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Sampling and Quality Assurance and Quality Control: A Guide for Scientists Investigating the Occurrence of Microplastics Across Matrices

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Abstract

Plastic pollution is a defining environmental contaminant and is considered to be one of the greatest environmental threats of the Anthropocene, with its presence documented across aquatic and terrestrial ecosystems. The majority of this plastic debris falls into the micro (1 µm–5 mm) or nano (1–1000 nm) size range and comes from primary and secondary sources. Its small size makes it cumbersome to isolate and analyze reproducibly, and its ubiquitous distribution creates numerous challenges when controlling for background contamination across matrices (e.g., sediment, tissue, water, air). Although research on microplastics represents a relatively nascent subfield, burgeoning interest in questions surrounding the fate and effects of these debris items creates a pressing need for harmonized sampling protocols and quality control approaches. For results across laboratories to be reproducible and comparable, it is imperative that guidelines based on vetted protocols be readily available to research groups, many of which are either new to plastics research or, as with any new subfield, have arrived at current approaches through a process of trial-and-error rather than in consultation with the greater scientific community. The goals of this manuscript are to (i) outline the steps necessary to conduct general as well as matrix-specific quality assurance and quality control based on sample type and associated constraints, (ii) briefly review current findings across matrices, and (iii) provide guidance for the design of sampling regimes. Specific attention is paid to the source of microplastic pollution as well as the pathway by which contamination occurs, with details provided regarding each step in the process from generating appropriate questions to sampling design and collection.

Keywords

Quality control, microplastics, sampling protocols, environmental fate, good laboratory practices, environmental matrix, standardization

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Introduction

Aquatic and terrestrial ecosystems are polluted with plastic waste on a global scale. Amounting to one of the greatest environmental challenges of the twenty-first century, plastic

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pollution has increased by several orders of magnitude since the 1970s, as production and use continues to outpace the capacity for proper disposal, recycling, or reuse.[1,2] A large fraction of this synthetic plastic debris is present across ecosystems as micro or nanoplastics in the form of fragments and fibers. These microscopic plastic items enter either from primary sources from industrial feedstock (e.g., nurdles or microbeads) or are secondary (see Table 1 for a glossary of research-topic-specific terms), resulting from the degradation of larger plastic pieces (e.g., plastic bags, containers, textiles).[3–5]

Microplastics (1 μm–5 mm) are known to be present in air, water, and sediment globally, from impacted to relatively pristine ecosystems, and are confirmed to be both directly or indirectly (e.g., from prey) acquired via ingestion, respiration, and adherence by both aquatic and terrestrial organisms.[6–8] Nanoplastics (1–1000 nm), although currently difficult to measure and thus not covered in the methods described in this paper, are assumed to be equally ubiquitous. In addition to ubiquity in the environment and wildlife, researchers estimate that humans may ingest upwards of 70 000 microplastic particles annually from food, water, and air combined.[9] It is estimated that the surface open-ocean currently contains between 7000 and 260 000 tons of plastics,[10–12] and that the volume of plastic input to the ocean is expected to triple by 2050.[13] On land, microplastics have been detected in soil and in terrestrial food webs.[14,15] When plastic is produced, it is usually packaged as powders or in pre-production pelletized form. During production these are melted down and molded into a product, producing scraps of plastics along the way. One well-known source of microplastics to the environment is primary pellets (i.e., nurdles), scrap, and powders from industry. In fact, some of the earliest records of microplastics in the environment are pre-production pellets on beaches and in the middle of the oceans.[16,17] Their presence in the environment, including in seabirds,[18] led to a voluntary initiative called Operation Clean Sweep in 1990. Because pre-production pellets have a distinct shape, they can be easily quantified and characterized in environmental samples as sourced from industry. Scraps and powders are less discrete but may still have distinguishing characteristics that link them to industrial plastics processes.[19]

Methods for quantifying and characterizing macroplastics (>5 mm) and primary pre-production pellets are relatively simple compared to smaller primary and secondary microplastics, and many citizen science and education efforts have been mobilized to remove these debris items from the environment.[20,21] The size of pre-production pellets generally ranges from 3 to 5 mm, and thus these items are easily sampled with a manta net or even simply via a transect on the beach. They are large enough to be seen with the naked eye and sorted during field sampling.[22,23] Because of their size, procedural contamination is generally not an issue. Moreover, to characterize them chemically, attenuated total reflection Fourier transform infrared (ATR FT-IR) or portable Raman spectroscopy can be used without the need for microscopy. However, these more easily characterized plastics have become the exception rather than what is most commonly encountered. In contrast, the majority of microplastics detected across terrestrial and aquatic habitats are secondary in nature and usually much smaller in comparison,[11,24,25] making them much more challenging to detect, identify, and classify without advanced approaches and instrumentation. The challenges inherent in isolating and accurately detecting microplastics from environmental samples are numerous. Not only must sediment, water, air, and tissues be sampled using the best available equipment and most appropriate techniques for a particular application or setting, but they must be processed using protocols that are highly protective of contamination from the point of collection through to extraction and analysis, which usually includes a combination of microscopy as well as spectroscopic approaches.[26–28]

As with any emerging field, protocols and approaches to data collection evolve over time and involve some degree of trial and error, as well as evaluation of multiple techniques and generation of best practices amongst numerous laboratories and research groups. To date, however, standardized approaches regarding the quality assurance and quality control (QA/QC) and collection of samples for the assessment of microplastics are not codified. Given the number of groups collecting these data and the need for comparability across laboratories, as well as the importance of controlling for background contamination to avoid false positives (e.g., counting cotton fibers shed from clothing as microplastics), it is imperative that as a field standard protocols and harmonized methods are settled upon to generate reliable and reproducible data sets. Some efforts have already been made to standardize protocols (e.g., Kershaw et al.[29] Gago et al.,[30] ASTM International[31]), but more work remains on this front. Collecting microplastic data according to an established framework of standards will greatly improve synthesis and meta-analyses across laboratories and geographical regions, leading to a more accurate global assessment of occurrence and risk.

Herein, we describe in detail a QA/QC framework for commonly used procedures for microplastic sampling, extraction, and identification that have been agreed upon by multiple lab groups and investigators to form the basis of what can become standardized protocols. The focus of this review centers on protocols for the collection of samples across a variety of sources, pathways, and matrices (e.g., wastewater, run-off, drinking water; Table 1, Fig. 1) that are summarized from over 200 studies published as recently as early 2020. We begin with an overview of the importance of study design and general recommendations for field and laboratory QA/QC practices. This is followed by an introduction to sampling with a focus on the main sources and pathways for microplastics into the environment, each with

Table 1 Glossary of terms and techniques.[a]

Additive	A chemical used during plastic production that confers increased stability (e.g., resistance to photodegradation), flexibility, and/or coloration to plastics. Examples are bisphenols, phthalates, flame retardants, dyes, pigments, and metals.
Background/ field control/check	Wet filter paper or open container with filtered water used during sample collection and processing to capture background microplastic contamination from the surrounding environment.
Biosolid	Sewage sludge that is further treated via digestion or composting to minimize disease-causing pathogens so that it may be used as a safe bulk soil amendment and/or fertilizer.
Biota	Animal, plant, or algal tissue; fresh animal feces.
Bulk sampling	Collection of water, sediment, or air samples without nets or filters, using grab sampling or auto-sampling approaches, accounting for a broad range of debris sizes (to 1 μm).
Composite	A sample created by combining at least two samples collected at different points in time or space for the purpose of being more representative of the study area.
Depth-integrated sample (r)	Water sample collected throughout the water column generally using a pump or plankton net.
Drinking water	Also known as potable water is water that is safe to drink or to use for food preparation.
Effluent	Treated wastewater (secondary, tertiary) that flows from an industrial or municipal treatment outfall or sewage pipe into a waterway.
Eolian	Born, deposited, reduced, or eroded by the wind.
Good laboratory/ field practices	GLPs, a set of principles put forth to assure the quality and integrity of non-clinical laboratory studies that are intended to support research for samples regulated by government agencies.
Grab sample	Sample of any matrix (e.g., water, sediment) collected instantaneously at one moment in time, usually from the surface.
Hydrograph	A graph showing the rate of flow (discharge) versus time past a specific point in a river, channel, or conduit carrying water.
Industrial feedstock	Virgin plastic in the form of pre-production pellets (also known as nurdles; small oval pieces of microplastic 2–3 mm diameter), scraps, or powder used as raw materials in the production of plastic products.
Influent	Untreated wastewater flowing into an industrial or municipal treatment facility, outfall, or sewage pipe.
Limit of detection	LOD, minimum number or mass of microplastics of a specified size range detectable with confidence by methodology used in a particular laboratory. In traditional analytical chemistry, the LOD is calculated as the mean of a number of blanks (minimum $n = 3$, EPA recommendation = 7) plus a minimum of two standard deviations. In the field of microplastics research, the LOD is used as a threshold for the number or mass of microplastics that can be measured with certainty above laboratory and/or field blanks. The LOD may be calculated for the sum of all particles within a blank, by shape, by type (e.g., film, foam, fiber) or other category deemed important.
Limit of quantification	LOQ, minimum number or mass of microplastics of a specified size range that can be reliably counted and that are statistically distinguishable from the study blanks with a higher degree of precision and accuracy. In traditional analytical chemistry, the LOQ value is equal to or higher than the LOD plus three standard deviations (accounts for 99.7% of variability) from the mean of a number of laboratory and/or field blanks (max $n = 10$).
Lower troposphere	The lowest region of the atmosphere, extending from the earth's surface to the lower boundary of the stratosphere, a height of about 6–10 km (3.7–6.2 miles).
Matrix	Environmental compartment from which a sample is taken (e.g., air, water, sediment, tissue).
Macroplastic	Synthetic polymer sized greater than 5 mm.
Manta net/trawl	A net used for surface sampling of a waterbody, which resembles a manta ray given its metal wings and broad mouth, to which is attached to a thin mesh net with a collection cup at the end (cod end). A flow meter can be attached for a rough volume estimate; however, this net is commonly used to sample a known surface area.
Microplastic	Synthetic polymer sized between 1 μm and 5 mm in any dimension.
Micro/nanofiber	A natural or synthetic fiber (e.g., cotton, nylon, polyester) having a diameter falling into the size ranges described above for plastics.
Nanoplastic	Synthetic polymer sized between 1 nm and 1000 nm in any dimension.

(continued)

Table 1 Continued.

