c (17 mW)	1800 1/mm
488 nm	1800 1/mm
229 nm	3600 1/mm, UV optics

^a Start at 50% power and adjust intensity depending on spectrum. ^bNumber of times sample is excited by laser for a given exposure time. ^c633 nm used in this study.

Renishaw software version WiRe 3.4 (Windows-based Raman environment) generated spectral data as *.wxd and *.txt files. A spectral library search to identify the samples was performed in KnowItAll® software (Bio-Rad), Vibrational Spectroscopy Edition with IR & Raman Spectral Libraries. The spectral data files (*.txt) were loaded into the program, which automatically performed baseline and noise corrections, if needed, and ran a single component search of the Raman library. The program reported the top three results based on a score from 0 to 100 and generated a report (*.pdf file). High quality matches (>50%) were considered confirmatory.

6.3 Samples

A variety of candidate polymers were selected for this study, primarily based on their environmental and commercial prevalence. Selected polymer materials included pure standards, commercial pellets, and grocery bags and food containers, some of which were

recycled plastics. The plastic items collected for this study and their respective polymer groups are listed in Table 6-3. Additional information on the items analyzed and methods used is provided as supplemental material (Table S6-3).

Table 6-3. Sample items and respective polymer groups.

Polymer Type	Sample Name	Sample Type	Polymer Type	Sample Name	Sample Type
Polyethylene Terephthalate	Polyester Scarf	Clothing	Polypropylene	PP Pellet	Plastic Resin
	Water Bottle	Food Container		Black Food packaging	Food Container
	Blue Food Packaging	Food Container		Opaque Food packaging	Food Container
	Clear Bottle	Food Container		Reusable plastic cup	Food Container
	Clear Green Bottle	Food Container		Yogurt Container	Food Container
	Clear Food Packaging (Recycled)	Food Container		Yogurt Container Lid	Food Container
				Soup Container	Food Container
High-Density Polyethylene	HDPE Pellet	Plastic Resin	Polystyrene	PS Pellets	Plastic Resin
	HDPE White Bottle	Food Container		PS Pellets	Plastic Resin
Polyvinyl Chloride	PVC Powder	Plastic Powder		Clear food packaging	Food Container
				Calibration Film	Plastic Standard
				Expanded polystyrene foam	Polymer Foam
Low-Density Polyethylene	Store #1 Plastic Bag	Shopping Bag	Additional Polymer types	Polylactic Acid (PLA) Pellets	Plastic Resin
	Store #2 Plastic Bag	Shopping Bag		Acrylonitrile Butadiene Styrene (ABS) pellets	Plastic Resin
	Store #3 Plastic Bag	Shopping Bag		Polyurethane foam	Polymer Foam
	Store #4 Plastic Bag	Shopping Bag		Nylon 6	Plastic Resin
	Store #5 Plastic Bag	Shopping Bag		Dryer Lint (containing various polymer fibers)	Fiber
	LDPE Pellet	Plastic Resin			
	Disposable food baggie	Consumer Product			

The main polymers analyzed were those commonly used as food packaging/containers. These included polyethylene terephthalate (PETE or PET), high and low density polyethylene (HDPE and LDPE), polypropylene (PP), and polystyrene (PS). Other materials examined included polyvinyl chloride (PVC), polylactic acid (PLA), acrylonitrile butadiene styrene (ABS), and polyester (PES).

6.4 Results and Discussion

6.4.1 Spectral identification

Example ATR-FTIR spectra of PP packaging for food products are presented in Figure 6-5. These results demonstrate successful ATR-FTIR identification of the base polymer in two

black plastics that showed peaks attributed to the dark additive(s). Although several large peaks (below about 1050 cm^{-1}) are present in the spectrum (black trace) for a black PP packaging material, and a black PP lid (red trace) had a peak (non-polymer) at 700 cm^{-1} , the polymer matrix was correctly identified by the spectral library search. In contrast to the dark plastics, the spectrum (blue trace) for a clear PP container had no potentially confounding peaks.

Figure 6-5. ATR-FTIR spectra of polypropylene food packaging.

Figure 6-6. Micro-Raman spectra of polypropylene food packaging.

Raman spectra for the same items are presented in Figure 6-6. In this case, Raman analysis had an obvious limitation with black food packaging. While characteristic peaks are visible in the spectrum (black trace), high background due to the additive(s) obscured the polymer spectrum. However, in this study, all samples were polymer standards, purchased materials (knowns), or plastic items marked with a resin identification code. A focus was the potential utility of IRMS as a complementary tool for studies of environmental plastic debris, (e.g., tracking/weathering studies). Thus, we selected known materials for accurate data interpretation. In the case of contaminated field samples, sample preparation steps (e.g., biofilm removal) may be necessary prior to spectroscopic analysis.

In this study, all samples were correctly identified, including a mislabeled material (pellets) from one vendor, incorrectly labeled as PP but was actually PE, based on ATR-FTIR analysis (Figure 6-7). Although additives can cause high Raman background and additional peaks in the FTIR spectra, all spectral matches agreed with the polymer resin codes, confirming sample identities as necessary for the IRMS evaluation. Thus, spectroscopic analyses were important in this work, not only to better understand their potential limitations, but also to confirm the identity of all polymers (and provide information on additives). Representative FTIR and Raman spectra are provided in the supplemental material.

Figure 6-7. FTIR identification of a PE product mislabeled as polypropylene PP.

6.4.2 IRMS

As mentioned earlier, higher (less negative) $\delta^{13}\text{C}$ values indicate a higher abundance of ^{13}C , while lower (more negative) values indicate lower amounts. Higher values are expected for plant-derived materials (e.g., bioplastics), while lower values are expected for petroleum-based plastics. Local variations in isotope ratios occur due to selective enrichment/depletion of the heavier isotopes (Muccio and Jackson 2009). For example, plants use atmospheric or dissolved CO_2 as a carbon source, and different factors affect their ability to enrich or deplete ^{13}C from these sources, by a process called ‘fractionation.’ Both genetic and environmental factors affect fractionation. The C_4 plants (monocotyledons such as sugar cane, millet, corn, tropical grasses) use the Hatch–Slack photosynthetic cycle, and typically have $\delta^{13}\text{C}$ values from -8 to -20. In contrast, most C_3 plants (dicotyledons such as wheat, rice, rye and cotton) employ the Calvin–Benson cycle and have $\delta^{13}\text{C}$ values ranging from -22 to -35. Crassulacean acid metabolism (CAM) plants (e.g., pineapple, cactus, orchids) can use either metabolic pathway (C_3 or C_4) and have $\delta^{13}\text{C}$ values between -10 and -34 (Muccio and Jackson 2009). Duarte et al. (2018) applied multi-element IRMS to monitor primary producers, trace sources and cycling of organic matter, and characterize the physiological status of photosynthetic organisms in the Red Sea. They reported data on carbon (and nitrogen, where possible) isotope ratios of phytoplankton, macroalgae, seagrasses, mangroves, and salt-marsh plants. The isotopic signatures differed for plant types across a north-south gradient. In continental plants, the carbon isotope composition depends mainly on enzymatic paths of primary production (e.g., C_3 , C_4 , or CAM) and WUE (Water Use Efficiency) (Duarte et al. 2018). Environmental factors (e.g., temperature, rainfall, sunlight) influence fractionation as these factors impact the kinetics of processes such as CO_2 diffusion into the stomata of leaves (Muccio and Jackson 2009).

Suzuki et al. (2010) demonstrated that stable carbon isotopic composition can be applied to discriminate plastics derived from C_4 plants from those derived from petroleum. Berto et al.

(2017) extended this work to assess the suitability of the technique (stable carbon IRMS) to discriminate a range of plastics and natural materials, considering their use in plastic packaging items (e.g., shopping bags, water bottles). They further investigated degradation of different types of MPs in marine environments. Specifically, they examined the variation in the $\delta^{13}\text{C}$ values of plastics subjected to physical or biological processes that occur in the marine environment. Based on a preliminary field survey, they found evidence of biotic and abiotic degradation of food packaging (Berto et al. 2017).

