Pitfalls and Limitations in Microplastic Analyses

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Contents

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Abstract The rising plastic production in the last 70 years led to an increase in
plastic waste in the environment. Intensive research activities about macroplastics
and microplastics (MPs) started some years ago. Different sampling strategies,
sample preparations and analysis methods have been described in the literature for
different environmental compartments and biota. Until the present, many papers
have been published about the quality and quantity of MPs in different matrices.
Pitfalls and limitations in MP analyses are often missing or not discussed. Therefore,
this chapter summarizes the present methods for sampling, sample preparation and
analysis, discusses the related limitations and outlines the complexity regarding MP
loss or contamination during sampling and laboratory work.

Keywords Airborne contamination, Analytics, Environmental compartment,
Spatial and temporal variation, Sample preparation

1   Introduction

Our modern daily life would not be possible without plastics – a relatively young
group of materials that accompanies mankind since the second part of the twentieth
century. Meanwhile, global plastic production rises almost exponentially and
reached approximately 360 million tons in 2018 [1]. Associated with this is an
increased loss of plastic into environmental compartments. The first description of
plastic particles in the sea dates back to 1972 [2, 3]. After four decades of silence, the
topic microplastic (MP) was discovered again. Since that time, intensive research has
been conducted in marine environments [4–6], whereas a focus on freshwater
environments was only set some years ago (e.g. [7, 8]).

   Already in 2012, Hidalgo-Ruz et al. [4] pointed out the necessity to establish a
common standard operation procedure (SOP) for MP sampling, extraction, purifi-
cation and analysis to facilitate comparability of data between studies, but still no
such standard exists. Thus, many reviews address sampling, sample processing and
analyses [9–15] in marine [4, 16], freshwater [7, 17–20] or terrestrial environments
and soils [21, 22] or biota [23–25].

   These reviews clearly show that different methods are used for sampling, prep-
aration and analysis of MPs from different matrices like water, aquatic sediment,
beaches, soil, air or biota. The reviews furthermore reveal that – even if there is a
common sense for the need of SOPs – still the report of different units and size

classes, categorization of synthetic polymer types, forms or shapes additionally to different sampling and analytical methods often hamper comparability of results for a distinct environmental matrix.

A global suggestion for standardized methods is still missing. However, the ISO/TC 61/SC 14 is currently working on a suggestion for standardization [26]. Meanwhile, first attempts concerning SOPs for MP sampling, sample preparation and detection were made in different national and international research projects [27, 28] and by regional action plans [29]; however, the future will show if they gain a broad acceptance. Until that time, comparability of data suffers from a missing consistent definition of MPs [30] and standardized methods.

Besides these general issues, everybody involved in studies on the contamination of the environment with MP faces various methodological specialties and difficulties. However, even if the careful consideration of these pitfalls and limitations occurring during MP sampling, sample preparation and analysis is inevitable and ultimately determines the quality and reliability of data on MPs, such general obstacles are sparsely mentioned in MP literature.

To fill this gap and raise the awareness for researchers, the goal of this chapter is to present and resume challenges, pitfalls and limitations that can occur when dealing with MP sampling, sample preparation and analysis/identification. For clarity of reading, the chapter is subdivided according to the aforementioned topics.

2 Sampling

Generally, MPs are an extremely heterogeneous group of particles covering different synthetic polymeric materials, sizes, forms and shapes that are furthermore subject to ageing and biofouling in the environment. These characteristics determine also their distribution in the environment, because particles behave differently in comparison to, for example, solvable analytes which may be relatively homogenously distributed in water. Therefore, sampling is the first crucial step when assessing MP abundances. The sampling strategy depends on the aggregate state of the target (solid, liquid, gaseous), the sampled environmental compartment (e.g. aquatic, terrestrial and atmospheric systems) or research item of interest (e.g. tissue, food, drink, sewage sludge, etc.) and of course the respective research question. Different sampling strategies are required for water, sediment/soil, air, biota or other samples. Therefore, a clear and concise strategy concerning location, time, compartment and sampling equipment is necessary.

2.1 Spatial and Temporal Variations in the Environment

Environmental factors lead to site-specific and temporal variations in MP concentrations and compositions. Overall, environmental distribution of MPs can be very

heterogeneous which makes representative sampling challenging. For instance, the strong spatial variation in current speed, with backflow areas, whirlpools, etc., strongly influences transport, fast export or temporary retention of MPs in rivers and may create hot spots and areas with low concentrations. The same is true for river banks and beaches where floating MPs usually concentrate at the high-water line. In lakes additionally, prevailing wind direction might also have a strong influence at which lakeshore MPs are found in higher concentration as especially larger MP particles are susceptible to wind drift.

In general, the proximity to anthropogenic activities is supposed to affect MP concentrations and compositions [31, 32]. Secondary MPs are generated via abrasion during usage as well as during building and construction activities, by fragmentation of larger plastic items as a consequence of ageing, or are directly produced as primary MPs and enter the environment via different routes. In this context, transport by wastewater originating from households or industries, wind transport, runoffs from roads, urban regions or agricultural areas with agricultural plastic use, fragmentation of littered plastic waste directly in the environment as well as unintentionally spilled pellets are considered as important pathways and sources, respectively [33, 34].

Potential sources and pathways can be analysed prior to sampling in order to choose sampling sites with informative value. However, spatial and temporal variations hamper the significance of findings derived from single measurements. Therefore, replications as well as repeated measurements are needed to display heterogenic MP distributions in the environment. Up to date, no standard for a representative sampling of different environmental compartments is available, and a definition of minimum replication and number of samples is lacking as standardized procedures are missing [4, 21]. Especially environments that are characterized by a strong heterogeneous contamination of MPs can hardly be sampled representatively with reasonable sampling effort.