Pathway	Route by which primary or secondary micro- or nanoplastics are delivered to a particular location where they become mixed or entrapped in one or more environmental matrices.
Positive control	Actual or artificial samples spiked with known plastics or other debris that are treated in the same way as unknown samples, also referred to as spiked recovery.
Persistent organic pollutant	POP, an often hydrophobic chemical that persists for years in the environment, usually having toxicological properties. Examples are legacy chemicals such as Polychlorinated biphenyls (PCBs), brominated flame retardants, oil-associated chemicals such as Polycyclic aromatic hydrocarbons (PAHs), and pesticides (e.g., Dichloro diphenyl trichloroethane (DDT)), as well as current-use refractory chemicals.
Plankton tow	A net used for collecting samples of plankton from a waterbody at various depths. It consists of a towing line and bridles, nylon mesh net, and a cod end. A flow meter is used to estimate sample volume.
Primary plastic debris	Plastics derived from industrial feedstock in a form that is already microplastic in size, e.g., pre-production pellets, microbeads, or powder.
Procedural blank	A sample which is ideally absent of microplastics (for example, distilled or filtered water) that is treated in the same manner as an environmental sample, for the purposes of comparison and detection of background contamination following processing.
Pump	A sampling device used to collect or transport liquid for collection. Common types of pumps used for microplastic collection include auto-samplers, which can be programmed to collect liquid samples at specific times or flow rates, or subsurface pumps, which continuously withdraw and transport liquid from below a surface using either suction lift or positive pressure.
Quality assurance (QA)	A series of steps or activities put in place in a systematic way to ensure that data that is generated is accurate and reliable.
Quality control (QC)	The process of verifying or checking all data, results, or reported methods to ensure their validity and correctness and to prevent erroneous conclusions; is a fundamental part of a quality assurance system or program.
Secondary plastic debris	Plastic fragments and/or fibers formed from the degradation of larger debris items, both during the use of larger plastic products or following disposal.
Sediment	Natural material made up of particles found in terrestrial or aqueous matrices, broken down via weathering and erosion; terrestrial sediments are also referred to as soil.
Sediment sampler	Refers to a device that is manually or automatically controlled to collect sediment samples, such as a petite Ponar, Peterson grab, Ekman sampler, or box core for grab samples; gravity or piston cores can also be used to obtain sediment cores to examine historic trends.
Sewage sludge	The semi-solid and solid organic material retained during the primary and secondary settling phases of industrial or municipal wastewater treatment.
Source	Origin of plastic, such as a factory, consumer and commercial products, litter and debris.
Stereoscope	Dissecting or optical microscope (or light microscope) that uses reflected light to provide three-dimensional magnification (up to 100×) of solid items, such as suspected microplastics.
Surface water sample	Water sample collected from the upper 1 m of the water column using various sampling collection devices, including grab or trawls.
Transect	A line or grid used in environmental surveys used to measure or account for the distribution of samples (sediment, water, biota, air); data are recorded at marked intervals along each line.
Vacuum filtration	Usually completed using a Buchner funnel holding a paper, polycarbonate, or glass fiber filter, placed in the top of a side-arm flask connected to a laboratory benchtop vacuum valve with tubing. This approach is used for separating solids (e.g., plastics) from a solid–liquid mixture.
Wastewater	Liquid waste resulting from industrial, domestic (i.e., sewage), or commercial activities.

[a]A more comprehensive spectroscopic nomenclature is available at https://www.s-a-s.org/spectroscopic-nomenclature/.

specific considerations and recommendations for gear selection, field sampling, study design and replication, and matrix-specific QA/QC. Finally, we close with suggestions on the QA/QC for analysis and data reporting of microplastics from all sample types considered. While we acknowledge that nanoplastics are undoubtedly also in need of further investigation, the detection and extraction of particles in this size range is beyond the scope of this manuscript since most limits of detection for visualizing particles are in the 50–200 μm range. Our overall goal is

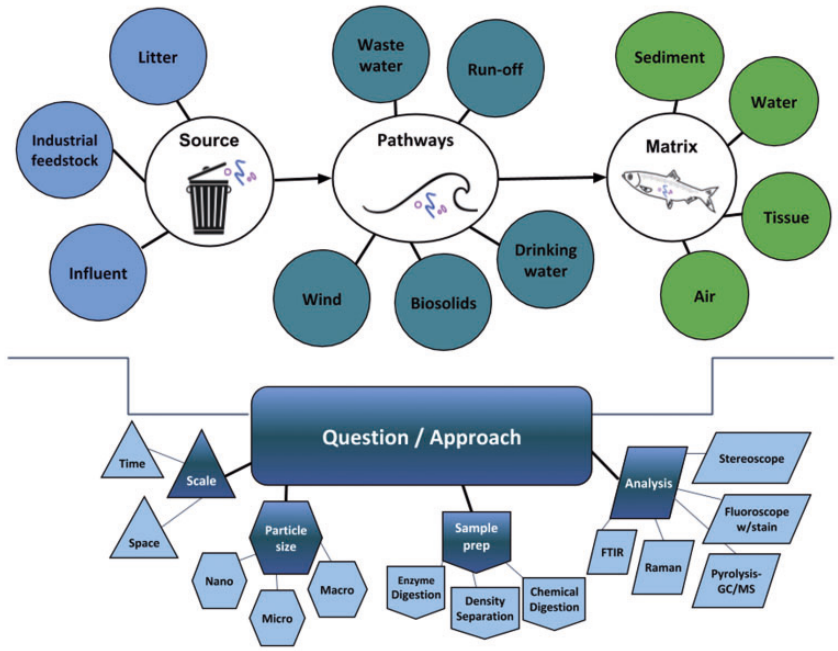


Figure 1. Sampling design should be informed by the source or origin of microplastic debris (e.g., litter, influent, industrial feedstock, or nurdles), the pathway by which it traveled to a particular location or facility, which could be via wastewater or biosolids, run-off from urban or agricultural areas, drinking water, or even via transport by wind, as well as the matrix (air, water, biota, sediment) of interest. Research questions and approaches are best rooted in standardized techniques that simultaneously address scale (across time and space), targeted particle size (nano, micro, or macro), plans for eventual sample preparation (enzyme digestion, chemical digestion, and/or density separation), and analysis (identification via a combination of stereoscope, fluoroscope with staining, FT-IR, Raman, or py-GC-MS).

to provide scientists with a general QA/QC guide and associated checklist to employ when approaching sampling and experimental design for a wide range of challenges and settings necessitating the detection of microplastic pollution, as well as an in-depth consideration of the issues and recommendations specific to each sample type.

Importance of a Well Thought-Out Study Design

Microplastic sampling is performed across an increasingly varied and expanding set of sources, pathways, and matrices (Fig. 1). It is also conducted to achieve a number of diverse objectives, from discovery for basic research to satisfying emerging regulatory requirements.[32] Each of these variables calls for a different set of considerations regarding gear selection, level of replication or number of study sites and coverage, as well as controlling for background contamination through general and matrix-specific QA/QC measures (Fig. 2). While initial studies in the field mainly focused on occurrence in biota and sea surface water,[33,34] this has expanded to include considerations of microplastic fate in freshwater, air, and sediment (terrestrial and aqueous), as well as more complex evaluations of transfer and fate between environmental compartments or within organisms.[14,35–37]

Similar to early studies across other contaminant types, discovery-based research to establish a baseline for presence across environmental compartments is imperative, and investigations into occurrence, fate, exposure, and distribution must be initiated before more complex questions are generated. Now that a large body of work on microplastic pollution exists, it is reasonable to expect that sampling regimes and the questions they are targeted to answer are carefully designed and controlled. As the science behind the study of microplastic pollution evolves, investigations center less on whether debris is present or absent and more on the assessment of risk, the modeling of microplastic movement through food webs, and the connection between plastic contamination, mitigation, and the need for regulation. For example, if the question is: "What is the risk of microplastic ingestion to ecosystem A or species B?", meaning that risk assessment is the reason for and the end goal of sampling, care should be taken to select an ecosystem and/or a representative indicator organism(s) for which ecological or biological endpoints representative of its ability to continue functioning or surviving can be measured. Responses would ideally be measured across several ecological or biological scales, on endpoints that directly influence organism fitness, such as swimming ability, reproduction, stress response, or sex ratio.[38–40] If the

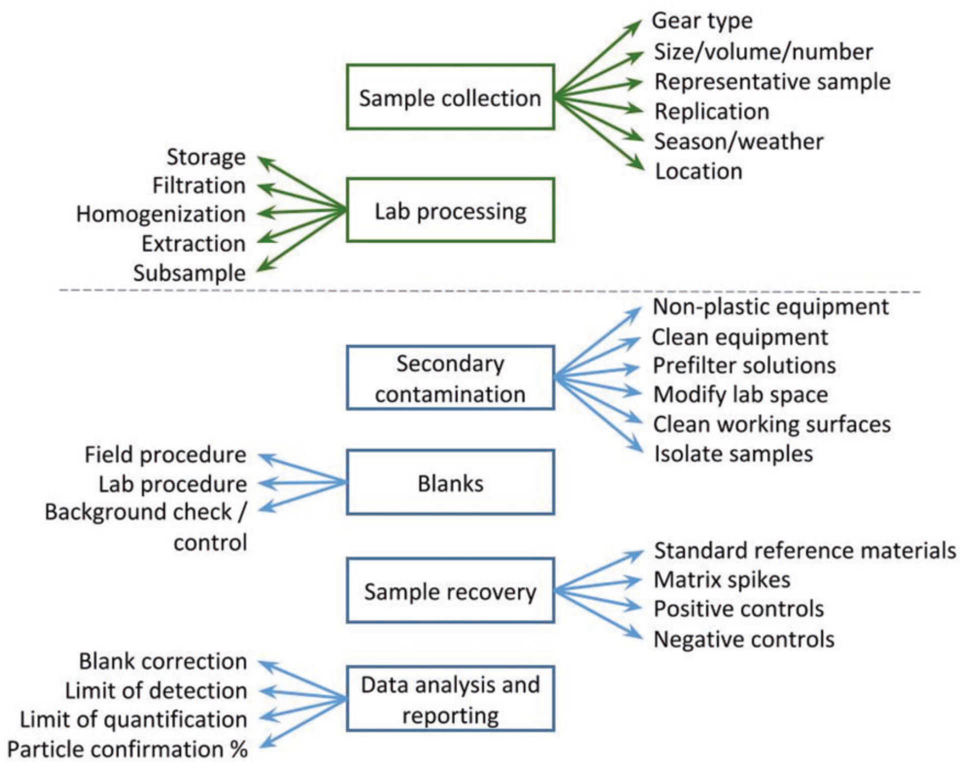


Figure 2. A guide of considerations for QA/QC measures associated with sample collection through processing and analysis, to ideally be determined at the onset of a study. Boxes above the dashed line indicate items to consider that are based on matrix and analysis technique; boxes below the dashed line refer to considerations to be undertaken for purposes of QA/QC.

question is: "What is the effectiveness of a proposed mitigation strategy?", understanding the effectiveness of an approach relies on knowledge of the status of microplastic contamination in that particular ecosystem or geographic area before mitigation. It also requires being able to measure what is captured by the mitigation strategy for the relevant size, shape, and type of microplastics. Research questions and approaches must be specifically curated to the challenge at hand and tailored to each sample type.

Regardless of the matrix or setting being examined and question(s) to be addressed, the majority of studies involving the detection and measurement of microplastics require similar initial steps and planning to ensure accurate estimation of microscopic debris as well as sufficient prevention and protection from procedural contamination of samples, including airborne synthetic debris (Fig. 2). For example, as with any scientific experiment it is imperative that sampling design includes adequate replication. When sampling sediment or water to determine microplastic loads, obtaining multiple samples per site to allow for compositing or averaging between pseudoreplicates is recommended.[41] If samples are being collected to estimate average occurrence or internalization across a geographical area or region, it is important to consider sampling at multiple points to represent each site, for biota to take range size and migratory patterns into account, and to calculate estimated variability or confidence limits.[42,43] If comparing across matrices (e.g., sediment and fish), it is important to co-locate sample collection as much as possible so the pathways and sources can be linked to the sinks and receptors.[44] It is also essential to factor in seasonal differences and shifts in weather across matrix types, particularly if the region being studied has large fluxes in precipitation (e.g., rainy versus dry season), or if influent received at a treatment facility tends to have time-dependent shifts in composition or volume.[44–47] Of critical importance across all matrix types is the inclusion of good field and laboratory practices, appropriate background controls, and procedural blanks to limit and account for airborne plastic debris or the introduction of plastic particles from equipment and personnel. Specific examples for sampling matrices are provided in Fig. 2, and range from microplastic sources such as plastic production and municipal influent to sampling of microplastics along the pathways they travel by in water, air, sediment, and biota via wastewater treatment plant (WWTP) discharge, stormwater input, air deposition and the breakdown of macroplastic, along with considerations specific to each.