The IRMS results of our study are presented in Figures 6-8 through 6-14. Figure 6-8 presents a comparison of the results of this study (orange bars) and a study by Berto et al. (2017) (blue). Our results represent pooled data for replicate analyses of two sample sets analyzed 16 days apart. As expected, PLA, had a higher (less negative) $\delta^{13}\text{C}$ result than petroleum based-products. The $\delta^{13}\text{C}$ value (-8.93) for PLA is consistent with a C4 plant origin ($\delta^{13}\text{C}$ range of -8 to -20), being derived mainly from corn starch (C4) in the US (or tapioca root, a cassava species with C3-C4 character, in Asia; and sugarcane [C4] elsewhere). Overall, results showed reasonable agreement with Berto et al. (2017), except for ABS and PLA, which were lower (more negative) than our results. In one case, for a PVC powder, our result was lower than that reported by Berto et al. (2017). The result was for triplicate analysis of a single PVC powder, while Berto et al. (2017) analyzed six samples that may have been from different sources.

Figure 6-8. Comparison of IRMS results from this study and Berto et al. (2017). Data from this study (blue bars) are pooled results for sample sets analyzed 16 days apart.

Figure 6-9. IRMS results for HDPE disposable bags of different origins.

Figure 6-9 presents the IRMS results for HDPE bags from major retail stores. The result on the right was for a plastic bag produced in Thailand, while the other two results were for bags produced in the United States (US). The difference between the two countries, about 0.9 (‰) higher for Thailand bag, suggests that the country of origin (manufacturer) may play a role in the $\delta^{13}\text{C}$ value, possibly due to differences in manufacturing processes. Differences between LDPE bags of different origins were much larger. Figure 6-10 presents results for LDPE bags from the US, Thailand, and China. The $\delta^{13}\text{C}$ results for US products were about 2-4 lower relative to a bag from Thailand and 2-5 lower relative to bags from China.

Figure 6-10. IRMS results for LDPE bags of different geographical origins.

Results for recycled PETE products, represented by the lighter blue bars on the right in Figure 6-11, were either comparable to (product 1) or significantly higher (product 2) than nonrecycled products. The recycled items were a water bottle (product 1) and fresh fruit packaging (product 2). The mean $\delta^{13}\text{C}$ value for the fruit packaging was 1.1 higher than the mean for the nonrecycled PETE samples, which may relate to use of bioplastic(s) in the

recycling process. The higher result for a recycled sample differs from results of a previous study (Berto et al. 2017), which found a lower mean for recycled PETE samples relative to nonrecycled samples.

Figure 6-11. IRMS results for PETE and recycled PETE used as food and other packaging.

Figure 6-12 presents IRMS results for PP food packaging/containers. The far right result was for a container manufactured in New Zealand in 2013, while the other results were for products made in the US in 2019. The result for the PP container made in 2013 is 4 higher than that for the highest mean (packaging 4) for the 2019 products. However, given the different origins of the samples, it is not possible to determine whether both factors (age and origin) affected the results. Differences between the other samples, all from the US, were smaller, but these items also could be distinguished due to the high measurement precision.

Figure 6-12. IRMS results for polypropylene food packaging/containers. Items from different US sources were collected in 2019. One from New Zealand was dated 2013.

To further address the potential of IRMS for environmental tracking of plastics, plastic straws from 15 sources were collected and analyzed. Results for replicate analyses ($n \geq 3$) are presented in Figure 6-13. The bar colors indicate the color of the straws, with grey representing a clear material. Straws 2, 7, and 14 were white with red stripes, and straw 11 was white with green stripes. In these cases, the white portion of the straw was analyzed. Straw 13 was half blue and half clear, and the clear portion was analyzed. The $\delta^{13}\text{C}$ values varied over a range of -31.07 to -24.63. Where known, the country of manufacture is listed. No trend in the $\delta^{13}\text{C}$ values was noted based on country of origin. One straw (orange) from China had a much lower value (-31.07) than three others from China. The straws likely contain varying levels of plasticizers as some were noticeably more flexible than others. Additional samples may help discern whether any trends related to geographical origin exist. But given the significant differences between the different straws, IRMS shows promise for studying transport of this pervasive plastic debris.

Figure 6-13. $\delta^{13}\text{C}$ results for 15 plastic straws with different points of origin (specified where known). See text for details.

In addition to tracking the origins of organic matter in the environment, stable carbon IRMS results may be a useful marker of changes in polymer structure due environmental weathering/aging, evidenced by increasing $\delta^{13}\text{C}$ values (Berto et al. 2017). As discussed in previous chapters (1.2.1, 2.2.1), continued weathering/aging can generate secondary N&MPs through breakdown of larger plastic debris. Breakdown occurs through hydrolysis, photodegradation (ultraviolet light), mechanical abrasion, temperature changes, and biological and chemical degradation (e.g., Thompson 2004, Browne et al. 2007, Barnes et al. 2009, Andrady 2011, Browne et al. 2011, Imhof et al. 2012, Lehner et al. 2019), and these environmental stressors can act in concert. For example, prolonged UV exposure causes brittleness due to changes in polymer structure, which increases mechanical degradation (Mattsson et al. 2015). Some polymers are more susceptible to certain stressors than others. In particular, PS and common polyolefins such as PE and PP are more prone to UV breakdown (Feldman 1984, Gijsman et al. 1996, Song et al. 2017).

We conducted a preliminary study to examine the short-term exposure effects of common environmental stressors such as heat and ultraviolet (UV) light. Plastic items of varying polymer types (PP and PS food packaging, white PS foam, and a PETE water bottle) were exposed for a one-week period under the following conditions: UV light, UV light + water (stirred at room temperature), water only (30 °C), and heat only (40 °C). As shown in Figure 6-14, the IRMS results for four samples, a PP candy container, PS foam, and two PP yogurt containers (white and clear) showed statistically significant increases (less negative) in their $\delta^{13}\text{C}$ values relative to unexposed samples, especially for UV exposure. A PS black food tray also showed a slight increase, but less than that for a white PS foam sample. The relatively modest increase in the black PS tray may reflect increased stability due to the black additive. Changes in the $\delta^{13}\text{C}$ results for PS and PP are consistent with the reported UV sensitivity of these polymers (Feldman 1984, Gijsman et al. 1996, Song et al. 2017). The trend of increasing $\delta^{13}\text{C}$ values also is consistent with a previous study by Berto et al. (2017).

Figure 6-14. $\delta^{13}\text{C}$ results ($n = 3$) for plastic packaging items before and after exposure to different environmental stressors (simulated conditions).

6.5 Methods: advantages, limitations, and potential applications

6.5.1 FTIR and Raman

Infrared instruments permit identification of polymers and other organic species based on the vibrational frequencies of chemical functional groups. Advantages of ATR-FTIR include fast, nondestructive analysis; polymer identification (chemical information at a minimum); and little or no sample preparation. Disadvantages include its sensitivity to water, manual sample loading, and the relatively large (about 500 μm) particle size required. These limitations were not an issue in our study as we used larger samples for the purposes of this work. However, samples with high numbers of relatively small MPs require alternative spectroscopic methods.

As discussed in Section 4, ideally, all suspect N&MPs in a sample should be confirmed analytically (Tagg et al. 2015, Dyachenko et al. 2017). However, for practical reasons, most Raman and FTIR methods have relied on a relatively small sub-set of particles for MP confirmation. Fast analysis and processing methods that cover a larger sample area can greatly improve N&MP detection. For example, a μ -FTIR system with an FPA detector can provide automated FTIR imaging of MPs down to about 20 μm (Löder and Gerdt 2015, Primpke et al. 2017, Simon et al. 2018). Relative to μ -FTIR, the main advantage of μ -Raman is higher resolution, important in identifying very small ($< 20 \mu\text{m}$) MPs (Käppler et al. 2016, Elert et al. 2017, Ivleva et al. 2017, Araujo et al. 2018) not detectable by IR techniques. Micro-Raman instruments with current generation detectors and fast spectral processing can extend analyses to the sub-micrometer range, not possible with current FTIR techniques. Automated particle selection, Raman mapping, and comprehensive reference libraries can expedite analyses and improve spectral matching. Unlike FTIR, Raman spectroscopy is insensitive to water. A disadvantage of Raman relative to FTIR is that the polymer spectrum can be obscured by high background (e.g., fluorescence and/or dark additives). Advancements in instrumentation are expected to resolve these issues and provide even smaller size resolution. For example,

Gillibert et al. (2019) described a Raman instrument that optically traps particles from tens of μm to as small as 90 nm and confirms their chemical composition (see Section 4). It provides particle sizes and shapes and has potential applications to particles coated with biofilms and other organic substances. These emerging spectroscopic techniques are expected to advance studies of N&MPs, including studies of the processes by which plastics degrade.