Limitations

Sampling with a high spatial and temporal resolution is time-consuming and requires intensive sampling campaigns. In addition, a certain degree of flexibility and mobility is needed to adequately address the influence of environmental factors (e.g. heavy rain falls, storm events, floods or low tides) on the MP abundance. Although anthropogenic factors have been linked to site-specific MP concentrations, the identification of relevant sources is biased by the varieties of materials and pathways.

2.2 Sampling Devices in Different Environmental Compartments

In the following section, currently applied methods and sampling strategies are discussed in detail. The MP research focused for a long time on water samples;

hence, a plenty of publications is available. By contrast, sediment, soil, air and biota are also targets of more recent MP research interest, with less published articles.

2.2.1 Water Sampling

The applied sampling device used for water samples predefines the detectable size range of MPs. Surface waters are frequently sampled, filtered and concentrated with plankton and neuston catamaran nets (optimized for higher wave conditions) or manta trawls. Here, mesh sizes >300 μm facilitate large sample volumes for representative MP counts [35]. However, MPs <300 μm are not addressed quantitatively, and, thus, the amount of MP particles in this size class is underestimated [36]. While sampling with smaller mesh sizes (e.g. 100 μm, 80 μm and below) increases the downward size range of quantitatively sampled MPs, simultaneously rapid clogging at higher seston concentrations limits the flow rate, reduces the sampling volume and leads to errors in sample volume measurements. For preventing the latter, a net cascade with decreasing mesh sizes (e.g. 500 μm, 300 μm, 100 μm) may provide a solution. These methods facilitate large sample volumes by simultaneously considering a higher size range of MPs and may be suitable at different sites under different conditions (e.g. lake, wastewater). A similar approach is the sampling of water via pump-based filter cascades with different mesh sizes. Here, defined volumes of water are either piped through connected filter cartridges [37, 38] or stacked steel sieves [39, 40]. By applying isokinetic sampling, i.e. water is withdrawn at the same speed than the water flow, a non-selective and uniform sampling of particles is guaranteed. Additionally, cascade-based filtration enables the on-site separation of size classes down to 10 μm [39]. A continuous flow centrifuge in which all particles with a density >1 g/cm^3 are retained is another potential alternative. However, it has to be tested if MPs can be sampled quantitatively with this approach [41, 42]. In general, the sampled water volume strongly depends on seston concentrations and, thus, may range from several litres to hundreds of cubic metres.

Limitations
Pumps with attached filter cascades are less mobile than nets. Additionally, the risk of fragmentation, particle loss (e.g. hose system) and contamination (e.g. abrasion of components) during sampling has to be acknowledged and validated [38]. However, the comparison of different methods clearly shows that quantitative and representative sampling with a net is only possible for samples of larger MPs.

Another difficulty occurs with the comparability of results. Sampled volumes differ and so do extrapolated MP concentrations (MPs per litre, m^3, mass units or particle numbers). Moreover, reporting sampling-related limitations (e.g. size range) and appropriate units is mandatory for comparable data. In the future, a combined sampling approach might be probably the most practical solution to sample a wide

size range of MPs quantitatively. For instance, MPs >300 μm are sampled by nets, and simultaneously the size fraction <300 μm is sampled by a pump-filter combination.

2.2.2 Sediment and Soil Sampling

Sampling site characteristics and the respective research question necessitate application of different methods. Aquatic sediments include samples from river beds, lake bottoms or from the bottom of the sea and terrestrial (sediment and soil) beaches, alluvial plains, river banks or agricultural areas. Plastic-free utensils like metal shovels or spoons are usually used to sample terrestrial sites and beaches [21, 43]. Sediment/soils are sampled with grabbers (Van Veen grab) and corers for reaching deeper layers [21, 35] or in soil profiles. In contrast to sampling with grabbers that allow for sampling of a disturbed mixture of the sediment/soil in the upper layers only, core sampling can provide undisturbed sediment cores. Thus, information for depth-specific MP deposition and age analysis of the respective layer is potentially preserved.

Reported sample volumes range between 0.5 and 10 kg, and sampling is mostly done randomly at many locations (e.g. with transects perpendicular or parallel to the water or in single squares on beaches) [4]. First proposals for standardized procedures were published by Frias et al. [28] and the MSFD Technical Subgroup of Marine Litter [44] for beach sediments. In these protocols, a monitoring time frame of once per season is suggested within a 30 cm^2 area and a sampling volume of about 4.5 l. Sampling depths may range between 5 and 300 cm. The use of a minimum number of replicates (at least 5) [44] has been recommended for sampling every 100 m on a beach [45].

Limitations
Undisturbed sampling may be achieved with cores, but the overall number of samples collected is limited. Corers often have rods of 1 m length and a diameter of 5 cm. Compaction or loss of sediment by drilling in a higher depth (each 1 m has to be drilled separately with a 1 m extension rod) is likely. In addition, bioturbation may limit the validity of age-dependent MP accumulation. Grab sampling includes larger volumes but disturbed sediments. Besides device-related limitations, different sampling approaches hamper the comparability in terms of different mass or sampling depths as well as reference units. Thereby, standardization of these strategies and a definition of replication and sample amount are urgently needed for the generation of representative and comparable data.