General QA/QC Considerations

Implementation of consistent QA/QC practices should be considered early and throughout the study process including during study design, sampling and collection, extraction, and analysis to strengthen the reliability and comparability of microplastic data. Although there are many facets to QA/QC, one of the most important elements when studying microplastics is the control and documentation of contamination. Microplastics are ubiquitous in the built and natural environment, including indoor air, and thus samples taken for quantification of microplastics are prone to secondary contamination during collection, transport, processing, and analysis;[48–50] this is particularly true for smaller microplastic particles (<500 μm). Microplastic contamination can stem from air deposition on samples or equipment, plastic sampling equipment and tools, water used for cleaning equipment and sample processing, working solutions, reagents, and synthetic clothing worn by field staff.[50] For example, 50–280 microplastic particles were detected per kg of sodium chloride salt, which is used in density separation of microplastics from sediment.[51] There are three approaches for reducing the high potential for secondary contamination: (i) implement good field practices and good laboratory practices (GLPs) that minimize procedural contamination of microplastics in air and chemicals and on surfaces and equipment; (ii) quantify the amount of contamination introduced to samples with background checks and field and procedural blanks, and implement blank subtraction/adjustments to sample data; and (iii) use procedural blanks to apply limit of detection (LOD) and quantification methods typically used in analytical chemistry, to see if data from environmental samples are sufficiently higher and thus usable, or simply flag samples below a threshold determined by the average contamination in field and/or laboratory blanks.[41,44,52,53]

Good Field and Laboratory Practices

A number of good field practices and GLPs for minimizing secondary contamination of samples for microplastic analysis have been recommended, applied in the scientific literature, and will be summarized here (e.g., Wesch et al.,[49] Prata et al.[54]). To start, regardless of the matrix in question, the use of plastic sampling and laboratory equipment should be eliminated, wherever possible, and glass or metal used in its place. Inconspicuous items, such as plastic lids on glass storage jars can degrade and contaminate samples. In situations where plastic cannot be avoided, appropriate procedural blanks are required to quantify and correct for any contribution from the equipment. For example, Klein and Fischer[55] utilized polyvinyl chloride (PVC) pipe and connectors for their bulk atmospheric deposition samplers. In order to compensate, Klein and Fischer generated a Raman spectrum of the PVC components of the pipe and any microplastic particles, with matching spectra were removed from their results. In the end, five particles out of 53 analyzed using μRaman matched the original PVC spectra. Similarly, net samples can be taken from sampling devices during biota collection. Fish may interact with nets during collection, so it is important to rule out net feeding from samples. FT-IR spectra of specific nets can be added to spectral libraries and particles matching color and polymers can be removed from the results.[8]

First, GLPs include procedures for removing any plastics on the surface of field or lab equipment prior to use. More stringent cleaning practices are important when working with or concerned about contamination from plastic-associated persistent organic pollutants or additives include baking or furnacing Pyrex glass at a high temperature (>350 °C) or other materials at lower temperatures,[56,57] or acid washing (e.g., 10% nitric acid). At a minimum, glassware should be soaked/washed with a concentrated detergent (e.g., Contrad 70 or Alconox) and rinsed three times with MilliQ or reverse osmosis (RO) water.[49,54] Once equipment is clean, it is important to store it appropriately. The equipment must be covered or sealed away from the field or laboratory environment due to potential contamination from microplastics in the air (see the Air section below) for aerial deposition measurements indoors. Take note that certain field locations may have elevated deposition rates that need to be evaluated and avoided. These may include synthetic rope on a research vessel or microfiber cloths used for wiping down surfaces and equipment in the field. When sampling from a boat, in particular, there are multiple potential sources of microplastics (e.g., boat hull, life vests) that cannot be removed. Thus, cleaned equipment can easily become contaminated without proper storage. The effectiveness of cleaning and storage procedures can be cursorily checked by examining tools and equipment under a stereoscope,[8,50] but procedural blanks are required for more extensive equipment checks.[58]

Second, both good field practices and GLPs include prefiltering all working solutions used during sample processing with clean vacuum filtration equipment and storing the filtered solutions in tightly sealed clean glass bottles. This includes digestive and density separation reagents, such as potassium hydroxide (KOH), hydrogen peroxide, and zinc chloride, as well as distilled or MilliQ water used for rinsing equipment.

Third, GLPs include modifications to the lab space and routines that minimize the sources of secondary contamination. At all times, personnel should wear only natural attire in the laboratory space, even when not processing microplastic samples. Clothes should be cleaned with a lint roller or similar to capture any loose fibers. Whenever possible, any furnishings or carpeting comprised of synthetic fibers should be removed from the laboratory space. If there is suspicion that microplastics are being tracked into the laboratory space from other areas of the building, a sticky mat can be placed at the entrance to the lab to remove particles from foot traffic and an air filter can be installed.[59] During sample processing, attire should include a cotton lab coat and gloves, and safety goggles (optional) if conditions are deemed hazardous or are irritating to the eyes, with minimal synthetic ribbon fasteners.[8,54] One additional precaution is to dye cotton lab coats a unique color (e.g., neon orange), so sample contamination would be notable and easily tracked back to the source. Some laboratories have implemented the use of clean suits made of a less common polymer in a bright color such as orange or purple (Moore, Horn, personal communication, 2019).

Fourth, some level of GLPs that physically prevent secondary microplastic contamination from reaching samples is needed. The most basic GLPs in this category are: (i) cleaning all working surfaces with MilliQ water or ethanol (including adjacent walls) prior to use and (ii) keeping samples isolated from the field or laboratory environment as much as possible (i.e., sample isolation). Sample isolation can take on many different forms. Some examples include sieve covers when fractionating samples, promptly closing sample and reagent lids, covering samples that are digesting with a watch glass, efficiently working through microscopy analyses, and covering samples with a lid or foil promptly when pausing or finished.[60,61]

Other, more sophisticated GLPs that physically isolate samples are use of laminar flow cabinets or use of a cleanroom during sample processing and analysis.[49,62,63] Note that fume hoods pump in lab air under the sash, constantly bringing in new contaminants. On the other hand, laminar flow cabinets often contain high efficiency particular air (HEPA) filters and push pre-cleaned lab air gently across the working surface. Two large-scale GLPs in this category include utilizing heating ventilation and air conditioning (HVAC) systems and HEPA filters to remove microplastic contamination from laboratory air.[49]

Overall, an evaluation of each lab's situation and logistics should be carried out to establish the most appropriate GLPs. Wesch et al.[49] compared airborne contamination of a wet filter paper in four different environments: indoor laboratory, mobile laboratory, fume hood, and laminar flow clean bench. They reported that a clean fume hood alone reduces airborne contamination by 50%, while a laminar flow clean bench or hood brings secondary contamination down by 96%. Using sample isolation methodologies and hermetic enclosures, Torre et al.[61] reduced secondary contamination by 95%.

Blanks and Background Controls

To quantify secondary contamination in the field and lab, field and procedural blanks, along with background checks or controls are necessary. There is no standardized methodology for blanks in the scientific literature, so examples of different approaches will be provided here. A field blank should mimic the sampling procedure as closely as possible. This may comprise an empty sampling container that is opened the same amount of time as the container used for sampling or running MilliQ water through a set of sieves or net (e.g., Sutton et al.[44]). Field blanks should be returned to the lab and evaluated for microplastics by rinsing and vacuum filtering any microplastics that accumulated in the sample container. The resulting filter paper should be evaluated for the presence of microplastics alongside environmental samples.[58] A procedural (or laboratory) blank for water may be run by taking pre-filtered MilliQ water through the sample processing and analysis steps alongside environmental samples. For example, Wiggin and Holland[64] filtered 20 L of MilliQ water alongside their river water samples and later quantified the microplastic on the filter via light microscopy (stereoscope) and Nile Red. An example involving sediment samples would be running an empty beaker containing all acid, oxidant, or catalyst reagents used for digestion alongside other digesting sediment to evaluate equipment, reagent, and airborne microplastic contamination during the digestion and analysis process.[65]

Along with field and procedural blanks, a check of field and lab background deposition helps to quantify and evaluate the risk for secondary contamination. One common background check or control is exposing a wetted filter paper in a petri dish to the work area during sample collection in the field or sample processing and analysis in the lab.[50,63,66] The wetted filter paper is later analyzed for microplastics using microscopy or spectrographic methods alongside environmental samples.

Sampling and QA/QC Considerations for Each Matrix

Drinking Water

Concern regarding microplastic contamination in the environment by government agencies, water providers, and consumers has ultimately led to further investigation into microplastic concentrations in bottled and drinking water. Currently, no standard methods exist for microplastic analyses in drinking water (but regulatory requirements are emerging).[32] Microplastics concentrations have been reported as high as 4000 particles per liter in surface water,[67] 600 particles per liter in finished drinking water [67], and over 10 000 particles per liter in bottled water;[68] however, drinking water sourced from groundwater has shown negligible microplastic concentrations.[69] Many microplastics studies in clean water matrices such as drinking water have used different size ranges and analysis methods for the same matrices and very few have reported particle recoveries.[70] The importance of quantifying contamination via laboratory and field blanks cannot be understated as microplastics are prevalent in indoor air[71] and outdoor air.[58] For this matrix in particular, it is vital that accurate blank concentrations as well as recoveries are reported so that researchers and managers can best understand and limit the risk to the public.[72]

Depending on the complexity of the matrix and the size of the particles being analyzed, the required sample volume will vary. Preliminary samples should be collected to obtain an estimate of particle size distribution and counts per liter. Enough volume should be collected to confidently identify microplastics at concentrations at least three times higher than in the field and lab blanks. Smaller particles (1–10 um) have been reported to be more prevalent than larger ones in drinking water samples,[67,68] thus allowing for a smaller sample volume.[51] For water treatment facilities that use groundwater or lakes as sources, grab samples should be sufficient as levels of plastics would not be anticipated to change rapidly. For those receiving river water, composite samples may be required, depending on the variability in water quality as well as seasonality (e.g., wet versus dry season).

It is also important to consider the use of plastic and plastic coatings throughout the entire drinking water treatment train. Added chemicals, polyacrylamide coagulant aids, plastic coatings, sampling lines, chemical addition lines and piping are commonly employed in drinking water treatment plants, as well as in the municipal distribution system and in homes, and may or may not contribute to microplastic contamination of drinking water. Therefore, it is advantageous to sample at multiple points throughout the treatment train to determine where microplastic is added (i.e., contamination) or effectively removed. Sample volume may need to change depending on where samples are collected within the drinking water treatment and conveyance system and how sample concentrations compare to lab and field blanks. Drinking water treatment plants can perform an audit of the use of plastic types throughout the system and determine if these plastic types correlate to microplastics found in finished water.

The growing consumption of bottled water and point of use treatment processes that employ RO and other filtration schemes makes it difficult to fully account for or manage all potential microplastic contamination sources in drinking water. It is likely that some fraction of the plastic residues in potable water originates from drinking water plants or from other points within the distribution system. During the collection and analysis of potable water samples, it is recommended that all steps be taken to avoid inadvertent contamination by following strict decontamination and cleaning protocols, since false positives might have a disproportionate effect on public safety concerns.

Future work is required to determine how existing drinking water treatment plant operations can be optimized to further remove microplastics through coagulation, sedimentation, and filtration, especially in the 1–10 μm size fraction, and how new plants can be designed to completely remove them. Bench and pilot-scale work should be completed to further examine the mechanism of removal of microplastics. It is essential to determine how conventional, advanced, and biological treatment systems remove (or add) microplastics and to examine the effluent of each unit process. An evaluation of current treatment methods along with examination of the mechanism of removal will allow for optimization of drinking water treatment.