6.5.2 IRMS

Unlike spectroscopy, small amounts of additives do not appear to affect the IRMS results significantly. In addition, IRMS can distinguish samples composed of the same polymer when their isotopic signatures differ (e.g., plant- and petroleum-derived plastics). Other advantages include high sensitivity; relatively small sample mass requirement; fast, automated analyses; and low cost. Disadvantages relative to spectroscopic techniques are that IRMS is not a confirmatory technique and is destructive. Thus, when applied to MPs, prior characterization is necessary if the sample amount is insufficient for separate analyses. Another disadvantage relative to spectral analyses is that sample preparation is somewhat tedious and time consuming (especially with replicates). However, proper sample preparation, including accurate weights and careful sample packing, is essential to accuracy and precision. And sample mass may be a limiting factor in some studies. Analysis of low sample masses (e.g., small particles) was unnecessary in this study and may require modification of a conventional EA-IRMS instrument. Highly precise, accurate measurement of the $\delta^{13}\text{C}$ values of μm -sized organic particles traditionally has been limited to secondary ion beam techniques, which are not suitable for analyses of large numbers of individual particles due to the substantial processing time required and instrumental factors (e.g., rough surfaces at the nanometer scale (van Roij et al. 2017)). Advances in instrumentation over the last 10 years have greatly reduced the minimum sample requirements.

Measurement of the $\delta^{13}\text{C}$ values of organic solids has commonly been by EA-IRMS (i.e., combustion in an EA and detection of resulting CO_2 by IRMS). In general, a minimum of about 20–25 μg of carbon is required, depending on the blank associated with the sample cup and carousel. The use of cleaning protocols and low-blank autosamplers have reduced the blank response. The minimum carbon mass depends on the instrument but at least several micrograms have typically been required (van Roij et al. 2017). Using a conventional EA-IRMS instrument, the minimum mass was further reduced by decreasing the EA to IRMS split ratio and use of a cryotrap, which allowed measurement of $\delta^{13}\text{C}$ values on about 0.5 μg of carbon (van Roij et al. 2017). New designs can have even lower limits. A design combining laser ablation, nano combustion gas chromatography, and IRMS substantially reduced the lower limit. A deep UV (193 nm) laser allowed optimal fragmentation of organic substances, with minimal isotope fractionation effects. An accuracy and precision better than 0.5‰ were reported for single spot analyses when measuring at least 42 ng of carbon (van Roij et al. 2017). This approach shows great promise for analysis of very small particles, provided individual particles can be isolated.

In some cases, a similar range in isotopic $\delta^{13}\text{C}$ values could mask different potential sources, limiting the utility of IRMS. However, multi-element isotope analysis could overcome this limitation. For example, an evaluation of deuterium (D) confirmed its promise in this regard, through source apportionment using mixing models to discriminate between isotopically-overlapping plant types. In a study by Duarte et al. (2018), an overlap was found in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for seagrasses and macroalgae. However, the δD values were significantly different ($-56.6 \pm 2.8\text{‰}$ and $-95.7 \pm 3.4\text{‰}$, respectively). This considerable difference demonstrates the potential of multi-element IRMS for ecological/environmental studies.

6.6 Conclusions

Our study showed IRMS to be a promising tool for tracking the sources and transformations of plastics in the environment. As a complementary technique for plastics characterization, its advantages include high sensitivity, automated analysis, and relatively low cost. Further, unlike FTIR and Raman techniques, IRMS could distinguish items composed of the same polymer, and colored additives had no discernable impact on the $\delta^{13}\text{C}$ results. Additives and other contaminants can alter FTIR and Raman spectra, but all spectral library matches were accurate in this study. However, little or no sample preparation was necessary, while environmental samples typically require pretreatment. For complex samples, multi-element analysis may extend the specificity of IRMS in tracing plastic sources. Disadvantages relative to spectroscopic methods are that IRMS is not a confirmatory technique and it is destructive.

The $\delta^{13}\text{C}$ results for petroleum- and plant-derived polymers reflected their sources, being higher for plant-based materials. Differences between plastics from different countries also were found. The $\delta^{13}\text{C}$ results for HDPE and LDPE bags from the US were significantly lower than those for bags made in China and Thailand, possibly due to differences in materials and manufacturing processes. High variability in the results for PP straws from different countries was observed but no trend in the country of origin was apparent. Significant differences between two recycled PETE products, and between nonrecycled PETE products, also were found. The $\delta^{13}\text{C}$ value for one recycled product was comparable to that for unrecycled products, while that for another recycled product was much higher. The much higher $\delta^{13}\text{C}$ result for a recycled material may relate to use of bioplastic(s) in the recycling process. This result differs from those of a previous study (Berto et al. 2017), which found a lower mean for recycled PETE relative to unrecycled PETE samples.

In addition to tracking environmental plastics, IRMS could be a useful indicator of polymer degradation due to biotic/abiotic processes. It is especially suited to laboratory studies under controlled conditions. Studies of interest include testing polymers classified as ‘biodegradable’ and assessing the durability of new, weather-resistant materials. Our preliminary results indicated a trend of higher $\delta^{13}\text{C}$ values for PS and PP items exposed to UV light, consistent with the UV sensitivity of these polymers. A PP food container manufactured in 2013 also had a much higher $\delta^{13}\text{C}$ value than those for products made in 2019, but this difference could not be attributed to age as product origins also differed. High measurement precision allowed several nonrecycled PETE products to be distinguished. Several PP products from the same country (US) also could be distinguished. Further research is needed, on samples globally, to evaluate the utility of IRMS for investigating the origins, transport, and transformations of plastics in the environment.

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Supplemental material

Additional studies: freshwater, sediments, and wastewater treatment plants.
(published 2016 or earlier)

Table S3-1. Additional Studies of Microplastics in Freshwater Systems: Freshwaters.

Study	Sample type(s)	Collection and particle size	Sample preparation	Analysis	MP abundances and types		Comments
					reported units	converted ^a units and/or MP types	
Estahbanati and Fahrenfeld 2016 United States Raritan River, NJ	River water, up and downstream of 4 WWTPs	Plankton net (0.2 m diameter, 0.51 m long) with 153 µm mesh size. Vol. = 1.3-13.5 m ³	30% H ₂ O ₂ + Fe(II). NaCl density separation. Sieved into 3 quantitative size ranges (500-2000, 250-500, and 125-250 µm) and 1 semi-quantitative (63-125 µm).	Visual (microscope) Classified primary or secondary based on shape and texture.	24 ± 11.4 MPs/m ³ upstream, 71.7 ± 60.2 downstream	0.024 MPs/L upstream, 0.072 downstream	Moderate correlation between MP level and distance downstream from WWTP
McCormick et al. 2016 United States 9 streams in Chicago, IL metro area	Upstream and downstream of tertiary WWTPs. Also monitored bacteria, O ₂ , and nutrients. Summer, 2014	Neuston net (333-µm). Adapted marine protocol (see McCormick et al. 2014).	See McCormick et al 2014.	Visual (microscope). 5 categories (Eriksen et al. 2014). Subset of particles (2 streams) analyzed by Pyr-GC-MS.	Mean (par/m ³): Upstream = 2.355 (±0.375) Downstream = 5.733 (±0.850) Higher downstream in 7 of 9 streams.	Mean (par/L): Upstream = 0.0024 (±0.0004) Downstream = 0.0057 (±0.0008)	Assuming similar levels over seasons, a minimum of 5.6 million and maximum of 1.7 billion MPs discharged annually. Pellets, fibers, fragments dominant. Main polymers were PP, PE, and PS.
Baldwin et al. 2016 United States Great Lakes	Water Great Lakes and tributaries	333-µm neuston net 0.2-0.35 m depth	125-µm sieve substrate 30% H ₂ O ₂ + Fe	Visual	range: 0.05–32 par/m ³ mean = 4.2 par/m ³	0.00005–0.032 par/L	
Fischer et al. 2016 Italy Lakes Chiusi and Bolsena	Water and sediments (Table S3-2)	6 Manta trawls (0.3 mm, 60 x 18.5 cm) total.	Density separation (Thompson et al. 2004) in NaCl solution (d = 1.2 g/mL) in sedimentation column. Removed supernatant and treated with HCl (48 h) at room temperature, then 1 h at 70 °C. Filtered and applied Nile Red.	Covered filters with counting grid. Counted under UV-microscope Small subset (20 fibers) by SEM	Range (par/m ³): Chiusi: 2.68-3.36 Bolsena: 0.82-4.42	Range (par/L) Chiusi: 0.0027-0.0034 Bolsena: 0.0008-0.0044	
Su et al 2016 China Lake Taihu	Water, benthic sediment (Table S3-2), Asian Clams 3rd largest lake in China (2,250 km ²)	Surface water with 333-µm plankton net, 0.3 m deep. Preserved in 5% methylaldehyde. Bulk water (5 L)	30% H ₂ O ₂ WPO. Filtration with 100-µm (net samples) and 5-µm PC filter (bulk water)	Visual (microscope) µ-FTIR or SEM-EDS (113/1805 particles) 81/113 confirmed by µ-FTIR	Bulk water (par/L): 3.4–25.8 par/L Net sample max: 6.8 x 10 ⁶ par/km ²	Fibers dominant (48-84%) Main MPs: cellophane, followed by PET, PES, terephthalic acid, PP	Shallow lake with history of pollution. Most MP-polluted freshwater lake worldwide Blue items prevalent in water samples (50-63% of MPs)
Dris et al. 2015a France Seine and Marne Rivers, Paris	River water (5 sites) upstream and downstream of Paris	0.1-0.35 m depth 80-µm plankton net and 0-0.3 m depth 330-µm manta trawl	Filtered onto glass-fiber filters (1/6 µm). Only particles > 100 considered.	Visual (microscope)	3–108 par/m ³ (plankton net) 0.28–0.47 par/m ³ (manta trawl)	0.03–0.108 par/L 0.0028–0.00047 par/L	Plankton net collected mostly fibers. 48% > 1000 µm. Atmospheric fallout may be source of fibers in surface water.