2.2.3 Air Sampling

Air is an important vector for transportation of MPs, e.g. MPs may be transported up to 95 km [46]. Dris et al. [47] investigated indoor and outdoor air in Paris and

counted atmospheric fallout of 2 to 355 particles per m^2 and day. Samples were taken with a pump over several hours at a height of 1.2 m indoor or using passive samplers (filters) outside [48]. Filter papers or discs moistened with ultrapure water placed in Petri dishes and exposed to the air are one of the strategies used in passive sampling and monitoring potential airborne contamination in the laboratory. Allen et al. [46] counted more than 350 MPs per m^2 (fragments, fibres, films) in a remote mountain catchment in the French Pyrenees. The authors used two collectors (rain sampler and particulate fallout collector) for a combination of wet and dry sampling. Besides MPs, outdoor sampling may also include sampling pollen or mineral dust. Vianello et al. [49] investigated the exposure of humans to indoor MPs with a breathing thermal manikin and detected 2–16 MPs per m^3.

Further work is needed to focus on the analysis of MPs in the air for the standardization of sampling and analytical methodologies. The environmental topic 'fine dust' and related sampling standards provide guiding principles that should be considered when developing sampling strategies for airborne MPs [50–53]. Airborne MPs are also a relevant source of contamination during sampling, preparation and analysis of environmental matrices. Here, lab blanks often reveal high numbers of fibres [54, 55]. The previously mentioned sampling methods may be used to monitor levels of contamination and develop prevention strategies.

Limitations

Pump-based active air sampler systems with a constant flow rate are energy-intensive and expensive to maintain. However, isokinetic sampling has been used a lot for PM 2.5 and PM 10 (particulate matter, particles with a diameter < 2.5 μm (PM 2.5) or 2.5–10 μm (PM 10)) measurements. The performance of these samplers will also be influenced by environmental conditions. The same holds true for passive sampling strategies with moistened filters. Here, the deposition of particles is strongly affected by local air flows and weather events. However, filter-based passive samplers are cheap and can be deployed easily but will require long-time durations of sampling. However, a wet and dry deposition sampler could be a good alternative [46]. Furthermore, the number of polymer-based particles in indoor air samples may be far outweighed by particles of inorganic origin (e.g. glass, ceramic [56]). Thus, plastic-free laboratories or laminar flow boxes are needed. However, a spare room is not available in every laboratory due to limited space.

2.2.4 Biota Sampling

The ubiquitous presence of MPs implies interactions with a diversity of species inhabiting aquatic and terrestrial environments. Thus, in theory, sampling of biota can encompass an enormous spectrum of species. The applied sampling strategies depend on the respective compartment and biocoenosis. For instance, pelagic and benthic invertebrates are commonly sampled with nets and grabbers, respectively. The sampling scale and methodology depend on the size of the organisms. Most studies have focused on several aquatic species such as planktonic invertebrates,

mussels and fishes [57–59]. Evidence for MP ingestion in larger organisms is rare due to extensive laborious procedures and ethical issues. Here, stranded carcasses or faeces of larger organisms such as birds, cetaceans and seals are commonly examined for MPs [60–64]. Data for field-collected specimens point to variable levels of contamination (see, for example, compilation by O'Connor et al. [65]). Therefore, sampling of large numbers of especially invertebrates will improve the representativeness of the findings. This indicates that only species with high local densities are appropriate targets for investigation of MP contamination in biota. Considering the dynamic structures of biocenoses, temporal and spatial variations have to be acknowledged. On-site fixation of the collected specimens with preservative chemicals (e.g. ethanol) is recommended to prevent egestion of MPs during transport and storage. Prior to sample preparation (e.g. digestion of biota), specimens should be examined for attached MPs. Overall, sampling of biota complements data on MP abundances in abiotic compartments. Representative easily accessible key species should be chosen and investigated along with other monitoring activities.

Limitations

Given the diversity of the biotic environment, only a small fraction of species has been sampled and analysed for MP contamination. For instance, contamination of bacteria, algae, macrophytes, terrestrial plants and vertebrates especially with nanoplastics (NP) remains largely unknown. As MP ingestion depends on the one hand on the feeding type and size selectivity of biota and on the other hand on the bioavailability of MPs, results from biota reflect only selected (e.g. size, shape) and time-dependent (e.g. egestion) MP abundances in the environment. A direct relation of ingested microplastics to environmental MP contaminations in terms of numbers is difficult to impossible. Characterizing the MP uptake via laboratory studies may help to identify these limitations.

The usage of fixatives can lead to spontaneous evacuation of the gastrointestinal tract due to extreme stress. This should be considered and tested prior to sampling. In general, species-specific potentials to ingest MPs impede the analysis of pooled samples of different species. However, laboratory and environmental results are hard to compare as concentrations used during laboratory experiments with biota often exceed MP concentrations in the field by orders of magnitude.

2.3   Summary

- MPs are heterogeneously distributed in the environment. Particulate properties (e.g. polymer type, size) as well as anthropogenic (e.g. WWTPs) and environmental (e.g. weather) factors lead to spatial and temporal variations in MP abundances. Thus, replication and repeated measurements are needed for representative results.
- The diversity of sampling strategies hampers the comparability of data. For instance, applied devices (e.g. size range) and sampled volumes strongly affect

the results of monitoring studies. Documentation of these limitations is mandatory for cross-study comparisons.