Wastewater

Municipal WWTPs represent one possible pathway of microplastic to freshwater, marine, and terrestrial environments.[4,73–75] Microplastics in the untreated wastewater influent come from a variety of industrial, domestic, or commercial sources. Industrial and commercial sources of microplastics may include particles used in airblasting,[76] pre-production pellets spilled during manufacturing,[77,78] plastic dust or shavings from construction activities, and fibers from synthetic textile fabrication.[79] Domestic wastewater can contain an abundance of synthetic and natural fibers from the household washing of clothing,[80] as well as microbeads from some personal care products and household and industrial cleaning products.[81] Although there is an abundance and diversity of microplastics entering municipal WWTPs, the vast majority of studies have demonstrated removal efficiencies from the influent into the sludge of 88–97% using secondary and tertiary treatment technologies. Studies have reported concentrations of microplastics ranging from 1 to 10 044 particles/L in influent and 0–447 particles/L in effluent.[82] The plastic polymers detected most commonly in influent and effluent include polyester, also known as polyethylene terephthalate, polyethylene, polypropylene, and polyamide.[4,82–85]

The methods and equipment used to sample microplastics in sewage or wastewater will depend largely on the plant characteristics, available sampling points, and objectives of the study (Fig. 3). If the goal is to understand peak flows and transport or flux of microplastics at specific times, instantaneous grab samples[83,86] or flow-paced samples during specific periods of time[87] may be appropriate. If the goal is to collect average daily information on influx, flows, and transport of microplastics then a 24 h composite will be more representative of the sample stream.[44,88,89] For combined sewer facilities (i.e., stormwater and wastewater), recent rainfall may also affect sample composition and should be considered during collection. Bulk samples may be collected manually using containers, or with pumps including time- or flow-paced autosamplers and subsurface pumps, or diversion of waste streams into collection equipment. Low volume samples (e.g., <30 L) may be collected and further filtered and processed in a laboratory, whereas higher volume samples may require the setup of filter assemblies so that calibrated flows can be directed directly through a series of sieves in the field. This can most easily

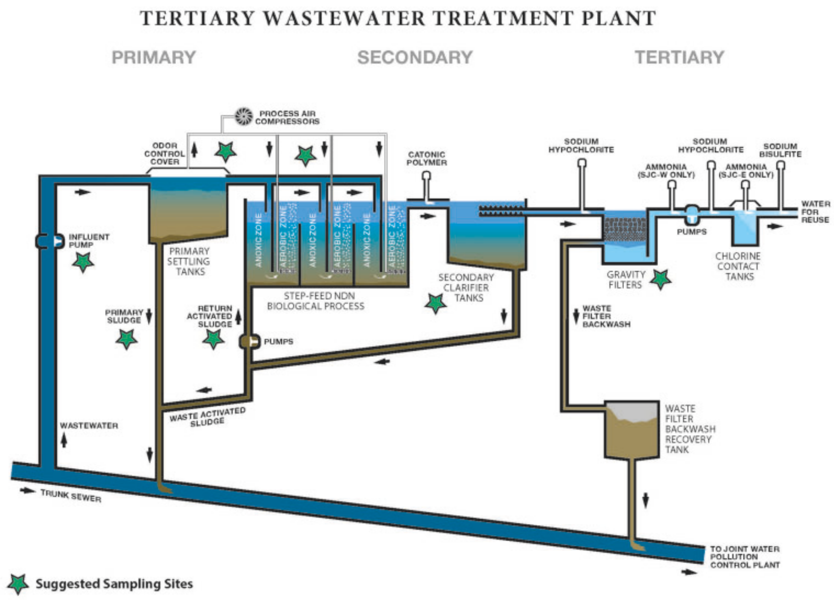


Figure 3. Typical processes of a tertiary wastewater remediation/treatment plant. Primary, secondary, and tertiary processes are indicated. Green stars denote recommended sampling locations, arrows denote the flow of wastewater, sludge, and solids. Figure modified from Carr et al.[81]

be accomplished by utilizing existing compliance sampling streams that are usual fixtures at WWTP facilities. Care should be taken to ensure that intakes for automated samplers are placed appropriately to ensure well-mixed, representative flows for particles of varying buoyancy/densities. Alternatively, wastewater evaluations may be conducted via surface skimming and weir filtration[81] (Fig. 4). Regardless of sampling equipment or techniques, it is important to determine and record flow rate for the duration of collection to establish total volume processed. When prolonged filtration studies are conducted, it is important to carefully establish the duration of collection. Filtration times will vary as a function of water quality, flow rates, and the capacity of the sieve assembly.

Sample volume is an important consideration when processing wastewater. For cleaner samples, including secondary and tertiary effluent, minimum volumes of 20–30 L are often necessary to provide reliable counts above minimum detection limits.[90] Several notable studies have increased representativeness by sampling relatively high volumes of secondary or tertiary effluent from 285 L,[73] 1000 L,[84] and up to 189 000 L.[81] Maximum volume is also an important consideration because of the variability in solid loads as effluent is processed during progressive wastewater treatment stages. Many studies reduce the sample collection volume of more complex samples such as influents (e.g., 1 L) because the high quantity of organic solids (e.g., fats, oils, grease, and cellulose fibers) of these types of sample matrices can greatly increase sample processing times. Due to the high variability of the wastewater matrix over time and treatment processes, it is important to homogenize samples if not already composited and increase replication (minimum $n = 3$) whenever possible, particularly for grab samples. Additional factors to consider and document in the design and interpretation of wastewater treatment studies include the types of treatment processes used, contact or residence times for different treatment processes, polymers or reagents used in wastewater treatment, population served, and any additional inherent variability produced by wastewater treatment processes.

Wastewater collection methods may have unique contamination sources that should be assessed with field or procedural blanks when possible, with an understanding that some limitations may exist within plant operations. For example, samples may be susceptible to contamination from tubing in subsurface pumps or autosamplers. Plant processing equipment such as piping and belt press filters may also contribute to sample contamination and should be carefully considered. While contamination from the plant will be captured in the samples and blanks, it is useful to know the degree of contamination from within the plant when developing management actions for microplastics entering the plant from upstream sources.

Sampling at a WWTP can be a very daunting experience. Developing an acceptable sampling work plan requires an intimate knowledge of the plant's operational processes and accurate information on its flow design. Sampling from or at pre-disinfected plant stages can pose serious infection risk, so caution should be observed, and sample collection should only be performed with adequate protective gear including gloves and face masks to minimize exposure to aerosols. Choice of appropriate sampling locations should only be made after consultation with experienced plant operators who know and understand the WWTP.

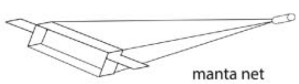
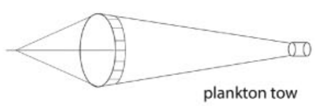
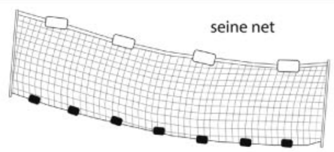
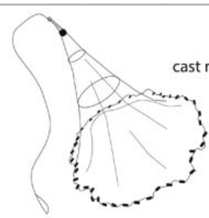
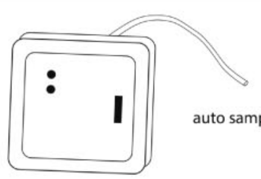
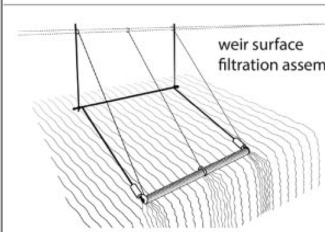
Sampling equipment	Sample type	Description
 surface water grab sample	drinking water surface water wastewater	Aqueous grab samples are generally taken directly off the side of a boat or in a river/stream with a bottle [44, 105]. The sampler will start by rinsing the bottle with sample water 3x to "clean" the bottle, and then dip the bottle to fill it completely with sample water. Grab samples tend to range from 1L to 10L in volume due to limited size of a sampling bottle and ease of returning it to the laboratory. Grab samples can be used if sampling needs to be inclusive of sizes smaller than the mesh size of a plankton tow or manta net, and if a small volume suffices for a particular study design or method, however the small volume can be limiting.
 stainless steel mesh sieve	drinking water surface water wastewater slude, biosolids sediment biota	Stainless steel mesh sieves are versatile and can be used during sample collection (e.g. filtering water through) to reduce sample volume, or after a sample has been taken to remove excess matrix material (e.g., water, sediment) from the sample to avoid bringing too much material back to the lab.Sieves are used following manta trawl sampling [11] or on the beach to collectmicroplastics [29, 149], as well as to sort through homogenate following the digestion of tissues [173]. The size of the mesh varies and is chosen based on the size of the mesh taken to collect a sample or the detection limit in the laboratory.
 manta net	surface water biota	Manta nets are one of the most common sampling devices for microplastics or zooplankton potentially containing microplastics in surface water [22, 23]. They are called manta nets because the device holding the net is held by a metal box with metal wings that help keep the device afloat. Off of the box is a long nylon net, typically with a 333µm mesh, with a cod end at the end where the sample is collected. The trawl is towed behind a vessel, gen-rally for 15-60 mins, two speed below 3 knots with a consistent heading (GESAMP 2019). Thisallows collection from a large water volume in a quantitative fashion. Mantas have also been modified to have smaller mesh sizes for plastic retrieval [122, 161].
 plankton tow	surface water biota	Plankton tows are also of the most common sampling devices for microplastics in surface water [179, 180]. They have been in use for decades for the collection of plankton, and are available in a wide range of mesh sizes. A flow meter is normally attached to the mouth to allow measurement of the water volume passing through the net over a particular time period. Like the manta net, the cod end is where the sample is collected and the tow collects samples from a large volume of water in a quantitative fashion. A modification of plankton tows are bongo nets, which consist of two connected tows pulled horizontally up through the water column.
 seine net	biota	A seine net requires two individuals, one holding the pole at each side (e.g. beach seine), or a boat trawling the net (e.g. purse seine), to be used. A longer beach seine may require a third individual in the middle of the net as it is dragged into shore. The net hangs vertically in the water column, held down by weights at the bottom and buoyed by floats along the top, often a pocket made of a smaller mesh size is sewn into the middle. Depending on the mesh size, a seine can be used to collect fish or macroinvertebrates across a wide range of sizes [179, 180]. These nets are easily used by novices.
 cast net	biota	A cast net, also referred to as a throw net, is circular in shape with weights around the bottom edge of the net. It is cast by throwing it into the air so that it spreads out prior to sinking onto the water surface. Fish or other surface dwelling animals are caught as the net is pulled in. Small bait or forage fish can be captured in this manner using a variety of mesh sizes [181], however throwing technique and ability may influence catch success. The net can be cast from a boat, from the shore, or by wading into the water, depending on the taxa being targeted.
 auto sampler	drinking water surface water wastewater	These pumps can be used to sample water from freshwater, marine, or industrial environments via a programmable peristaltic or vacuum pump. Sampling can be integrated over time. The benefits of using an auto-sampler are that a composite can be made across time and that there is no lower limit on the size of debris sampled. Samples must be carefully filtered using sieving or other approaches following collection [44, 104].
 bench-scale gravity filter	wastewater	Gravity filters are used to perform tertiary treatment at wastewater treatment plants and typically contain ~24 inches of anthracite, ~12 inches of sand, and ~54 inches of gravel. It is a physical process that relies on the force of gravity to remove solid impurities from solution. A bench scale filter can be used to simulate the removal efficacy of these substrates and to track the movement of microplastics through the tertiary treatment process [81].
 weir surface filtration assembly	wastewater	A novel device specifically designed to sample surface water within a wastewater treatment plant, this assembly is constructed using strips of stainless steel mesh (125 um) connected to polyethylene ring supports that are supported by bamboo rods. A hemi-cylindrical scoop is made from the mesh wrapped around a cylindrical framework, and can be adjusted in length depending upon the width of the channel being sampled [81].