Study	Sample type(s)	Collection and particle size	Sample preparation	Analysis	MP abundances and types		Comments
					reported units	converted ^a units and/or MP types	
Faure et al. 2015 Switzerland Lakes and rivers	Water: 6 largest Swiss lakes, and rivers. Sediments.	Manta trawl (300 µm). 320-430 m ³ surface water	Stored at 4 °C in salt-saturated water.	Visual (microscope)	Mean (rivers): 7 par/m ³ Range: 0.10–64	Mean (rives): 0.007 par/L Mostly fragments and foam.	Pellets small fraction by number but 32-52% by mass. Also examined adsorbed organics, dissected fish, and water birds.
Mani et al. 2015 Germany Rhine River	Water	18-cm sampling depth. 300-µm manta net. Mean filtered = 150 m ³	Stored at 5 °C in 10% NaCl. Sieves (separation substrate) Enzyme + H ₂ O ₂ . Density separation in 23% NaCl/H ₂ O (density 1.16 g/mL)	Visual (microscope) FTIR (118 suspect MPs)	Mean = 892,777 par/km ² (max: 3.9 x 10 ⁶)	Mean ^a = 0.005 par/L Spherules (45.2%), fragments (37.5%), clear spherules (13.2%), fibers (2.5%) and others (1.1%).	MP levels diverse along and across river. PS (29.7%) dominant, then PP (16.9%), other (13.6%), acrylate (9.3%), PES (5.1%) and PVC (1.7%).
Zhang et al. 2015 China, Three Gorges Dam	Water	Trawl, 112-µm net	1.6 mm stainless steel sieve. Liquid allowed to settle 1 week. Large particles removed with tweezer.	Visual (microscope) ATR-FTIR on 50-100 suspect MPs	Mean = 8.47 x 10 ⁶ par/km ² (max: 1.36 x 10 ⁷)	-	PE, PP, and PS “hotspot” for MP pollution
McCormick et al. 2014 United States N. Shore Channel, Chicago, IL	Water upstream and downstream of WWTP. Also examined microbial assemblages. Sampled on 9/13/2013	2 neuston nets (333-µm), behind stationary boat. 20 min samples (n = 4). Rinsed material into 1-L containers. Shipped on ice. Stored at 4 °C.	Sieved and stored 0.330–4.75-mm fraction in oven (75 °C) until digestion by WPO (0.05 mol/L Fe(II) + 30% H ₂ O ₂ at ≤ 75 °C). Density separation in NaCl (6 M). Filtered on glass-fiber filter (0.7-µm pore size).	Visual (microscope). Counted 5 random subsamples of filter, each 3% of area. 4 procedural blanks. SEM on some particles.	Means (±SD) par/m ³ : Upstream = 1.94 ±0.81 Downstream = 17.93 ±11.05	Means (±SD) par/L: Upstream = 0.002 ±0.001 Downstream = 0.018 ±0.011 Samples corrected for fiber background.	AS WWTP. Treats domestic wastewater. Ave. flow = 927 million L/day. No effluent disinfection. Foam and pellets found only downstream at low levels relative to fragments and fibers.
Yonkos et al. 2014 United States 4 estuarine rivers, Chesapeake Bay	Water Papatsco, Magothy, Rhode, and Corsica rivers	Manta net (330 µm) trawl, 15-cm sampling depth	Rinsed samples into 0.3 mm sieves and transferred to pre-weighed glass beakers to dry. Weighed and digested (75 °C) by Fenton's reagent. Density separation (300 g/L table salt in DI water).	Visual (microscope) µ-Raman on 10 small (≤ 2 mm) (likely to be misidentified) fragments	Mean, par/km ² (g/kg sample): Papatsco = 155,374 (102) Magothy = 112,590 (74) Rhode = 67,469 (18) Corsica = 40,852 (9)	Mean ^a , par/L: Papatsco = 0.001 Magothy = 0.00075 Rhode = 0.00045 Corsica = 0.00027	Watershed properties ^b : area (km ²), % developed ^c , and population: Patapsco: 1,637, 54%, 899,000; Magothy: 92, 59 %, 32,350; Rhode: 67, 12%, 4,300; Corsica: 97, 13.5%, 3,500
Free et al. (2014) Mongolia Lake Hovsgol	Water	16-cm sampling depth manta trawl, 333-µm	Tyler sieves (0.355-0.999 mm, 1.00-4.749 mm, >4.75 mm) 30% H ₂ O ₂ , density separation (density = 1.62 g/mL)	Visual (microscope)	20,264 particles km ⁻² (max: 44,400)	Mean ^a = 0.00012 par/L Most common: 40% fragment, 38% film, and 20% line/fiber	Range of sizes: 0.333 to 5 mm. MP density decreased with distance from southwest shore
Sadri and Thompson 2014 Southwest England, Tamar Estuary	Water	Manta trawl (300 µm)	Sieved (3 mm, 1 mm, and 270 µm)	Classified as: as > 5, 2-5, and <1 mm FTIR on 50% of counted pieces	Overall mean: 0.028 par/m ³ 84% of 204 suspect MPs confirmed	Overall mean: 0.000028 par/L 40% PE, 25% PE, 19% PP, 8.2% PVC and nylon	MPs black and yellow PP only in 1-3 and 3-5 mm size fractions Nylon in < 1 and 1-3 mm fractions Most MPs 1-3 mm
Zhao et al. 2014 China Yangtze Estuary and East China Sea	Water	Collected estuary water (0.2 m deep) with pump and sieved (32-µm). Sea water with neuston net (333 µm). Fixed in formalin.	30% H ₂ O ₂ digestion of samples with high organics, followed by density separation in saturated ZnCl ₂ . Floating particles filtered onto 1.2-µm cellulose nitrate filter	Visual (microscope) Counted particles > 500 µm	Means (par/m ³) - Estuary: 4137.3 ±2461.5 (max: 10,200) - Sea: 0.167 ±0.138	Means (par/L) - Estuary: 4.1 (max: 10.2) - Sea: 0.0002	Most MPs clean and colors. Small fraction black and white. Max size was 12.46 mm. >90% 0.5 to 5 mm