- For water, combined approaches using nets for larger MPs and filter cascades for smaller MPs are recommended. Contamination (e.g. abrasion of sampling equipment) has to be considered and validated.
- For sediment and soils, corers are advisable for deeper profiles (stratified layers) and shovels for surface samples. Here, the volume of the sample should ensure representative MP analysis.
- For air, active and passive samplers might be used for monitoring studies. Monitoring airborne MPs is mandatory during sample preparation.
- For biota, applied sampling devices depend on the targeted species. Here, large numbers are usually needed to compensate for high variabilities.
- Overall, parallel sampling of aquatic, terrestrial and aerial compartments is beneficial for a better understanding of the environmental distribution and fate of MPs. Standardized and harmonized sampling strategies are needed for cross-study comparisons.

3 Overview and Comparison of Sample Preparation Techniques

3.1 Complexity of Matrices

Sample preparation is an important task in MP research. Whereas macroplastics may be easily spotted in the environment, detection of MPs requires an extensive processing of the native sample due to an unfavourable target-nontarget ratio.

The complexity of matrices varies between and within environmental compartments. For instance, concentration and composition of suspended solids differ among water bodies and among locations (e.g. groundwater, lake, stream and ocean). The same is true for the composition of sediments (e.g. marine beach, lakeshore), soils (organic- or mineral-rich), biota samples (biomass, content of protein, fat, carbohydrates, biological structures, etc.) or air. As particulate materials (e.g. minerals, organic matter) interfere with MP identification, these abovementioned variations imply different demands on sample preparation. Here the complexity of sample preparation increases with increasing proportions of particulate matrix matter. However, the first step towards representative counts is achieved by reducing the volume of the native sample. This is done by separating MPs from natural components based on material properties. Several methods have been proposed including sieving, density and oil separation, electroseparation as well as digestion of organic material. Depending on the investigated matrices, single or multiple combined techniques (e.g. sieving, density separation, digestion of organic material) are applied for sample preparation.

Limitations

Processing of native samples most often implies manipulations of the original conditions. For instance, MP sizes, polymer composition, hetero-aggregates, biofilms, MP degradation state as well as sorbed pollutants can be altered by sample preparation [66]. In addition, the diversity of methods limits the comparability between studies. Detailed information on each method is presented in the following section.

3.2   Size Fractionation

Size fractionation via sieving may allow for an easier processing of environmental samples in the following extraction and purification steps and is applied on aqueous and sediment samples.

Variable and wide particle sizes limit the detection of MPs via microscopy and spectroscopic analysis. For instance, multiple particle sizes require different magnifications, and large particles may overlay small particles. For an optimized analysis via microscopy and spectroscopic analysis, stacked sieves with different mesh sizes may be used to fractionate MP size classes. Here, wet or dry sieving is possible. Closed wet sieving units prevent formation of dust (e.g. particle loss) and contamination.

Limitations

Clogging of sieves may result in particle loss and unprecise fractionation. In addition, abrasion or fragmentation during sieving may alter numbers, sizes and shapes of MPs. During wet sieving with a small mesh size, small particles get washed away if the washing fluid is not filtered for a recovery of small fractions.

3.3   Density Separation

Plastics are lightweight materials and may be separated from natural components by utilizing differences in densities. This circumstance is broadly utilized for separating MPs from sediment or soil matrices. For instance, the density of commodity plastic polymers varies between <0.01 and >1.4 g/cm^3 (e.g. foamed PS and PVC), whereas mineral materials are typically denser (e.g. >2.5 g/cm^3 for quartz, feldspar and calcite) [4]. If environmental matrices are suspended in high-density liquids (>1.2 g/cm^3), MPs will float to the top, and minerals sink to the bottom, respectively. Depending on its density, selection of the separation fluid determinates the spectrum of recoverable polymers (Table 1). In addition, monitoring the density of separation fluids is imperative to ensure reproducibility. Density separation is conducted with several devices ranging from simple conical flasks and funnels to custom-built equipment (e.g. [67]). For instance, the Munich Plastic Sediment

Table 1 Density separation

Chemical	Sodium chloride (NaCl)	Zinc chloride (ZnCl)	Sodium iodide (NaI), sodium bromide (NaBr)	Sodium tungstate dihydrate (Na$_2$WO$_4$·2 H$_2$O)	Sodium polytungstate (Na$_6$H$_2$W$_{12}$O$_{40}$)	Potassium formate K (HCOO)
Density [g/cm^3]	Up to 1.2	Up to 1.8	Up to 1.8 (NaI), medium density 1.4 (NaBr)	Up to max. 3.25	Up to max. 3.1	Up to 1.6
Advantage	Low price, eco-friendly	Low price, high density	High density	Low price, high density	High density, non-toxic	Low price, not hazardous
Disadvantage	No recovery of dense polymers (PVC, PET)	Hazardous and corrosive	Expensive, reacts with cellulose filter, hinders visual analysis		Expensive	Hygroscopic
References	[28, 44]	[28, 69]	[68, 70, 71]	[44, 72]	[73, 74]	[75]

Separator allows processing of large sample volumes in a single run (max. 6 l sediment) [67]. Recovery rates of 95.5% and 100% for small and large MPs, respectively, as well as the closed construction are further benefits. After density separation, the supernatant is filtered (pressure or vacuum) and/or centrifuged and further processed. Large MPs in the filtered supernatant may be individually picked with tweezers while sorting under the stereomicroscope. Another possibility is fluidization. Nuelle et al. [68] decreased the mass of a sediment sample by inducing air-generated turbulent gas bubbles into a density solution and adding the sediment. Lighter particles were transported to the top and transferred to another glass vessel.