Figure 4. The selection of the proper equipment or gear based on a combination of plastic source, pathway, and matrix/matrices being investigated is imperative to generating reliable data. Many commonly used gear types and devices have been adapted for use in the collection of samples for microplastic analysis. A selection of some of the more commonly used as well as examples of sampling devices designed for specific applications (e.g., wastewater plant sampling) are described here.

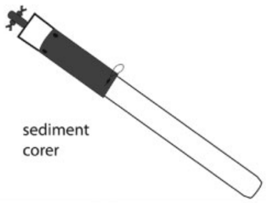
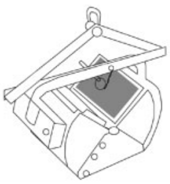
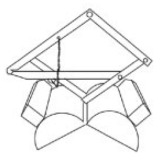
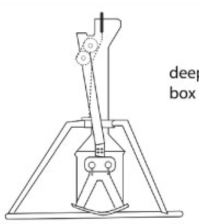
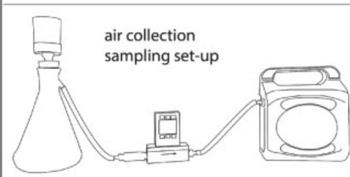
 sediment corer	sediment biota	Sediment corers work by boring a large tube into the benthos to allow retrieval of a column, or core of sediment inside the tube. This allows for the sampling of sediments and the organisms that live within them, leaving the structure of the sediment intact across depths. Cores accounts for stratification, allowing evaulation of plastic deposition over time [137, 143, 153]. Corers range widely in size and length, some are handheld and some are designed to be deployed from a ship. Gravity corers, for example, are made from carbon steel and use the pull of gravity to penetrate the seabed. Piston corers which operate via a mechanical trigger can obtain even longer and larger samples. Core samples are discrete compared to ponar, peterson, or van veen samples which are composites.
 petite ponar	sediment biota	The petite ponar is a smaller sediment surface grab sampler (under 30 lbs) that can be deployed without a winch and crane. It is mainly used to sample stream, lake and river bottoms [152] as well as the seafloor in relatively shallow areas [143, 153], across a diversity of hard bottom types (e.g. sand, gravel, clay). It also comes in a larger standard ponar size. A downside is that it does not account for stratification or distribution of organisms and / or plastic debris across different depths. As such, samples collected with a ponar are considered to be composites.
 peterson / van veen grab sampler	sediment biota	Similar to the ponar, a peterson or van veen grab sampler is used to scoop sediment as well as benthic fauna into a clamshell bucket constructed from stainless steel or another durable metal. It operates like scissors attached to a bucket on each side, which are locked in an open position until the sampler hits the bottom. Pulling upward closes and envelopes the sample. It is also mainly used to sample freshwater environments or the seafloor in relatively shallow areas, across a diversity of hard bottom types (e.g. sand, gravel, clay) [152]. A downside is that like the ponar, it does not account for stratification or distribution of organisms and / or plastic debris across different depths, and such samples are considered to be composites.
 deep ocean box corer	sediment biota	Box corers are used for the sampling of soft sediments at the bottoms of lakes and oceans. These large samplers must be deployed from a research vessel and can be used at any depth. Samples can be retrieved with minimal sediment surface disturbance, allowing quantification of benthic fauna and the study of sediment stratification [137]. The box is normally about 1/2 meter deep and constructed of stainless steel. Similar to other sediment samplers, the corer is held open until hitting the bottom, at which time it is triggered to close around the sample. The benefit of a box corer is that the penetration depth is less variable than that of petersen, van veen, or ponar grab samplers, but it can only be used from a larger research boat or ship.
 air collection sampling set-up	air	This simple vacuum filtration setup is used to sample microplastics from ambient air - to evaluate human exposure and atmospheric loading of microplastics. It pumps air through a filter membrane secured in a vacuum filter holder (top left) using a light duty vacuum pump. A airflow totalizer (middle) is included in the setup to accurately track the amount of air pulled through the filter membrane and subsequently report microplastic counts per cubic meter of air. Sampling can take somewhere in the range of 2-8 hrs depending on the microplastic density in the ambient air. Sites with lower density take longer sampling windows to collect sufficient microplastic material on the filter membrane. A corded or battery powered pump may be utilized, as long as consistent pumping is achieved over the sampling window [124].

Figure 4. Continued.

Operators will also provide real time details on the plant's operations or if changes in the normal plant processes occur.

Future research on microplastics in wastewater is greatly needed to better understand the effectiveness of different secondary or tertiary treatment processes and polymers/coagulants on removal rates, the role of source control in reducing microplastic in discharged effluent, and the overall contribution of wastewater to aquatic and marine ecosystems relative to other industrial or environmental pathways. However, for data to be comparable between studies the methods used to sample and extract microplastics from different wastewater matrices must first be standardized. Only then will developed treatment technologies, mitigation strategies, and regulations for microplastics in wastewater become effective.

Sludge and Biosolids

Sewage sludge is the semi-solid and solid organic material retained during the primary and secondary settling phases of industrial or municipal wastewater treatment (Fig. 3). Sludge is turned into biosolids when it is further treated via digestion or composting to minimize disease-causing pathogens so that it may be used as a safe soil amendment to fertilize agricultural crops. Several studies have shown that up to 80–90% of microplastics in raw sewage are removed after entrapment with grease or grit, or by settling, and end up in the solid sludge phases.[81,82,85,89] However, the relative amount of microplastics removed by these processes at different stages of treatment have been found to differ by shape, size, and density of different classes of microplastics.[82,83] For example, larger and/or high-density microplastics (e.g., PVC) may be more likely to be sent to the solid fractions when captured by preliminary treatment screens or by sinking to the grit fraction during settlement processes, whereas the majority of the lower density microplastics ($<1.0\,g/cm^3$) will float and be skimmed off the surface with grease skimmers.[83] Microbeads may be removed preferentially during grease skimming and end up almost exclusively in solid fractions rather than effluent.[83]

Fibers have been found to be one of the dominant types of microplastic in sludge samples[35,85] with polyester, polyamide, and polypropylene as the most commonly detected polymers types.[4,84,85] Ultimately, fibers of all types and composition become inextricably blended and inseparable from cellulosic fiber residues from toilet paper and other abundant organic waste products in the influent. Therefore, confirmatory steps should be taken to differentiate synthetic fibers from material that may be counted as false positives such as cellulose and cotton fibers.[85,88] Sludge and biosolid samples are generally collected as grabs in glass containers and transported back to a laboratory for further processing.[81,84] The high content of organic matter and solid material often prevents direct filtration in the field as is performed with other wastewater matrices. These complex sludge samples, in every case, will require application of aggressive digestion schemes such as catalytic wet peroxidation or enzymatic degradation to address stubborn organic matrices before any of the plastic isolation techniques can be effective.[82,91] Samples are commonly processed as one-time grabs, or multiple grabs may be combined to create a more representative composite. For example, a study by Lusher et al.[92] collected 5 to 10 grab samples of sludge of approximately 100 g each. Samples were collected on consecutive days when possible or over a period of three days to two weeks, depending on the plant characteristics.[92] Whether grab samples are used individually or combined into composites, it is important to ensure that each sludge sample for analysis has been thoroughly homogenized and is representative of the waste stream being considered. In general, sampling points for sludge should be well thought out to ensure that the sample will address the study question(s).

There are several other important aspects to consider and document when sampling for semi-solid and solid fractions in WWTPs. For instance, the residence time of sludge could theoretically affect biodegradation of certain plastic polymers and semi-synthetic fibers,[86] although this area requires further study. The technology used to process solids could also affect the composition or abundance of microplastics, such as the use of anaerobic digestion versus lime stabilization as solids treatment,[93] or the use of centrifuges versus belt presses used in sludge dewatering.[94]

Because the majority of microplastics entering WWTPs are retained in the solid fractions, sewage sludge and biosolids represent a potentially significant source of microplastics to agricultural and terrestrial ecosystems.[93,95] The application of treated sludge as a fertilizer to agricultural land is widespread because of the improvements to soil quality as well as its enormous economic advantages.[96] Few studies have examined the effects of microplastics in biosolids applied to terrestrial systems, but preliminary research has indicated effects on key organisms in soil communities such as earthworms.[14] Future research is needed to better understand the degree of effects to agricultural ecosystems including crops and the animals that may graze on them, as well as how microplastics in biosolids may be transported throughout terrestrial and aquatic food webs.

Bodies of Water

Microplastics found at all depths in bodies of water—streams, oceans, lakes, and rivers—can originate from multiple pathways, including air deposition, WWTP discharge, stormwater runoff, and the in situ fragmentation of macroplastics. Because of these multiple sources of microplastics carried in these pathways, there is a high diversity of microplastic types that are present in the environment. Surrounding land uses, e.g., urban, agricultural, and industrial uses, can influence the types of microplastics in surface water.[44,87] The shape, size, and density of microplastics will influence how they are distributed throughout the water column by physical forces such as currents, waves, and wind.[97] Currents can result in conversion zones where microplastics are concentrated;[10,98,99] winds alter the vertical distribution of microplastics;[100] and long water residence times in bays, estuaries, and lakes result in accumulation of microplastics over time.[101] For rivers and tributaries, seasonally driven runoff may increase the transport and delivery of microplastics.[102] In addition, storm size and hydrograph stage are important considerations for stormwater sampling that should be standardized across studies. For example, the San Francisco Bay Regional Monitoring Program determined that 70–75% of the annual load of small sediment particles was mobilized by the first 12.3 mm (0.5 inches) of rainfall of the rainy season.[103] Sampling on the rising stage of the hydrograph is generally preferred to the falling hydrograph, particularly in climates where rain is highly seasonal and first flush events tend to wash highly concentrated flows from the surrounding landscape. To estimate load, sampling across the entire hydrograph is necessary. Finally, having a method for measuring or estimating water flow in a stream or through a net at the time of sample collection is important for interpreting results, particularly if study questions involve calculating the volumetric quantification of loading.[44]

Factors affecting the delivery and depth distribution of microplastics should be considered when determining how water samples are collected.[104] Sampling depth (i.e., surface, fixed-depth, depth-integrated) and device should be reflective of your research question. Collection methods such as manta trawls or surface grabs may be biased towards positively buoyant microparticles, e.g., styrofoam, polyethylene, and polypropylene (Fig. 4). A surface grab will likely capture a smaller size range of microplastic particles than net-based sampling,[105] which is limited by the mesh size of the net. Most manta trawls, plankton tows, and bongo nets, for example, have a standard mesh size of 330 μm, limiting the collection of smaller microparticles