Study	Sample type(s)	Collection and particle size	Sample preparation	Analysis	MP abundances and types		Comments
					reported units	converted ^a units and/or MP types	
Lechner et al. 2014 Austria Danube River	Water	Stationary conical driftnets (500- μ m), 0.5 m depth	Samples suspended in water and particles removed.	Visual sorting by naked eye.	Mean (par/1000 m ³) 316.8 $\pm$ 4664.6 (max: 141648)	Mean: 0.00032 par/L	Pellets, flakes, spherules, others
Lima et al. 2014 Brazil Goiana Estuary	Water	Conical plankton net, 300- μ m	Floating particles sieved (45- μ m)	Visual (microscope)	Mean: 3.1×10^{-4} – 2.6×10^{-3} par/m ³ (max: 0.19 par/m ³)	Mean: 3.1×10^{-7} – 2.6×10^{-6}	Note: All results by Li et al. 2018 are mean and max values
Eriksen et al. 2013 United States Great Lakes	Water 21 stations over 1300 km expedition	16-cm sampling depth mesh manta trawl, (333- μ m)	2 M HCl, Tyler sieves for 3 size classes: 0.355–0.999 mm, 1–4.749 mm, > 4.75 MM)	Visual (microscope). SEM-EDS (counts adjusted based on SEM-EDS)	Mean: 43,157 par/km ² (max: 466,000)	Mean ^a = 0.00027 par/L (0.000043 par/L) ^d 20% of visually identified MPs < 1 mm were Al silicate	81% of particles in 0.355-0.999 mm range Colored spheres < 1 mm suspected microbeads
Faure et al. 2012 Lake Geneva, Mediterranean Sea (MS)	Lake water	Manta trawl with plankton sieve (300- μ m)	Sieved to 5 mm	Visual (microscope) Sorted, counted and weighed	par/km ² : 4.81×10^4 (Lake Geneva) 1.4 – 31.5×10^4 (MS)	par/L ^e 0.0002 (Lake Geneva) 5.7 – 126×10^{-5} (MS)	
Moore et al. 2011 United States 2 California rivers, 1 creek	Water	Hand nets (800 and 500 μ m), manta trawl (333 μ m)	Tyler sieves (4.75, 2.8, 1.0 mm). Dried samples (65 °C)	Visual 2 size ranges: 1– < 4.75 and $\geq$ 4.75	Maximum (par/m ³) 1.29×10^4 (Los Angeles River, 1–4.75 mm).	12.9 par/L (max): 71% foams, 14% fragments, 10% pellets, 1% whole items.	Sorted, counted, and weighed by each type. By mass: 37% whole items, 29% fragments, 13% pellets, 11% PS foam.

^aValues converted from particles km⁻² to particles L⁻¹ are from Horton et al. 2017 except where indicated. Sample volumes were based on sampling area and depth. ^b2011 values. ^cUrban/industrial and suburban/residential. ^dValue reported by Rezanian et al. 2018. ^eEstimated by authors. Abbreviations: DI = deionized, par = particles, Pyr-GC-MS = Pyrolysis gas chromatography with mass spectrometry, WPO = wet peroxide oxidation, WWTP = wastewater treatment plant.

Table S3-2. Additional Studies of Microplastics in Freshwater Systems: Sediments.

Study	Sample type	Collection	Sample preparation	Analysis	MP abundances reported/converted ^a	Types/sizes/comments
Ballent et al. 2016 Canada Lake Ontario and tributaries	Benthic and shore sediments	33 nearshore and beach using sediment trap, core and grab sampling.	Taylor sieve (5.6, 2.0, and 0.063 mm), except gravity core samples. Half of 0.063 fraction used for density separation. Gravity core samples treated by sodium polytungstate (SPT) density separation	5.6 and 2.0 fractions examined visually (microscope). Limit of about 0.25 mm. Subset by Raman and X-ray fluorescence.	Means (par/kg): - 980 lake benthic - 140 lake beach - 610 tributary benthic Mainly fibers and fragments < 2 mm.	Highest (>1000 par/kg) in Greater Toronto Area. MPs in beach samples decreased with distance from Toronto, and with sediment depth. Near-shore means varied with sampler (core, trap, and grab = 2130, 1070, and 730 par/kg, respectively). Both high and low density MPs found.
Imhof et al. 2016 Italy Lake Garda	Shore sediment from 5 beaches (3 replicates)	See Imhof et al. 2018 (Table 3-2).	See Imhof et al. 2018 (Table 3-2).	See Imhof et al. 2018 (Table 3-2). Metals by ICP-MS.	75 particles/m ² Converted values ^a : 1.2 par/kg	Identified plastic and paint particles.
Käppler et al. 2016 Germany (Warnemünde) Gotland Basin	Beach sediment at 6 locations, in triplicate. Marine sediment.	Beach: Top 1 cm of 10 x 100 cm area. Basin: Sediment traps	MPSS unit (Imhof et al. 2012). Density separation in SPT (d = 1.8 g/mL). Sieved (500-µm). Transferred suspect MPs with tweezers.	µ-ATR-FTIR (germanium crystal and MCT detector). µ-Raman.	Concentrations not reported.	User-generated and commercial spectral libraries used. PS, PE, PAN > 500 µm PE, PP, PVC, PC, PS, PTFE, PET, <400 µm
Fischer et al. 2016 Italy Lakes Chiusi and Bolsena	Shore sediment, 36 samples	Collected top 3 cm of sediment from 0.025 m ² area. Sieved (5 mm). < 5 mm fraction weighed.	Wet sieved: 1-5, 0.5-1, 0.3-0.5, and <0.3 mm. NaCl density separation, HCl and Nile Red treatments (see Table S3-1).	See Table S3-1	Means (par/kg dry wt.) Chiusi: 234 ±85 Bolsena: 112 ±32	Fibers and fragments dominant. Up to 40% of suspect synthetic fibers were cotton. Nile red stains PE, PP, PETE (best for white or opaque polymers). Not PVC.
Su et al. 2016 China Lake Taihu 3rd largest in China (2,250 km ²)	Benthic sediment, water (Table S3-1), Asian Clams.	Peterson sampler (3 pooled, 2-kg samples per site).	NaCl density separation of 1 kg wet sediment. Settled overnight and filtered supernatant (5µm). Processed and analyzed by same method used for water (Table S3-1).	Visual (microscope) Subset of 113 particles by µ-FTIR or SEM-EDS	11.0–234.6 par/kg sediment (dry wt)	Fibers dominant (48-84%). White and clear particles more common (29%-44%). Main MPs: cellophane, followed by PET, PES, terephthalic acid, and PP. Most polluted lake in China. Most MP-polluted freshwater lake worldwide.
Zhang et al. 2016 China Tibet plateau lake	Sediments High-altitude inland lakes	Collected 20 cm x 20 cm (top 2 cm) with shovel.	Sieved (1 mm mesh). Material passed by sieve used for density separation in potassium formate (d = 1.5 g/mL). Filtered on glass-fiber filters (1.2 µm). Dried in petri dish (60 °C).	Visual (microscope) Raman, SEM	8 ±14 to 563 ±1219 par/m ² Mostly 1-5 mm PE, PP, PS, PET, PVC	MPs can be high in inland waters without adequate waste management. SEM used to examine surface texture for oxidative and mechanical weathering. identified.
Corcoran et al. 2015 Canada Lake Ontario	Benthic sediment, beach.	Beach samples (surface and 5-cm depth) and bottom sediment core samples.	Dried, sieved. Added to water. Floating particles removed and remainder used for SPT solution (d = 1.5 g/mL).	Visual (microscope) µ-ATR-FTIR (Ge crystal). Analysis area 80-100 µm diameter, and 1-2 µm depth.	Station 403: 26 par/42.2 g Station 208: 9 par/103.2 g Or 616.1 and 87 par/kg	Bottom sediments: PE (74%) dominant. PP (17%) and nitrocellulose (9%). Some MPs were mineral-particle mixtures (from additives or adsorption). Humber River transports pellets into Lake Ontario.
Faure et al. 2015 Switzerland multiple lakes	Shore sediment and water (Table S3-1)	Shores: 5-cm sampling depth, 0.3 m ² quadrats.	Stored in 5-L PP buckets at 4 °C in salt-saturated water.	Visual (microscope)	Mean, all beaches: 1,300 ± 2,000 par/m ² Converted ^a = 20 par/kg	MPs found in all 33 beach samples PE (62%, mainly films), PP (15%, mainly fragments), PS (12%, mainly foams).