Limitations

Although differences in densities represent a promising and frequently applied method to separate plastic materials from natural components, several limitations have to be acknowledged. First, densities of organic matter are similar to those of plastic materials. Thus, organic matter as well as any other material with a similar density will float in dense liquids and will be included in the sample. Second, degradation of MPs and formation of biofilms and hetero-aggregates affect density of plastics. Furthermore, aggregation and disaggregation of particles during density separation might affect the fate of MPs (e.g. sedimentation of MPs attached to larger minerals). Third, method validation is mainly conducted with artificial sediments. Therefore, recovery rates are not directly applicable to complex environmental matrices. Fourth, comparability of results is hampered by the variety of methods (e.g. equipment used, sample volume and separation liquid).

3.4 Alternative MP Extraction Approaches

Centrifugal forces may also be used for separating MPs from other materials. Bauer et al. [76] published a paper on sink-float density separation of polyolefins from waste. Two-stage processing with a centrifugal force separator and a hydro jig for the first separation of larger waste has been conducted. Another possibility for separating MPs from environmental samples is the use of oil extraction due to the oleophilic characteristics of plastics. Crichton et al. [77] mixed a dry sample with water and canola oil and swirled several times so that each particle got into contact with oil. The sample settled; oil was decanted, rinsed and filtered; and the filter incubated in reagent alcohol in order to remove all the remaining oil on MPs. A recovery rate of >90% was achieved for expanded polystyrene (EPS), polyvinyl chloride (PVC), acrylonitrile butadiene styrene (ABS), vinyl, polyamide (PA) fibres and polyester fibres (PES). This method is cost-effective and easy to handle. Whereas sediments and particulate matter are conductive, plastics are nonconductive materials [78]. Thus, electroseparation may be used for separation of sediments and MPs. The method is based on electrostatic behaviour of particles [79]. Dry particles are charged with a corona electrode up to 30 kV and separated into conductive and nonconductive particles. In beach sediments, the recovery rate of pristine

microplastic particles >63 µm is close to 100%. This method allows processing of high sample volumes and reduces the remaining matrix to 10%. Due to the reduction of material, only small amounts of chemicals are needed. Moreover, it is a relatively cheap method as only the device is needed and no chemicals are required.

Limitations

The application of centrifugation, oil separation and electroseparation remains to be tested and validated for the separation of a diversity of polymers, size classes and shapes. In addition, further research is necessary in order to verify recovery rates for different matrices and environmental MPs (e.g. hetero-aggregates). For centrifugation, one can envision that this method will include expensive apparatuses and high maintenance expenditures as the rotating parts may be subjected to significant force and sensitivity towards the efficiency of the approach when applied to samples from different environments. For oil separation, high concentrations of organic matter may reduce the overall efficiency. Separating sediment components based on their conductivity does only work for completely dry samples. Thus, particle loss, especially in the smaller-size fractions due to the formation of dust, is possible and has to be considered for mitigation strategies.

3.5   Sample Purification

Synthetic polymers are relatively resistant against selected acids and bases. This is beneficial for MP purification. In theory, incubating environmental samples with acids and/or bases result in digestion of organic matter and, thus, in reduction of the volume. However, whether MPs remain unaffected depends on the applied chemicals and the polymer types (Table 2). For instance, polyesters like PET, PBT, PC, PLA and other synthetic polymers such as cellulose acetate (CA) and PVC might be degraded with 10 M NaOH [80, 81]. Application of HCl results in high digestion efficiency ($>95\%$) albeit with melting of synthetic polymers like PET [82]. Digestion with H_2O_2 and Fenton's reagent has been often used with promising efficiencies. However, a study revealed that only 70% of MPs were recovered after incubation with 30% H_2O_2 for 1 week [83]. In this regard, validation of the digestive protocols is mandatory. Enzymatic digestion with single or sequential incubation of enzymes is another gentle possibility to eliminate organic materials without affecting synthetic polymers [58, 84]. Depending on the study and validated workflows, samples are either treated before or after density separation. In general, the complexity of the sample (water, sediment, biota or air) affects duration and efficiency of digestion.

Limitations

Strong acids and alkaline solutions adversely affect synthetic polymers. Here, the degree of degradation and discoloration is related to the used chemicals and is polymer-specific (Table 2). Acid digestion with chemicals like HCl and HNO_3 is not recommended as synthetic polymers like nylon and PET have low resistance to

Table 2 Organic digestion

Digestion	Alkaline	Acids	Oxidizing	Enzymes
Chemicals	Sodium hydroxide (NaOH), potassium hydroxide (KOH)	Nitric acid (HNO_3) or hydrochloric acid (HCl)	Hydrogen peroxide (H_2O_2)	Cellulase (>30 U/ml), lipase (>15,000 U/ml), chitinase (>40 U/ml), protease (1,100 U/ml)
Advantage	Most organics destroyed, KOH: Most polymers resistant	HNO_3: most organics destroyed	Most organics destroyed	Most organics destroyed, not hazardous
Disadvantage	Some polymers degraded (e.g. PC, CA, PET, PVC)	HNO_3: dissolution of PS and PE possible, HCl Incomplete destruction of organics	Polymers might be affected	Time-consuming, partly expensive, different Enzymes for different samples
References	[5, 58, 85]	[5, 83]	[28, 83, 86]	[5, 15, 80, 84, 87]

these acids. Alkaline digestion may also damage or discolour plastic fragments [10]. However, in order to effectively remove organic matter, certain compromises have to be made. This includes concentration of chemicals, incubation temperature and time. Although enzymatic digestion does not dissolve and degrade synthetic polymers and is very effective in the purification result, the proposed protocols are time-intensive [80, 84]. For instance, several sequentially applied enzymes (e.g. lipase, chitinase, lignase and proteinase) are needed to effectively digest biological structures (e.g. lipids, chitin carapaces, lignin, carbohydrates and proteins). In addition, exposure conditions have to be monitored and modified for optimal reaction rates.