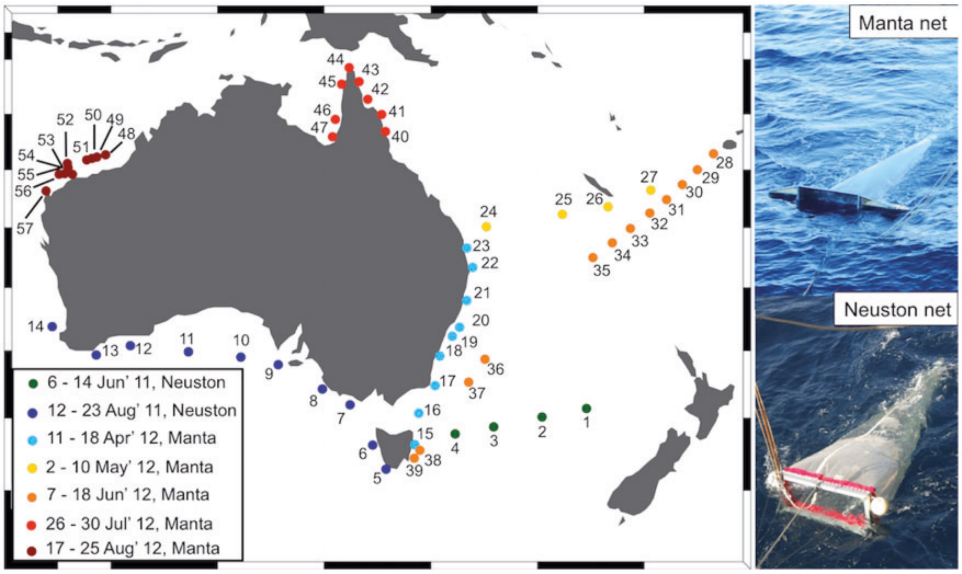


Figure 5. Map of comprehensive study conducted off the coast of Australia. The concentration of marine plastics in coastal waters was characterized and estimated using surface net tows (manta net, neuston net), and their potential pathways were inferred using particle-tracking models and real drifter trajectories. Mean sea surface plastic concentration was 4256.4 pieces km^{-2}, and after incorporating the effect of vertical wind mixing, this value increased to 8966.3 pieces km^{-2}. Dot colors indicate the voyage when the net station was sampled and numbers follow the chronological order of sampling. Pictures of the two types of net used are shown in the right panel. Reprinted from PLoS One.[95]

and fibers, although nets with a smaller mesh size can be custom ordered for particular applications and this is encouraged given that many microplastics fall below this size.[106] Depth-integrated sampling may be the best method for capturing a representative bulk sample in streams and stormwater channels.[102] Depth-integrated samplers used to collect suspended sediment in rivers are an example of a device that could be used to collect this type of sample.[107] A pump or autosampler (i.e., ISCO sampling pump) can also be used to collect depth-integrated or fixed depth samples.[44,108–110] In some instances, a pump or depth-integrated sampler may not be logistically possible. In these instances, water column samples could be collected using a stainless steel pail,[44,85,111] but microplastics may be over or under sampled depending on the major source of microplastics.

Representative samples should also be considered. Small volume samples (e.g., 1 L) may be difficult to extrapolate across broader scales, do not capture the variability of microplastics that may be in the environment, and are more easily compromised by secondary contamination, while large volume samples such as those collected via manta trawl may miss the smallest size fraction and be difficult and time consuming to process due to the large number of microparticles. For samples with high particle counts, it may be appropriate to subsample, but it can be challenging in the case of heterogeneous plastic to create truly homogeneous distributions from components that have varying densities, shapes, and sizes in a given volume. New recommendations from the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection include conducting shorter (i.e., time) trawls instead of trying to subsample from one longer trawl.[29] Ongoing research in San Francisco Bay suggests that 3–4 L samples are needed for most surface grabs to adequately analyze the smaller size fraction.[44] Manta trawls are thought to underestimate particles, especially fibers that may escape through the net in their longest dimension.[44,105,112,113] It may be strategic to collect paired manta trawl and surface water grab samples at the same monitoring site to analyze a wider range of particle sizes than with either method alone. Multiple field samples taken at the same monitoring site may also build confidence in the data by capturing the variability.[29,44,114]

For storm event sampling, the volume of water collected may vary based on storm duration and water velocity. If the pump intake is matched to flow, a greater volume of sample will be collected during higher flows than lower flows.[107] On the other hand, if the volume of the sample collected is held constant across storms, a smaller volume of water will be collected during larger flows than smaller ones. Sutton et al. collected sips throughout the hydrograph to obtain a representative composite sample of storm flow.[44] The total volume of water collected was the same across rain events, so smaller sips were taken during long-duration storms than short-duration storms. Regardless of the method used for stormwater sampling, practitioners need to anticipate issues that can affect sampling gear, representativeness, and safety, including very high flows and high amounts of large debris (e.g., trash, logs, branches, plant material) that could damage gear and block intakes. Results for surface water sampling are generally reported as microplastics per area (e.g., microplastics/km^2) for manta trawl or plankton tow collection methods[115] (Fig. 5), while grab and pump samples are usually reported as microplastics per volume of water (microplastics/liter or microplastics/m^3).[44] For consistency, it might be useful to report both in microplastics per volume.

Air

Microplastics in the atmosphere (lower troposphere, ground level) are now frequently reported and can originate from laundry dryer vents, non-exhaust road particulate pollution (e.g., tire treads, brake pads, PVC speed bumps, traffic cones), construction sites and activities, eolian transport of plastic from litter and landfills, industrial processes, and more.[116–118] The limited data available now indicates that the atmospheric compartment transports microplastics over long distances to remote areas contributing to microplastic pollution in terrestrial and aquatic compartments.[58,118] Microplastics in air may be inhaled and/or ingested (both in normal breathing and unintentional ingestion of settled dust[117]) and pose a health risk to humans.[9,36,79,119,120] Low micrometer and nanometer size plastics are thus of particular interest because these are inhalable and respirable and can deposit deep into the lungs.[36,79,119,121]

There are two types of airborne microplastics to consider when sampling: microplastics that stay suspended in the air, some of which can travel great distances,[8] and microplastics that settle out and deposit on land and water surfaces, thereby acting as an input of microplastics to these compartments.[55,122] Sampling for microplastics suspended in air requires a volume of air be filtered and the microplastics, along with other particulates, pulled onto a filter paper or net (e.g., a plankton net).[122–125] Hundreds to thousands of liters of air are required to collect enough microplastics on a filter paper or net depending on flow rate and microplastic levels in the study area.[122,124] Tracking the actual volume of air filtered is important for reporting microplastic counts per volume (liter or cubic meter) of air filtered. This can be accomplished with inline flow meters or totalizers.[124] Microplastics that fall out of the air via dry or wet deposition can be collected as they deposit. A moistened filter paper or a Petri dish with double-sided tape left in a study area for a specific amount of time (e.g., 24–72 h) that is collected and analyzed can suffice for dry deposition, whereas bulk sampling devices (Fig. 4) with funnels leading to collection bottles support both wet and dry deposition sample collection.[48,58,122] Note the area of the moistened filter paper, petri dish, or funnel used when collecting microplastic fallout is an important piece of data to record and is utilized in calculations to obtain the final microplastics/day/area values reported. Allen et al.[58] measured microplastic deposition rates (e.g., atmospheric fallout) in a remote area of the French Pyrenees Mountains. Atmospheric deposition collectors were used to obtain monthly composite samples of microplastics from dry and wet deposition. Rainwater from the collectors was vacuum filtered, digested to remove organic matter, re-filtered, and oven-dried.

Fibers[48,122] or fragments[58,98] are likely to be the most numerous depending on the study area. Airborne microplastic fragments and films tend to be in the submillimeter range down to micrometers,[58] while fibers have a larger range of 50–5000 μm in length, but widths between 7–15 μm.[48,58,122,126] All airborne microplastic fragments, films, and fibers reported share a similar size particle distribution pattern. The observed trend is an increasing number of particles at lower size ranges. For example, Cai et al.[126] found that fibers in the 200–700 μm range dominated, while there were very few fibers at 4000 μm. Many authors predict the trend of an increasing number of particles at lower size ranges continues down through nanoplastics; however, since most limits of detection for visualizing particles are in the 50–200 μm range,[48,126] nanometer particles are not commonly quantified in microplastic studies at present.

Most studies carried out on airborne microplastics thus far have focused on microplastics deposited on land surfaces from the atmosphere. In both urban and rural areas and in outdoor air, combined wet and dry deposition rates have been reported in the range of 2–512 particles/m^2/day. One study reported dry deposition rates of 1600–11 000 particles/m^2/day[48] for indoor air, which is markedly higher than all other reported outdoor deposition. Studies evaluating microplastics suspended in air can report highly variable levels. Dris et al.[48] found 0.3–1.5 microplastic fibers/m^3 in outdoor urban air (rooftop of an office building) and 0.4–59.4 microplastic fibers/m^3 in inside air (apartments and office buildings). On the other hand, Kaya et al.[125] reported 782–3891 microplastics/m^3 in outdoor urban air (bus terminal and university).

The levels and types of airborne microplastics are influenced by vehicle and foot traffic, attire (synthetic versus natural), location, time of day (as it relates to foot traffic indoors and weather conditions outdoors), and wind direction.[55,58,125] Another consideration is human height.[126] If inhalation and/or ingestion by humans is being studied in an area, then sampling height should be near the average human breathing height of 1.2 m.[48] If long-range transport of microplastics to an area is of interest, then rooftop or other higher altitude sample collection should be considered. This spatial and temporal variability should be noted and study plans designed with it in mind and appropriate metadata recorded. This is a new field of study and many more datasets are needed to evaluate the role microplastic size, shape, and composition, as well as wind and rain play in the regional and global atmospheric transport of microplastics from their origin to the site of deposition.

Sediment

Microplastics have been commonly found in aquatic and terrestrial sediment. Early reports from littoral regions included pre-production pellets on beaches[17,127,128] and fragments and fibers in subtidal sediment.[129] Microplastics have been found in regions of high human population density[59,96] and in areas remote from human influence, including polar regions[130] and the deep sea.[131,132] The accumulation of microplastics in sediment will be influenced by the proximity to pathways (ocean litter, sewage outfalls, landfills), as well as local processes (runoff, currents, waves) that influence transport and deposition of particles.[116] Sediment is likely a sink[10,11,132] for plastics denser than seawater, as well as less dense plastics where aggregation and biofouling decrease buoyancy.[133,134] Microplastics have been found in stream channel beds[135] and estuaries.[136,137] Microplastics in terrestrial sediment, although less widely examined than aquatic sediment,[138,139] have been reported in floodplain soils[140] and agricultural and urban soils.[135,141] Microplastic accumulations in terrestrial sediment are likely dependent upon land use type and proximity to sources. They may originate via several pathways including deposition from air and precipitation, irrigation practices, soil amendments, and the breakdown of macroplastics (e.g., tire dust, agricultural films, litter, mismanaged waste).[142]

The abundance and distribution of microplastics in sediment will be influenced by their innate properties, environmental conditions, and sampling location. Particle size, shape, and buoyancy combined with air and water currents will likely determine patterns of microplastic deposition in sediment. Distribution can be influenced by episodic storm events and seasonal high current flow.[135,143] Microplastics in sediment may be redistributed by tilling of soils, dredging of channels, grooming of beaches,[144] sediment dispersion,[141,143] and bioturbation.[145,146] The methods selected for sample collection must consider the sediment type and location, sample depth, area and volume, and should be reflective of the research question. Reflecting the broad variety of marine, freshwater and terrestrial sediment, there is considerable variation in sediment sampling approaches, which hinders comparison across studies.[147,148]

Beach sediment has been extensively examined for the presence of microplastics using a variety of sampling methods, including selective sampling from the beach surface, volume-reduced sampling (by sieving), and bulk sampling.[28,149] Bulk sampling has produced the broadest range of size classes of microplastics with studies reporting microplastic sizes between 1 μm and 5 mm, though the majority of studies report particle sizes of 10 μm and larger,[28] likely reflecting the limits of identification with microscopy and spectroscopy.[48,126] Patterns of microplastic distribution within beaches are not well understood; however, local waves and currents as well as geophysical characteristics of the beach likely influence the deposition and accumulation of microplastics,[80,150] thus the location of sample sites within beaches (swash zone, high tide line, supralittoral zone) should be considered. Transects from shore to highwater points and across the strand-line are often employed to obtain representative samples.[151] Given the majority of intertidal samples to date have been taken from the sediment surface, the Marine Strategy Framework Directive (MSFD) Technical Subgroup (2013) recommend that samples should be collected from the surface 5 cm of the sediment.