Study	Sample type	Collection	Sample preparation	Analysis	MP abundances reported/converted ^a	Types/sizes/comments
Klein et al. 2015 Germany Rhine, Main Rivers	Shore sediment Main River has industrial influence		Strungaru et al 2019	ATR-FTIR	228–3763 par/kg Up to 4,000 par/kg	630 µm–5 mm/kg PE, PP, and PS accounted for >75% of all MPs. PET, PVC, EA, EPDM, PA and acrylic-based polymers also present
Talvitie et al. 2015 Finland Helsinki Tertiary WWTPs (see Table S3-3)	Sediments near WWTPs and reference sediment at sea	Corer sampler. Top 30 cm placed in containers.	NaCl density separation (Browne et al. 2010). Supernatant filtered using custom device with 200, 100, and 20-µm filters. (see Table S3-3).	See Table S3-3.	Viikinmäki discharge site (par/kg sediment): 1.7 (±1.0) fibers, 7.2 (±4.9) synthetic Suomenoja discharge site: 4.7 (±3.5) fibers, 10 (±14) synthetic	Reference site (at sea): 1.7 (±1.3) fibers, 0 synthetic, and 1,220 (±160), 1,060 (±471), and 346 (±186) black carbon particles at Viikinmäki, Suomenoja, and reference sites, respectively. 70 (±20), 3.8 (±2.3), and 1.9 (±1.5) ring-shaped particles (respectively)
Castañeda et al. 2014 Canada St Lawrence River	Benthic sediment 10 sites	Sampling depth 10–15 cm. Petit Ponar grab (225 cm ² area) and Peterson grab (930 cm ²) samples	Sieved (500 µm mesh) and preserved in ethanol.	Visual (microscope) Microbeads identified based on color and shape. Type determined by DSC.	Median microbeads across all sites: 13,759 par/m ² Converted values ^a : 70.6–105.8 par/kg	Converted value reported as a range that depends on assumed sampling depths.
Imhof et al. 2013 Italy Lake Garda	Shore sediment collected by random grid sample technique.	Three, random-grid samples from a 20 cm grid (0.04 ²) at depth of 5 cm (volume = 2 L).	Density separation in ZnCl ₂ solution (d = 1.6–1.7 g/mL)	µ-Raman SEM Macro and MPs	par/m ² North shore: 1108 ± 983 South shore: 108 ± 55 Converted ^a : 17 par/kg and 1.7 par/kg	Mainly low-density MPs: PS (45.6%), PE (43.1%), and PP (9.8%). Small fraction (9-500 µm) also had PA and PVC. North shore had 483 ± 236 macro plastics/m ² and South had 8.3. Higher levels in north may be due to south-to-north winds. High levels on north shore may be due to south to north winds.
Vianello et al. 2013 Italy Venice lagoon	Sediments Duplicates at 10 sites	Up to 5 cm taken with box-corer and refrigerated.	Density separation in NaCl. Sieved (32 µm) supernatant. Repeated extraction 3 times. Resuspended pooled materials and filtered (0.7 µm glass-fiber filter).	ATR µ-FTIR (MCT detector). 12 unit areas (4.5 mm ² each) for chemical mapping.	2175-672 MP/kg	Of 10 polymer types, PE and PP > 82% of total. 93% of MPs 30–500 µm. Fragments at most stations: 87%. Fibers (10%) not detected at 2 sites. Occasional films (2%) and pellets/granules (1%).

^aValues initially reported as particles m⁻² were converted to particles kg⁻¹ by Horton et al. 2017. DSC = differential scanning calorimetry, ICP-MS = inductively coupled plasma with mass spectrometry. MCT = mercury-cadmium-telluride, µ-ATR-FTIR = Fourier transform infrared spectroscopy (FTIR) coupled with an attenuated total reflectance microscope objective (µ-ATR), SEM-EDS = scanning electron microscopy with energy dispersive X-ray spectroscopy, WWTP = wastewater treatment plant.

Table S3-3. Additional Studies of Microplastics in Wastewater Treatment Plants.

Study	Sample and plant/study details	Sample collection	Sample ^a preparation	Analysis ^b	Main findings ^c	Comments
Bayo et al. 2016 Spain WWTP with CAS process and 2 parallel AS bioreactors. PEq = 210,000	Sludge from urban WWTP: - Sludge C (from centrifuge after anaerobic digestion) - Sludge P (rejection of primary clarifier) Collected February-April 2015.	Collected in glass containers and stored at 4 °C	NaCl (120 g/L) density separation on duplicates (50 g wet wt.). Shook salt mix (500 mL) 60 min. (200 rpm). Sieved supernatants with 200 mesh for sludge C, and 20 and 200 for sludge P (particle sizes at 0.850 mm and 0.075 mm, respectively). Repeated procedure, dried extracted fractions, stored in jars.	Microscope showed MP fibers with associated organic debris. To remove it, ground samples and added 10 mL isopropyl alcohol (48 h at 60 °C). Centrifuged, dried - analyzed by microscope, FTIR, and DSC.	Levels not reported, but MPs found in both sludges, from primary clarifier, and after anaerobic digestion and centrifugation, the last step before disposal and use on soils Compounds identified by FTIR included: PP, Nylon [®] , transparent thermoplastic polyamides, norbornene, and ethyl acrylate.	Supplies 35,000 m ³ /day. MPs could be transported with organic matter in fertilizers. FTIR allowed measurement of oxidation, determined as peak area at 1650–1850 cm ⁻¹ divided by carbonyl peak area (1,720 cm ⁻¹). Ratio corresponds to % of polyolefin oxidation (e.g., photo, thermal) in polymer lifecycle.
Carr et al. 2016 Southern CA. USA Sanitation Districts of Los Angeles: operates 10 tertiary wastewater reclamation plants (WRPs) treating 681 million L/day, and 2 secondary WWTPs. Larger one treats 1.06 billion L/day. Smaller one treats 0.3 million liter/day	8 municipal plants: - 7 tertiary WRPs - Larger secondary WWTP (handles wastewater and solids). MP transport examined at 1 tertiary plant (WRP 1). Sampled primary, secondary, and tertiary stages. Also sampled different stages at secondary plant. Survey between 06/2014 and 01/2015.	Grab and stacked sieve unit (8" diameter, 400–20 µm) samples. Typically used 400, 180, and 45 µm sieves (20 µm clogged) at flow rates of 11.4–22.7 L/min. About 2 x 10 ⁵ L filtered at tertiary plants. Surface filtering unit (with 125 µm sieve) also used for final effluent. About 9.5 x 10 ⁶ L skimmed. Wrapped sampler in foil to shield from light and air.	Residues in sieves or surface filter removed with spray of DI water, centrifuged, and volume noted. Combined high-solids residues from secondary plant effluent. Added DI water, stirred (500 mL), and pipetted 5-mL into gridded petri dish for screening and counting. Over 20% of sample volume examined. Same procedure used for secondary effluent of WRP1. Grab samples (skimings, scum, sludge, backwash, biosolids) digested and disinfected with bleach. Examined by microscope in fume hood.	Residues and other processed samples examined under optical microscope, along with tactile and physical properties. Some isolated MPs confirmed by FTIR.	Tertiary influent: Could not count (complex) Tertiary effluent (3 plants): - Below-surface: None detected. - Surface-filtered: 1.1 x 10 ⁻⁶ MP/L (31 non-fiber MPs/28.4 million L) 99.9% removal efficiency Tertiary plant distributions (WRP 1): - Surface sludge (primary tank, 2 shifts): 5 MPs/g - RAS (ave.): 50 non-fiber MP/L (1/20 mL) - Gravity-filtered backwash: None/45.4 L. - Secondary effluent: 1.76 x 10 ⁻⁴ MP/L (1 non-fiber MP/600 mL residue from 5.68 x 10 ³ L). - Final effluent: None/1.93 x 10 ⁵ L Secondary plant distributions: - Influent: 1 MP/L - Effluent (ave.): 8.8 x 10 ⁻⁴ MPs/L (373 MPs/4.23 x 10 ⁵ L). - Grit (ave., n = 2-3): 1 MP/2.1g - 1° and 2° skimming: 20 MPs and 0 MPs/5g - Centrate thickening: 51 MPs/100 mL influent and 267 MPs/100 mL thickened centrate - Biosolids: 5 MPs/5 g	Secondary plant discharge = 9.30 x 10 ⁵ MP/day > 90% were blue PE fragments identical to those in toothpaste. Tertiary effluents not a significant MP source: 78–98% removed at primary stages by solids skimming and in sludge. Biofilms can affect density separations due to density or other changes. Blue MPs in autoclaved sample floated but distributed in solution in non-sterile sample. Counts for grab samples of surface scum in primary tanks are estimates due to complex matrix. Microwave digestion (110–120 °C) in strong acid altered MPs. Hypochlorite was milder.
Mason et al. 2016 United States Broad study of 17 municipal WWTPs across the U.S. PEq = 3.5 x 10 ³ –5.6 x 10 ⁷	90 effluent samples from 17 facilities Sampled just before discharge point. Sampled September 2013 through May 2015	Pumped (12–18 L/min) through stacked sieves: 355 µm on 125 µm used for shorter (2 h) periods. Only 355 µm sieve for longer periods, up to 24 h (125 µm clogged).	WPO (Fenton's reagent). Filtered with 125-µm sieve. Sieved particles transferred to glass petri dish for analysis.	Visual under dissection microscope. MPs removed, counted, and categorized.	Average effluent concentration (MPs/L): 0.05 ± 0.024 MP/L (range: 0.004 – 0.195) Fibers (59%) and fragments (33%) dominant. Some natural fibers. Films (5%), foams (2%), and pellets (1%) minor. No MPs in blanks. Smaller (125–355 µm) particles (57%) had similar amounts of fibers and fragments. Larger particles (43%) mostly fibers (80%).	Discharges (MPs/day): 0.05–15 million. Mean (17 facilities): > 4 million/facility. Estimated daily releases: 3–23 billion microbeads into U.S. waterways. Average efflux: 8.58 x 10 ⁵ –1.40 x 10 ⁸ m ³ /y. Tertiary filtration not effective.