3.6   Summary

- Varying complexities require matrix-specific and standardized procedures for water, sediment, soil, air and biota samples. Here, single or multiple combined techniques may be used.
- Sample preparation increases probability of contamination, particle loss and fragmentation. Thus, an extensive validation of the entire process is essential to prevent underestimations and overestimations of MP abundances.
- Size fractionation and density separation are commonly applied procedures to narrow the sample volume and to separate MPs from natural components, respectively. Alternative methods have been published for separation, but have not been tested in detail and are therefore not advisable at the moment.

- If organics prevail, samples must be purified (with alkaline or oxidizing solutions, acids or enzymes). All have advantages and disadvantages, and treatment with strong chemicals might affect polymers.

4 Loss of Analyte and Contamination

4.1 Sample Loss Due to Adhesion and Static Forces

Synthetic materials are characterized by their surface charge [88]. Plastic polymers are often easily charged statically. The cleaner the surface of MP particles from environmental samples, the more the particles are prone to static charge. Due to static forces, especially when being dried or when samples are processed, MP particles may be lost due to these forces when particles interact with one another or with surfaces such as glass used during laboratory work. High hydrophobicity of synthetic polymers such as nylon, polyester, PVC and PE, for example, results in the accumulation of positive or negative charges depending on the material. This suggests that MPs constituted of such synthetic polymers are at a greater risk of being lost due to the influence of static interactions [79].

4.2 Blanks

Airborne fibres and smaller MP particles represent the largest part of contamination. In order to receive representative results, it is imperative to produce blanks to trace the contamination with plastics during the whole process (from sampling to analysis).

Laboratory protocols include organic digestion, density separation and possible counting/photographing MP particles with a microscope. During all steps conducted in the laboratory, a blank sample with distilled water is treated with the same chemicals/solutions as used for the processing of the real samples. In the standardized protocol for monitoring MPs in sediments [28], one blank per day of laboratory work is suggested. Furthermore, the mean value of at least three blank samples allows for the more assured correction of the real samples.

Blanks collected on substrates (e.g. filters) must be analysed by means of FTIR, Raman spectroscopy, mass spectrometry, etc. commonly used for the detection and characterization of the synthetic polymer constituting MP particles in samples. The average amount of synthetic polymer found in blanks may be subtracted from the real sample values. To be conservative, mean values of the found plastic particles should be round up to the next integer (e.g. 2.3–3) prior to correction of the particle numbers in real samples.

4.3 Mitigation Strategies Against Contamination

In order to avoid MP contamination, an accurate clean workspace is absolutely necessary. Especially, the workspace in the laboratory may lead to contaminations due to several handling steps of samples. It should be cleaned before starting to work. The workspace could be cleaned with 70% ethanol and paper tissues; all apparatuses can also be washed with 100% ethanol. It is also of importance to filter all working solutions such as ethanol, hydrogen peroxide, acids, bases, enzymes, etc. prior to use. During the entire sample processing steps, the use of plastic material should be avoided and replaced by steel or glass devices [89]. Moreover, cotton cloths are recommended while handling samples [44]. Samples must be covered with aluminium foils or materials made from glass in order to avoid contamination from air. Furthermore, samples should be handled and processed under controlled air circulation; the use of a laminar flow box or clean bench is suggested [10].

5 Analytics

5.1 Visual Identification

Many researchers have used visual identification with microscopes for identifying MP particles in earlier studies. Most often, visual, light or digital microscopes are used for the analysis of larger plastic particles with a size of 300 μm to 5 mm (e.g. [90–93]). Smaller particles are not so easily recognizable as plastics; hence, they are first analysed under a stereomicroscope, and synthetic polymers are later identified by means of Raman, pyrolysis GC-MS or FTIR spectroscopy [94, 95]. Aqueous samples can be sorted with the help of sorting chambers like Bogorov counting chambers. Size limits for visual inspection without assistance from other characterization techniques are recommended to be >500 μm [87] or even >1 mm [4]. Norén [96] recommends a standard criterion for visual identification of particles to reduce chances of misidentification, such as absence of organic origin structures on MPs, equal thickness in MP fibres, homogenous coloration of the particle and finally application of fluorescence microscopy to exclude structures of biological origin. Advantages of visual identification are that relatively quick first quantification is possible and that each identified particle larger than 500 μm may be described, photographed and measured [15].

Limitations
Larger particles may be identified by their shape, size, degradation stage and colour as possible plastic fragments [4], but a 100% certainty of identification is not given. Smaller particles, especially <300 μm, are not very well identifiable as these fragments may also be of mineral or organic origin [4, 97]. Handling of particles below a certain size using instruments such as forceps can become unmanageable because of their minute size [96]. Moreover, the results of visual identification are

subjective, depend on the sample matrix and are very time-consuming [15]. There-fore, visual identification should only be used as preliminary evaluation of results coupled with another method for a higher accuracy and precision [98]. The approach by itself should not be applied to MP particles <500 µm as the probability of misidentification is very high. It is instead highly recommended to use assistance from spectroscopic approaches for the accurate identification of such MPs [99].