Riverbed and estuarine sediment have been sampled using the cylinder resuspension method,[135] and with benthic samplers (e.g., petite Ponar grab, Peterson grab,[152] box core;[137] Fig. 4). Samples of deeper marine sediment have been obtained with benthic grabs and cores.[60,104,130,131] In marine sediment,[104] beach sand,[148] and lake/pond sediment,[65,153] higher concentrations of microplastics are present at shallow depths. The vertical distribution of microplastics in dated sediment cores may provide a means for reconstructing historical inputs and understanding fate and loss of microplastics to marine and aquatic systems,[60,143,153] as the vertical distribution of microplastics in sediment is not well understood. Martin et al.[104] found microplastics were concentrated in the water-sediment interface and top 0.5 cm of benthic sediment. They recommend samples should be taken to at least 5 cm depth. Terrestrial soils have been sampled at the surface and to shallow depths (~5 cm) using quadrats and excavation with steel tools,[140,154] and to greater depth with cores and soil augers.[141,155,156]

Most studies express the number of microplastics per weight or volume of sediment.[79,148] Drying samples is recommended to eliminate variation in weight and volume measures due to moisture.[79,148] The sample may have to be rewetted in order to remove microparticles from the sediment. The number of replicates required will depend upon the density of microplastics and variability among samples. Higher variability in distribution might be expected in sites subject to frequent perturbation (e.g., beach sand) compared to sediment less frequently affected by waves and currents (e.g., deep ocean sediment). The number of samples will ultimately depend on the research question, but some useful guidelines have been established in previous studies. A minimum of five replicate beach samples was suggested by the MSFD Technical Subgroup;[157] Besley et al.[148] recommended approximately 10 samples per 100 m of beach, and Hanvey et al.[150] reviewed studies reporting between two and twelve replicates sampled in littoral and marine sediment.

Variable sizes and quantities of minerals and organic material contribute to the complex composition of sediment. Distinguishing microplastics from similarly sized sand and silt can be challenging. The organic material present in sediment may obscure visual identification of microplastics or hinder analysis via spectroscopy.[142] Separation of the mineral and organic phase through density fractionation and digestion may be necessary steps in the preparation of sediment samples for analysis. The methods used will be dependent upon the mineral type and quantity of organic material.[158]

Methodologies for sampling non-plastic contaminants in sediment are well established, and the sediment studies for microplastics highlighted above utilize many of these methods. Future studies of microplastics in sediment should ensure that the techniques used are not subject to artifacts that affect microplastics differently as for other contaminants, e.g., ensuring no preferential losses from the sediment water interface or when homogenizing grab samples. QA/QC steps for sediment sampling of microplastics can also be improved, due to the nature of background contamination, especially from fibers. Field blanks are necessary, especially mimicking the handling of samplers, and homogenizing and transferring sample materials to containers. In sediment cores, a deep core slice (pre-industrial) is one means of carrying a blank through the entire sampling, processing, and analysis protocols.

Biota

The effects of plastic debris internalization and entanglement are widely documented in aquatic, albeit mostly marine, organisms.[159–163] Occurrence is beginning to be assessed and detected in land-dwelling animals as well.[15,62] Although over 40 000 organisms are known to have encountered or ingested plastic debris to date, many remaining species, particularly those in freshwater or terrestrial ecosystems, have yet to be investigated. Furthermore, even though many studies in marine biota have been completed or are in progress, there remain large areas of coastline and ocean globally with relatively few data, particularly for commercial fishery species that have direct implications for human ingestion and exposure.[164–166] Biota across marine, freshwater, and terrestrial habitats can be used as bioindicators for microplastic pollution, although the choice of species and monitoring strategy is still under discussion.[167,168]

The proper quantification of microplastic debris internalization is an involved and sometimes laborious process necessitating great attention to detail in both the field and the laboratory. Although larger plastic items ingested by or entangled around organisms can be easily identified without concern for contamination from clothing or surrounding air,[169] the growing majority of plastic debris is microscopic in size and requires stereoscopic examination, as with other matrices containing microplastics, followed by analytical approaches such as FT-IR and/or Raman spectroscopy to confirm that a suspected debris item is actually synthetic.[84,170] For biota within a manageable size range, wild specimens (e.g., birds, reptiles, fish, zooplankton) are generally collected with nets or traps while using proper procedures to control for background contamination, including wearing cotton or non-shedding materials.[171–173] Preservation protocols should also be carefully evaluated. In general, freezing or drying is preferred since ethanol can sometimes interfere with digestion approaches (see Lusher et al.[91] this issue for more details), but in most cases specimens or samples preserved across a diversity of methods, including formalin, can be used.[111]

Replicating the number of organisms captured from each site to account for variability between specimens is key to effective and accurate sampling and internalization estimates. Recommendations range from 10 individuals per site or area to upwards of 50 depending on the organism and the size of the area the sample is meant to represent.[174,175] It is important to consider not only sampling multiple animals per site but also in collecting animals from several locations at each study site, in effect creating a composite or average internalization count for that location, and to take season into account when possible. For example, a recent study using archived samples of forage fish from the Baltic Sea found significantly higher ingestion of plastics during the summer months, presumably because feeding rates generally increase during this time of the year.[176] Additional considerations include taking range size and migratory patterns into account. If the range of an organism spans two sampling sites, they cannot be considered independent of each other. It is also recommended to calculate variability or confidence limits surrounding average internalization estimates using approaches such as the binomial proportion confidence interval because ingestion may be underestimated when sample size is small.[43,174]

If the main interest is in the most commonly investigated internalization routes such as ingestion and respiration,[173] a first pass dissection in a clean or protected area (e.g., hood) is commonly performed after removal of the tissue of concern (e.g., gills, lungs, digestive tract) to visualize any larger plastic items. Often suspected plastics larger than 1–2 mm in size are easily distinguished from prey items or bone. If trophic transfer is of interest and the animal contains whole mostly undigested prey items, these can be removed at this stage and then rinsed and digested separately.[177,178] However, partially digested prey items should be avoided since there may have been mixing or contamination from stomach fluids.

When sampling biota, it is important to consider the size and habitat type, as well as the behavior and life history of the organism of interest. For example, ecological questions regarding the depth at which they would be found as well as time of day at which activity levels are highest should be addressed early in the design of a sampling study. If sampling along the coast, it may be possible to use seine net, gill net, or plankton tow to remove organisms of interest such as small fish, shrimp, or jellyfish from the water column; a trawl net positioned at the depth at which the species of interest is normally found; or baited traps for larger fish and crabs.[179,180] At the surface, a cast or dip net can be used depending on the organism. Benthic invertebrates may be collected via grab sampling, in traps, or bottom trawl. However, sampling at a variety of depths is important if the aim of the study is to collect across a diversity of species.[181] Sessile biota such as bivalves are easily collected by hand, either from the wild or from farmed areas.[173,182] Sampling offshore and at depth requires hook-and-line fishing, plankton or manta nets, or bongo nets depending on the size and life history of the study species.[7] The collection of microzooplankton, such as ciliates, observed to ingest microplastics in the laboratory,[159,183] requires either taking multiple smaller grab samples or using a pump because the mesh size of most nets is too large to capture organisms of this size.

Interest in the study of archived samples, sometimes collected over multiple decades for the purposes of routine monitoring, is increasing as questions regarding the establishment of a baseline or starting point and the potential for increase across time or changes in the types of microplastics internalized or encountered arise. While archives can be an attractive means of obtaining large numbers of samples relatively inexpensively, caution must be taken in the assessment of how organisms were collected and stored, and also account for potential sources of contamination that would not have been controlled for at the time of sampling. Recent studies on plankton and forage fish have taken great care to thoroughly clean specimens externally prior to digestion or dissection, and to focus solely on whole organisms rather than on pre-dissected tissues, which carry a higher risk of contamination from the surroundings in which samples were processed, sometimes decades previously.[176,184,185]

Necropsies of deceased individuals can provide information on diet as well as exposure to microplastics. This approach has been applied to birds for many years and has been recommended for monitoring within Oslo/Paris Convention for the Protection of the Marine Environment of the Northeast Atlantic. Fulmars have proven to be suitable indicators of plastic within diets of the foraging sea bird, and the program has detected dietary shifts in the type of plastic pollution.[186–188] These methods are being adopted to include microplastics, as the current program uses 1 mm as the lower size limit. Similar approaches have been applied to sea turtles and marine mammals, where digestive tracts and stomach contents are isolated and sorted for plastic items, including microplastics.[189–191] In sorting digestive tract into dietary and anthropogenic particles, it is possible to see some differences between feeding types and areas of feeding, for example, offshore deep diving species appear to have a higher proportion of microplastics in their digestive tracts, but most particles are found towards the latter end of the intestines suggesting that marine mammals, irrespective of their stomach anatomical structure, are able to egest microplastics along with other unwanted particles. In these types of studies, lower size limits are imposed by the sieves used for sorting, but also dissection needs to be carried out in a controlled environment to limit contamination for microplastics.[192]

Another sample type that presents contamination challenges is scat. Scat should be collected fresh to ensure microplastic presence is from the animal that produced the feces rather than from contamination acquired from the air or a nearby water source.[192] Acknowledgment of possible external contamination should be made.[193] It can be incredibly challenging to determine microplastic internalization in large organisms such as marine mammals, due to extensive permitting and long periods of time per specimen for necropsy. Scat samples provide a means of estimating exposure without the need for necropsies.[193,194] The study of scat also provides information about trophic transfer. It is unlikely that larger animals, such as seals and dolphins are directly ingesting smaller microplastic particles and fibers; it is likely these come from their prey.[161,193,195] Other studies have demonstrated that smaller debris items can be ingested by predatory species via movement through marine food webs in the wild or in laboratory models.[183,196,197] One recent study conducted in the Celtic Sea on the predatory flatfish *Pleuronectes platessa*, which feed upon sand eels and are in turn fed upon by European otters, found evidence of transfer from the eels (*Ammodytes tobianus*), which feed primarily on zooplankton, to flatfish.[177] Zooplankton are known to indiscriminately feed on microplastics,[7,159] and small crustaceans are now confirmed to create an interface for the transfer of plastics from sea to land.[149] As such, further studies on scat and on the prey of larger animals will be highly informative, filling existing gaps in knowledge on marine, aquatic, and terrestrial organisms.

Far fewer studies have been conducted on terrestrial organisms. An area of emerging concern beyond documenting occurrence in additional species across a diversity of ecosystems is the assessment of land-dwelling biota. Given the annual estimate for plastic pollution on land is 4–23 times that of what is released to the global ocean, this concern is highly warranted.[198] Soils are contaminated from a variety of sources such as irrigation, compost amendments, biosolids from sewage treatment, and the simple act of littering and subsequent fragmentation.[142] A handful of studies focusing on terrestrial plastic ingestion have documented internalization as well as the potential for trophic transfer, with earthworms readily taking up microplastics from soil[14] and a variety of terrestrial and freshwater bird species documented to contain microplastic.[62,199] Recommendations on protocols for sampling biota from marine and freshwater ecosystems should be adapted to terrestrial environs, with careful consideration of replication based on organism type, variability in diet, digestive time, and range size among others. The same concerns regarding potential background contamination, use of archived samples and scat, as well as the potential for contamination from gear (covered in QA/QC below) apply across aqueous and land-based sampling regimes.