Study	Sample and plant/study details	Sample collection	Sample ^a preparation	Analysis ^b	Main findings ^c	Comments
Michielssen et al. 2016 United States (MI) 2 WWTPs: Secondary (Detroit) plant treated wastewater and stormwater. PEq = 2.4×10^6 Tertiary (Northfield) plant treated wastewater only. PEq = 9.9×10^3 Also had a pilot-scale AnMBR.	Raw wastewater; and preliminary, primary, secondary, and final effluents. Evaluated 3 processes in removal of 'small anthropogenic litter' (SAL 20 μm –4.75 mm): - Primary, secondary (AS) - Primary, secondary, tertiary (granular sand filtration) - Primary, pilot-scale AnMBR with microfiltration	Grab samples in plastic containers. Volume depended on wastewater type, from 1L (raw influent) to 38 L (final effluent). Blank (20 L DI water) collected and processed with field samples. Samples shipped and stored at 4 °C.	Effluents filtered with 5 stacked sieves: 4.75 mm–20 μm . Sieves processed separately. Different SAL size fractions processed in small subsets, poured into petri dishes for analysis.	Visual of different size fractions under stereomicroscope. SAL removed and counted based on shape.	Concentrations (SAL/L) for secondary, tertiary, and AnMBR, respectively: Influent = 133, 93, and 83 Effluents = 5.9, 2.6, and 0.5 Removal efficiencies: 95.6%, 97.2%, and 99.4%, respectively. Thin fibers most common MPs in effluents: 44% in secondary plant, 79% in tertiary, and 83% for AnMBR. Fragments second most common, then microbeads. Only 1 fiber in blank (no blank correction). Tertiary plant unique in having 2 secondary treatments: trickling filter followed by AS. Removal by filter not significant. Tertiary treatment improved removal by 7.4%. Final membrane filtration had best overall efficiency. Served as proof-of-concept design.	Secondary plant treated 660 million gallons/day (MGD) or 2.5 billion L/day ($9.1 \times 10^8 \text{ m}^3/\text{year}$). Discharge: 15 billion SAL/day. Tertiary plant treated 0.45 MGD (1.7 million L/day) or 1.7 million L/day ($6.2 \times 10^5 \text{ m}^3/\text{y}$). Discharge: 4.4 million SAL/day Secondary plant was representative of U.S. WWTPs (tertiary plants a small fraction). Retrofitting with granular sand or membrane filtration would increase efficiency. Environmental fate of retained SAL (e.g., in sludge) must be resolved.
Murphy et al. 2016 Scotland (Glasgow) Secondary WWTP with discharge to Clyde river. PEq = 6.5×10^5	Sampled at 4 stages: influent, grit and grease effluent, primary effluent, and final effluent.	10-L steel buckets attached to wire	Vacuum filtered wastewater (30-50 L) through 65- μm sieve. Mixed solid samples (grit, grease, and sludge) 1 min and took triplicate, 2.5-g subsamples. Dried (<50 °C) 2 h prior to analysis.	Visually (microscope). May have missed MPs with colors similar to background. μ -FTIR confirmation.	Average concentrations (MP/L) Influent: 15.70 (± 5.23), Effluent: 0.25 (± 0.04) 98.41% removal efficiency. MPs accumulated in grease ($19.67 \pm 4.51 \text{ MP}/2.5 \text{ g}$). Microbeads found only in grease. FTIR identified many non-plastics (e.g., plant debris) initially classified as MPs.	Calculated discharge of 65 million MPs/day. Final effluent = $9.52 \times 10^7 \text{ m}^3/\text{y}$ Efficient removal but even low MP concentrations could result in significant release due to large effluent volumes.
Talvitie et al. 2015 Finland (Helsinki) Tertiary WWTP in metro and coastal areas. PEq = 800,000 Discharge to sea (depth > 20 m), 8 km from south tip of Helsinki.	Influent ($n=1$) and ($n=3$) after sedimentation (1° and 2°), final effluent, surface seawater (as reference), and sediments (Table S3-2). Sampled between Oct. and Dec. 2012	Wastewater collected with pump and custom filter device with 3 internal filters (200, 100, 20 μm). Collected into containers in refrigerators. Volume depended on location and mesh (0.3 L influent and 1–285 L elsewhere).	Filtered wastewater received no additional treatment.	Visual under stereomicroscope (mag. $\times 50$). Selected particles analyzed by FTIRi (Talvitie et al. 2017a)	Average concentrations (MP/L): - Influent: 610 (180 textile fibers and 430 fragments) - Final effluent: 13.5 (4.9 ± 1.4 fibers and 8.6 ± 2.5) - After sedimentation (MP/L), fibers and non-fibers, respectively: Primary: 14.2 (± 0.7) and 290.7 (± 28.2) Secondary: 13.8 (± 1.6) and 68.6 (± 6.3) - Reference seawater (MP/L): 0.01–0.65 textile fibers and 0.5–9.4 non-fiber 97.8% efficiency	Effluent: 270,000 m^3/d ($9.86 \times 10^7 \text{ m}^3/\text{year}$) Discharge = $3.65 \times 10^9 \text{ MP}/\text{day}$ Fibers in effluent 25x higher than in reference water. Other MPs 3x higher. Fibers captured in sludge or otherwise trapped.
Dris et al. 2015a France Paris Seine-Centre WWTP. Secondary, with biofilters. PEq = 8.0×10^5	Raw, settled, and treated wastewater. River water (Table S3-1). Air Sampled 2014, on 3 consecutive days.	Collected wastewater with automatic sampler (24-h composite).	Filtered 0.05 L wastewater with glass fiber filters. Glass vessels and filters heated at 500 °C (4 h) before use. Covered with foil. Blanks processed also.	Microscope (visual) Wore cotton lab coats.	Except for 1 spherical particle, MPs were fibers. High levels of fibers. - Influent mean = 293 fibers/L (range 260–320) - After primary treatment: 50–120 fibers/L - Final effluent mean = 35 fibers/L (range 14–50) Fibers lengths mainly (45%) 1–5 mm Removal: 83%–95%.	Treated 240,000 m^3 daily. Discharge = $8.40 \times 10^9 \text{ MP}/\text{day}$ Influent pretreated by screening, grit and oil removal, then settled in 1° tank. MPs in atmospheric fallout mostly (> 90%) fibers: 29–280 $\text{m}^2 \text{ day}^{-1}$