5.2 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy is a vibrational spectroscopy approach which has been used for decades for the analysis and characterization of synthetic organic polymers and their products [15]. This technique involves excitation and detection of molecular vibra-tions of a sample, which leads to the acquisition of a characteristic spectral finger-print. FTIR spectroscopy is an absorption technique: the IR radiation absorption by molecule vibration depends on the change of the dipole moment of a chemical bond within a molecule constituting the sample. In this approach, the sample is irradiated with IR light (mid-IR range), a part of the IR radiation is absorbed by the exited molecule vibrations within the sample being probed and detected either by reflection or transmission mode.

Synthetic polymers with their ever-repeating molecule composition possess highly specific IR spectra with distinct signals that make it an ideal technique for the identification of MPs. They can be characterized based on their chemical structure and can be identified by comparing them to reference spectra. As a measuring variation, the surface technique attenuated total reflectance (ATR) FTIR spectroscopy allows for the fast and reliable single analysis of large MP particles >500 µm [15]; however, reliable results need a relatively clean surface, and potential MP particles with biofilm need to be cleaned by, for example, wiping with alcohol.

Micro-FTIR spectroscopy, the combination of FTIR spectroscopy with an IR microscope, facilitates the analysis of particles down to a size of 10 µm. Measure-ments in transmission mode require the use of IR transparent filters (e.g. aluminium oxide or silica) on which the sample is placed. The thickness of the sample that can be analysed with this mode is limited due to the total absorption of IR radiation through samples above certain thickness. Micro-FTIR mapping with a single ele-ment detector and focal plane array (FPA)-based FTIR imaging has been extensively applied for the identification of MPs [100]. The former is very time-consuming, whereas the latter allows for the acquisition of several thousand spectra within a short time frame and at a high spatial resolution. This helps in the sequential imaging of the whole sample filters and is currently the predominant method for the identi-fication and characterization of MPs. The use of reflectance mode in FTIR is also possible. However, this mode results in the generation of complex spectra difficult to interpret as particles with irregular shape lead to refractive error [12, 15, 101].

Overall, the advantage of FTIR analysis is that qualitative and quantitative information about each MP particle can be obtained and the sample is not destroyed

and is still present for further downstream analysis. The analysis requires relatively short measurement times, and large sample areas of up to 10 × 10 mm can be efficiently measured within less than 2.5 h. This procedure circumvents extrapolation of measurement results from sample filter subareas and potential uncertainties related to unequal particle distribution on the filter. However, measurement of whole filters results in large datasets with up to more than one million IR spectra which are best analysed via automated approaches [102, 103].

Limitations

Information about the mass of MPs is not accessible with this method. One of the major drawbacks of this technique is that very small particles <10 μm cannot be analysed due to the diffraction limit of light. Especially analysis of fibres having a diameter in that size range is very difficult. Furthermore, there are reports showing significant underestimation of MP particles <20 μm [12]. Thickness of the particles >50–100 μm leads to total absorption as well as black particles which absorb strongly in the IR range. This may lead to difficulty in data analysis and underestimation of MPs if such particles are present. Samples must also be thoroughly dried as water is a strong absorber of IR radiation. There are other limitations which are common for the complementary FTIR and Raman spectroscopy techniques. These will be discussed together in the section after Raman spectroscopy below.

5.3 *Raman Spectroscopy*

Raman spectroscopy is like FTIR a vibrational spectroscopy technique. It is a surface technique involving inelastic scattering of light to unveil molecular composition of a sample by probing vibrational and rotational frequencies of molecules. A monochromatic light source such as a laser is used to interact with the sample. Most commonly used lasers have wavelengths ranging from 500 to 1,064 nm. Radiation from this source interacts with the sample and one in a billion photons from the source is inelastically scattered revealing information about molecular vibrations of the sample. The differences in the frequency of inelastically scattered photons in comparison to the Rayleigh photons (photons which do not interact with the sample) are known as the Raman shift which forms the basis of the Raman spectrum [104, 105]. Like FTIR spectroscopy, this approach also provides a unique spectral fingerprint of different chemical structures, but the basic difference is that Raman spectroscopy depends on changes in the polarizability of the chemical bond within molecules constituting a sample. Hence, the two approaches are complementary; signals which are strong in IR may be weaker in Raman spectra and vice versa. The Raman spectrum of a synthetic polymer has several unique sharp signals corresponding to the chemical functional groups constituting the sample and can be identified by comparing them to reference spectra. Currently, micro-Raman spectroscopy approach (Raman spectrometer setup coupled with a microscope) is predominantly used. A confocal mode of configuration ensures a lateral and depth

resolution that allows the analysis of particles up to ∼0.5–1 μm depending on the type of sample being analysed. Thus, with respect to size, a higher percentage of MPs can be detected in comparison to FTIR spectroscopy. Raman spectroscopic imaging mode allows qualitative and quantitative estimation of small size-range MPs albeit with time duration being the principal constraint as Raman images are acquired by stepwise point measurements, the so-called mapping. Raman spectroscopy also allows the chemical analysis and localization of specific components like MPs within complex matrices such as biological cells and tissues. Additionally, it is a significant approach to monitor the alterations in the biochemistry of organisms when stressors like MPs are ingested. Micro-Raman spectroscopy has been applied worldwide for the analysis of samples ranging from single microbial cells to the analysis of MPs in recent years [106, 107]. This approach facilitates detection of even the smallest MP particles in environmental samples, but time-effective integration for MP research is yet to be demonstrated [36, 95].