Following dissection, most laboratories proceed to a homogenization and digestion step, placing the tissue of interest into a reagent made in filtered water, such as KOH or hydrogen peroxide. Other digestive agents such as hydrochloric and nitric acid have been used in earlier microplastics investigations, but these acidic reagents are now known to breakdown some plastic types and should be avoided.[192] If the tissue was contained within a specimen (e.g., digestive tract of fish, bivalve) and thus protected from external contamination, it can be placed directly in the digestive reagent if working in a clean space. For whole organisms such as zooplankton (e.g., small crustaceans, larval fish) or small terrestrial biota (e.g., worms) that can or are desired to be digested whole, the animal's exterior should be rinsed with filtered water (e.g., Milli-Q) first to ensure microplastics from the external environment are not adhered to the skin or exoskeleton. For extensive details and recommendations on extraction procedures, please see Lusher et al. (in this issue).[91]

Although many taxa such as invertebrates, fish, and even mammals have already been evaluated,[8,200–202] the combined unique influence of habitat type (e.g., marine, freshwater, terrestrial), trophic position, diet, and feeding strategy for each species that encounters microplastic debris makes it difficult to draw generalizations even across related groups, e.g., Gray et al.,[23] Welden et al.,[177] Brennecke et al.,[203] Watts et al.[204] The impetus for further investigation of internalization and the pathways by which micro- and nanoplastics travel through food webs is warranted because the presence of microplastics in digestive and gill tissues affects growth, fecundity, and physiological responses such as respiration. In some cases, it can also cause internal damage and heightened stress responses.[38,160,183,205,206] Effects on these endpoints, many of which contribute to individual fitness, are key to determining whether microplastic ingestion could be having an effect at the population level for a particular species. Furthermore, recent evidence suggests that smaller microplastic items can be translocated to the bloodstream and may be deposited in diverse tissue types.[207] In addition, terrestrial organisms exposed to micro- and nanoplastics are as vulnerable to the detrimental effects of plastic internalization as marine organisms.[14,208]

Thus, it is important to continue to assess and quantify the internalization of plastics across taxa, including terrestrial wildlife, as well as to explore tissue types beyond typical routes of entry such as the digestive tract and gills. This is of particular concern given that evidence for translocation implies that smaller microplastics and nanoplastic particles could be distributed throughout animal tissues consumed by humans, from fish fillets to steaks and chicken breasts. The current state of research and available methods are limited in their ability to detect small microplastics and nanoplastics in edible tissues of biota. As such, the study of microplastic internalization seeks not only to measure exposure and risk to wildlife but also aims to document the routes by which humans are exposed (e.g., Cox et al.[9]). To accurately develop risk assessments and hazard ranking of microplastics and nanoplastics for biota, including humans, we must first have sufficiently controlled methods with a thorough level of QA/QC from sample design to processing and the isolation of particles, identification and quantification.

QA/QC in Data Analysis and Reporting

Characterization and Assessment of Laboratory and Field Blanks

Following sample collection and processing, blanks and background checks can be quantified and characterized by color and morphology and omitted if analogous microplastic is contained in experimental samples (e.g., Vandermeersch et al.[209]), or at a minimum acknowledged alongside data at the time of publication. An additional option demonstrated by Kroon et al.,[66] is to collect items that may contribute to secondary contamination (e.g., neuston net, remotely operated vehicle (ROV) paint chips, coral skeleton, human hair, clothes, gloves, lab coats, rubber bands), analyze the items using ATR FT-IR, and construct a customized spectral library. Any microplastics in their samples with a >90% spectral match to their customized library were omitted from sample tallies.

Quantified procedural blanks may be used to set a LOD (also referred to as a method detection limit or MDL) and a limit of quantification (LOQ). These are typical parameters that would be defined in a quantitative study's QA/QC plan. The U.S. Environmental Protection Agency (EPA) sets standardized procedures for determining LODs, requiring a minimum number of seven blanks (for non-plastic, usually water-soluble contaminants).[52] van Buuren[210] defines four common QA/QC terms: MDL (minimum measured concentration of a substance that can be reported with 99% confidence that the sample is higher than the blank), minimum level (lowest point on calibration curve), practical quantitation limit (three times the lowest point on the calibration curve, or minimum level), and reporting limit (lowest concentration that an analyte can be detected and quantified). An example of the use of LOD and LOQ has been applied to a study of microplastics in bivalves for biomonitoring[41] and also preliminary interpretation of microplastics in drinking water.[53] Both studies assessed the use of LOD and LOQ when investigating the suitability of sample sizes and quantification of microplastics in two very different environmental matrices. Bråte et al.[41] attempted to identify the uncertainties behind the database on LODs and LOQs for fibers and fragments separately and suggested that the relatively high LOD and LOQs can highlight the uncertainties in data. On the other hand, Uhl et al.[53] used LODs and LOQs on all particles, irrespective of particle type which suggested very little quantifiable microplastic contamination in their drinking water system. Interestingly, the lack of quantification in this study is likely indicative of the low sample volumes (1 L per sample versus 10 000 L recommended) used in the assessment.[70]

Powerful tools for systematically accounting for secondary contamination of samples, LOD and LOQ are successfully applied within analytical chemistry.[211] However, their application to microplastics may not be as straightforward as proposed by Uhl et al.[53] Steps to differentiate between sample types are required.[41] Unlike a chemical with a known composition or group of congeners, microplastics are highly diverse in color, size, morphology, and composition. This means the LOD for a brightly colored 200 μm red fiber may be very different from that of a 200 μm translucent film or a 50 μm blue particle. Further, the equipment used to quantify microplastics is inconsistent, with different types of microscopes with varying magnification limits and techniques and many microplastics manually observed. This means that LODs will be equipment and operator specific, and it will be difficult to come together with a community LOD or LOQ. The composition of blanks can be very different from that of the actual sample. For example, Klein and Fischer[55] found that procedural blanks comprised 51% fibers, while samples yielded only 5% fibers. Lastly, although it is suggested that EPA guidance be applied to the estimation of LOD and LOQ wherever possible, microplastics behave differently from the analytes and organic compounds these protocols were designed to evaluate. For example, smaller sized microplastics and nanoplastics are subject to Brownian motion, or random motion throughout a solution, making it difficult to generate repeatable measurements.[212,213] As described above, systematic correction for secondary contamination of microplastic samples is important in producing robust data; however, the most accurate procedure for such a correction is still under development.

Sample Recovery

For chemical analysis of organics such as pesticides or flame-retardants, matrix spikes are generally included to test the recovery of a method. For microplastics, matrix spikes have generally not been used to assess the recovery of various methods in a laboratory, even though using matrix spikes is considered best practice in analytical chemistry. Given that many of the labs working to institute early protocols for microplastics extraction and analysis did not necessarily specialize in analytical chemistry, it is not surprising that matrix spikes have yet to become common practice for this subfield. The future of microplastics research should consider the need for matrix spikes to be able to measure the recovery for individual methods and in individual laboratories. This may include creating representative standard reference materials with microplastic particles in them that can be used to spike into representative matrices to be carried through the extraction and preparation process leading to quantification and characterization of microplastics in a sample. Like other methods for other analytes, recovery around 80% or higher is recommended. Recovery may be lower for some matrices, such as wastewater influent and sediment because they are complex mixtures.

Lares et al.[85] conducted matrix spikes on influent after preliminary screening and digested sludge. Each sample was spiked with seven different plastic polymers of varying density and properties, with 10 particles per polymer. Recovery rates and standard errors were calculated for each plastic type or shape based on the number of recovered particles. When considering drinking water, positive controls in RO water and known matrices should be implemented to ensure lab methods are achieving acceptable or known recoveries. Highly recognizable particles of various colors, shapes, densities, and sizes should be used as a positive control, as different particles will have varying recoveries.[70]

Matrix spikes can include plastic particles, films, and fibers with varying size, polymer type, and color to comprise a positive control,[64,85,135] and organic particles such as algae, cotton, fibers, and wood fragments to target false positives. Digestive procedures coupled with selective dyes (e.g., Nile Red) should separate organic matter from the target microplastics,[214,215] but some recalcitrant organic matter can remain, such as lipids, and be falsely counted as microplastics unless more advanced polymer characterization methods are utilized[175] (e.g., FT-IR and Raman). Spiking with organic materials will allow the effectiveness of the digestive and selective dye methodologies to be quantified. Maes et al.[214] stained three algal cultures with Nile Red and observed subsequent fluorescence. While algae could also be stained with Nile Red and falsely identified as plastic, their Nile Red solution contained low levels of solvent, resulting in very light staining of algae and dark staining of plastics. The Nile Red-exposed algae showed low-grade fluorescence that required greater and higher intensity exposure to a fluorescent source than the stained plastics. In this case, the algae were not falsely counted as plastic. Spiking a sample with both polymeric and organic materials simultaneously, in future studies, would give a better indication of potential interferences and incidence of false positives.

Analysis

While considerations such as level of replication, number, and spacing between sampling sites, and prevention of contamination are critical to designing an effective sampling regime, it is also important to evaluate options for, as well as the cost of, analysis on extracted items that are suspected to be microplastics. Although in the early days of microplastic research, even as recently as five years ago, it was acceptable to identify plastics using approaches such as the "hot needle test" (e.g., Vandermeersch et al.[209]) or merely by visualization (e.g., color, consistency), it is now common practice and expected that a minimum amount of suspected synthetic particles across sample types are confirmed using Raman, μFT-IR spectroscopy, or pyrolysis–gas chromatography–mass spectrometry (py-GC-MS).[26,91,216] At the lower end, studies with samples having low variability between replicates (e.g., similar plastic type throughout, such as microplastics from toothpaste[81]) may be able to justify confirming lower percentage, while studies with higher variability between samples or smaller average particle size may aim for a larger fraction of debris items, but there is still debate as to the exact number. The question should also be considered when determining a subsampling strategy for chemical identification, given that trying to understand the success rate of the researcher in properly identifying anthropogenic materials is different from questions around identifying the source of the materials to the environment. The time and cost involved in analytical confirmation should also not be underestimated at the onset of sampling, as data may not be publishable without minimum confirmation. Further analytical considerations and reporting recommendations will be covered in greater depth elsewhere in this issue in papers led by Cowger et al.[57] and Primpke et al.[217]

Conclusion

The field of microplastics research continues to grow at an exponential rate as concerns are fueled by increased production and associated contamination, as well as demonstrated biological effects. While both sampling and QA/QC procedures are well defined for most environmental contaminants, the diversity of types, sizes, and shapes of microplastics makes it difficult to directly apply these methods to the field of microplastics. As such, here we have described the current state of the field, gathering examples representative across sample types and approaches to the collection of accurate, background-corrected data. Although some of the above-described methodological recommendations for sampling and QA/QC may shift slightly over time, the protocols described herein represent agreed-upon approaches used by numerous laboratories across countries and sectors, signifying a major step forward in the codification of methods for this now prominent area of research. Adoption of standardized procedures and harmonized methods by the global research community will make possible the generation of more reliable and reproducible data and will also permit better comparisons across studies, allowing for much-needed larger scale meta-analyses to be conducted.[218] Given the large body of work that now exists, better harmonization across research groups will make it possible to effectively address some of the most pressing challenges to date, such as assessing the risk of microplastic exposure to organisms and entire ecosystems, designing effective mitigation strategies, evaluating the need for truly biodegradable plastic alternatives, and developing appropriate regulatory frameworks.

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