Study	Sample and plant/study details	Sample collection	Sample ^a preparation	Analysis ^b	Main findings ^c	Comments
Magnusson and Norén 2014 Sweden Secondary WWTP: relatively small, with chemical and biological processes. PEq = 12,000.	Influent, effluent, sewage sludge. ($n = 2-4$) Recipient seawater at distant site used as background. Sampling completed in 1 day.	Collected 2 L influent with Ruttner sampler and filtered (300- μ m filter in holder). Collected 1000 L effluent with filter holder and pump, 1.1 g sludge (dry wt.), and 6,600 L recipient water.	Stored filter samples in Petri dishes and sludge in PE containers (dark at room temp.) No further processing. Collected slightly dewatered sludge. Dried (70 °C, 4.5 % dry wt.). 25 g wet sludge sieved with 300- μ m filter cut from plankton net. Analysis same as for water.	Visual (optical microscope). Sub-sample by ATR-FTIR Note: Could not obtain spectra for any fibers.	Mean concentrations (MPs/L) Influent: 15.1 ± 0.89 Effluent: 0.00825 ± 0.00085 Ref. seawater: 0.00045 MPs/L > 99 % of MPs retained in sludge. Fibers retained more than other shapes. Sludge (MP/kg): 0.720 ± 0.112 (wet wt.), 0.0167 ± 0.00196 (dry wt.) Only fibers in recipient water.	Estimated discharge: 1,770 MPs/h. Discharge = 4.25×10^4 MP/day, MP discharge to environment may be substantial but relative importance difficult to assess due to lack of quantitative studies. Higher levels near mouth of discharge than 200 m downstream.
Martin and Eizhvertina 2014 Canada	Final effluent 2 WWTPs PEq = 450,000	1 L effluent, 12 μ m mesh size	WPO	Visual (optical microscope)	Effluent: 3.32 MP/L	Results from Gundogdu et al 2018
HELCOM 2014 Russia (St. Petersburg) Central WWTP of Vodokanal PEq = 5 million	Influent after mechanical purification and final effluent. HELCOM pilot study (2012-2014) (report authors: Talvitie J. and Heinonen M.) March 3-7, 2014	Manual effluent collection in plastic containers. $n=3$ for each of 3 sampling days. 24-h composite samples.	Collected wastewater in containers. Poured into device with mesh filters (300, 100 and 20 μ m) (Talvitie et al. 2015). Filtered 50 L with 300- and 100- μ m and 1 L with 20- μ m. Also, automatic composite (24-h) samples. Filtered 100 mL influent to 8 L effluent.	Visual under light microscope (filters in petri dishes). See Talvitie et al. 2015)	Influent: 467 fibers, 160 synthetic particles, and 3,160 black particles per L After mechanical purification: 33 fibers, 21 synthetic particles, and 302 black particles Final effluent: 16, 7, 125/L Purification %: 96.57, 95.63, 96.04 %	Treats 350 million m ³ wastewater annually. MP release substantial due to high discharge volumes. Contribution relative to other sources needs to be assessed. FTIR and other confirmation methods improve accuracy. Recommended composite sampling over different seasons.
Dubaish and Liebezeit 2013 Germany 2 STPs	Effluents (and surface waters) Plants: 1) Wilhelmshaven 2) Zetel	Collected 1 L effluent Container, 40 μ m Pump, 10 μ m	Sieved 1 L (40- μ m). Rinsed solids into 50-mL bottles. Dried and digested overnight in 30 % H ₂ O ₂ . Added 40% hydrofluoric acid to remove inorganics (may cause PS, PC loss). Filtered on grey, gridded cellulose nitrate filters (1.2- μ m).	Visual (microscope up to $\times 80$ mag.). Grey filters aided view of clear particles. Counted MPs and black carbon.	1) Effluent = 80.4 MPs/L (32.7 granular, 23.7 fragment, 24 fiber) 2) Effluent (Zetel) = 31 MPs/L (12 granular, 19 fiber)	Wilhelmshaven Efflux (m ³ /year): 1.13×10^4 Discharge (MPs/day): 2.5×10^6 High variability may relate to small sample volume.
Browne et al. 2011 Australia (NSW) 2 tertiary STPs and reference sites	- Effluents of STPs (and 3 domestic washers). - Marine sediments at sewage disposal and reference sites. - Beach samples (8 sites, 6 continents)	Collected effluents in 750-mL jars. Sediment, 5–10 cm (deep) from surface (van Veen grabs), and beach samples 1-cm deep.	Filtered 750-mL effluents with glass-fiber filters. Extracted 50-mL subsample of sediments and beach samples with saturated NaCl solution Target particle size < 1mm (defined MPs as plastic debris as < 1 mm)	MPs on filters identified and counted by transmittance FTIR. Used synthetic fibers ATR library (Bruker I26933)	- Effluent mean ($n = 5$): 1 MP/L. PES (67%), acrylic (17%), and polyamide (16%) fibers dominant. - Beach sediments: 82-31 fibers/250 mL. Fibers dominant: PES (56%), acrylic (23%), PP (7%), PE (6%), and polyamide fibers (3%). - Marine disposal: PES (78%) and acrylic (22%). Two former disposal sites (no disposal in >10 years) had over 250% more MPs than reference.	More MPs in densely populated areas. 1 garment shed >1,900 fibers per wash. Similar proportions of PES and acrylic fibers in sewage-effluent and sediments suggests MP fibers derived from sewage via clothes washing.

A²/O = anaerobic, anoxic, oxic (aerobic), AnMBR = anaerobic membrane bioreactor, AS = activated sludge, BAF = biologically active filter, CAS = conventional activated sludge, DAF = dissolved air flotation, DF = discfilter, DSC = differential scanning calorimeter, FPA = Focal plane array (detector), HCl = hydrochloric acid, H₂O₂ = hydrogen peroxide, MBR = membrane bioreactor, μ -ATR-FTIR = Fourier transform infrared spectroscopy coupled with attenuated total reflectance microscope objective (μ -ATR), NaCl = Sodium chloride, par = particles, PEq = population equivalent, RSF = rapid sand filtration (filters), STP = sewage treatment plant, WPO = wet peroxide oxidation, WWTP = wastewater treatment plant.

List of plastic items and analysis methods

Table S6-3 Selected polymer groups, items, and analytical techniques

Group	Sample	IRMS	FTIR	Raman	Group	Sample	IRMS	FTIR	Raman
(ABS)	(ABS) Acrylonitrile Butadiene Styrene Dajac Labs	✓	✓	✓	(PLA)	(PLA) Polylactic Acid Ballyhoo	✓	✓	✓
(PC)	(PC) PC Bead NIOSH	✓	✓		(PP)	(PP) Black food package		✓	
	(PC) Polycarbonate Ballyhoo		✓	✓		(PP) Black food packaging	✓	✓	
(PE)	(PE) "Polypropylene" Ballyhoo		✓	✓		(PP) Green Lid		✓	
	(PE) Blue lid liner		✓			(PP) McCafe Cup	✓	✓	
	(PE) Brown Lid		✓			(PP) Opaque food packaging	✓	✓	
	(PE) Canes Plastic Bag	✓	✓			(PP) PP Bead AWWERC	✓	✓	
	(PE) Clear Blue Lid Liner		✓			(PP) Sanitizing wipes	✓	✓	
	(PE) CRG Plastic Bag		✓			(PP) Sistema cup	✓	✓	
	(PE) HDPE - opaque bead		✓			(PP) Soup		✓	
	(PE) HDPE - white bottle		✓			(PP) Yogurt		✓	
	(PE) HDPE Bead UC	✓	✓	✓		(PP) Yogurt Container		✓	
	(PE) Kroger Plastic Bag		✓			(PP) Yogurt lid black		✓	
	(PE) LDPE - blue lid		✓		(PS)	(PS) Calibration film		✓	
	(PE) LDPE - white lid		✓			(PS) Clear bead		✓	
	(PE) PE Powder AWWERC	✓	✓			(PS) Clear food packaging		✓	
	(PE) Sour soup container	✓	✓			(PS) EPS		✓	
	(PE) Target Plastic Bag	✓	✓	✓		(PS) Polystyrene Ballyhoo		✓	✓
	(PE) UDF Plastic Bag	✓	✓	✓		(PS) PS Bead AWWERC	✓	✓	
	(PE) Walmart Plastic Bag		✓			(PS) PS Clear		✓	
	(PE) White CM Container	✓	✓		(PU)	(PU) Foam - aged		✓	
	(PE) Ziplock bag		✓			(PU) Foam - new		✓	
(PETE)	(PETE) Blue Container	✓	✓			(PU) Pu pellet		✓	
	(PETE) Blue food packaging		✓		(PVC)	(PVC) PVC Powder AWWERC	✓	✓	
	(rPET) Blueberry Container	✓	✓		(Misc.)	Cellulose Acetate		✓	✓
	(PETE) Cantaloupe Container	✓	✓			Dryer Lint		✓	✓
	(PETE) Clear blue water bottle		✓			Nylon 6		✓	
	(PETE) Clear bottle - inside		✓			Soda can liner		✓	
	(PETE) Clear bottle - outside		✓			Soda can lining		✓	
	(PETE) Clear food packaging		✓						
	(rPET) Clear food packaging		✓						
	(PETE) Clear green bottle		✓						
	(PETE) Coke Bottle		✓						
	(PETE) CW Strawberry Container	✓	✓						
	(PETE) PETE (Ice Mountain Bottle)	✓	✓						
	(PETE) Polyester Scarf	✓	✓	✓					
	(rPET) PurAqua	✓	✓						

FTIR and Raman Spectra

FTIR

ABS

PC

PE

PE

PETE

PLA

PP

PS

PVC

Raman Spectra

ABS

PC

PE

PETE

PLA

PP

PS

Appendix A: Quality assurance plan

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