Limitations

One fundamental drawback of the Raman technique is that it is a very weak phenomenon, which leads to long measurement times with Raman images of whole filters requiring 24–48 h or even longer depending on the resolution and sampling area. Another major drawback is that the Raman signal can be severely affected by the presence of coloured and noncoloured organic or inorganic debris or contaminants which result in fluorescence that swamps the entire Raman spectrum. Hence, environmental samples must be processed thoroughly to have least possible impurities on the samples for a clear identification of MPs from the environment. Lasers with longer wavelengths such as 1,064 nm can also be applied to overcome fluorescence, but the Raman intensities are drastically reduced with the use of longer wavelength excitation sources due to loss in energy. Hence, the focus of the future for the realization of a time-effective and fluorescence suppression-based micro-Raman spectroscopic analysis of MPs depends on testing of the alternate approaches such as the wide-field imaging method and the non-linear Raman spectroscopic approaches like coherent anti-Stokes Raman scattering (CARS), stimulated Raman scattering (SRS), time-gated Raman spectroscopy (TGRS) and shifted excitation Raman difference spectroscopy (SERDS), respectively [108–112].

Limitations Prevalent in FTIR and Raman Spectroscopy

FTIR and Raman spectroscopic MP analysis utilize spectral libraries comprising of data acquired from a large collection of mostly pristine synthetic polymers for comparison to carry out identification of MPs in samples being investigated. But a critical issue is that the spectra of environmental MPs tend to be sometimes different from those included in the spectral library which hinders MP identification rates. FTIR and Raman spectra of environmental MPs are influenced by factors such as the presence of additives, plasticizers, colouring agents, residence times in different environmental conditions, etc. [107]. Hence, it is important to include spectra of environmental MPs in the libraries to improve the MP identification efficiency, and the process of establishing such a comprehensive spectral library inclusive of all this data in laboratories worldwide can be highly time-consuming. Thereby, establishing

a comprehensive open-source spectral library which includes FTIR and Raman spectroscopic data acquired from a diverse range of synthetic polymers; biodegradable polymers including those which contain additives, colourants and plasticizers; and aged polymers obtained from exposure to different environmental conditions is necessary. These libraries should also include the parameters used for spectral acquisition. Generation of such an open-source spectral library may be of great help to research groups to avoid creation of repetitive databases and encourage more MP identification studies worldwide especially in groups with limited access to reference polymers [107]. Agglomeration of MPs on filters and substrates used in these approaches is another critical issue which may be overcome by using small aliquots of samples for analysis. The estimated MP quantity from such small aliquots can then be extrapolated to the total investigated sample volume [113]. However, it is important to note that more concrete studies are required to investigate the feasibility of this approach to achieve an accurate estimation of MPs [114]. Furthermore, identification of aged MPs using these techniques is a challenging task as long residence times in the environment expose these particles to factors like sunlight which may alter the chemistry of the polymers. This results in changes in the spectrum of the MPs potentially hampering their identification.

5.4 Pyrolysis Gas Chromatography Mass Spectrometry (Pyr-GC-MS)

In contrast to FTIR and Raman spectroscopy, pyrolysis GC-MS (Pyr-GC-MS) allows quantification of masses of MPs in environmental matrices. This analytical approach involves thermal degradation of large molecules into smaller ones in an inert atmosphere to assess the chemical composition of samples like MPs. Tuning of the pyrolysis temperature leads to the generation of unique volatile degradation products which are similar or can be traced to their precursor synthetic polymers [12]. These pyrolysis products can be separated on a gas chromatography (GC) column and mass spectrometry (MS) measurements then make identification of these products possible. Pyrolysis fingerprint or pyrograms facilitate the identification and characterization of the synthetic polymer constituting the MPs. These pyrograms can be compared with reference pyrograms of known virgin synthetic polymer samples. For synthetic polymers with polar subunits like polyesters, thermochemolysis – which is thermally assisted methylation and hydrolysis – is applied. This procedure is known to improve chromatographic separation, increase sensitivity and give additional structural information. Plastic additives can also be simultaneously determined during Pyr-GC-MS analysis if a thermal desorption step is used before pyrolysis. Pyr-GC-MS has been previously applied for the analysis of MPs from environmental samples like marine sediments, river sediments, sea water surface and marine organisms [12, 115, 116]. Fischer and Scholz-Böttcher [117] recently showed the concentration of a whole environmental MP sample on filters

after purification and the subsequent Pyr-GC-MS analysis in one run. This approach is promising as it was carried out without the need for optical or mechanical presorting [118]. Furthermore, mass-related quantification was obtained at trace levels using calibration procedures. Dierkes et al. [119] showed that the mass of the most common polymer types PE, PP and PS might be measured with limits of quantification down to 0.007 mg/g sample.

Limitations

However, there are certain limitations for quantification of MPs using Pyr-GC-MS. Firstly, the analysis is destructive as samples are pyrolized. Secondly, quantification is mass-based meaning that no further information about particle number or shape can be obtained. Analysis of copolymers would result in pyrolysis products of each comonomer instead of a specific pyrolysis product for copolymers. Furthermore, additives, fillers and dyes contribute to the mass of MP particles but are not specifically included in mass-based quantification.

In fact, identification of single MP particles via specific pyrolysis products is relatively fast and simple. However, mass-based quantification of MPs in environmental samples, hence, a large variety of MP particles, is far more complex. Specific pyrolysis products for common synthetic polymers are well described in the literature [120, 121]. Nonetheless, abundance of organic substances such as natural polymers (cellulose, keratin, chitin, lignin) and hydrophobic compounds (fats and waxes) results in similar or identical pyrolysis products possibly leading to an overestimation of MP concentration. For instance, polystyrene (PS) is widely evaluated via its monomer styrene which, however, also results from the pyrolysis of, for instance, lignin. Besides, styrene is also a part of other synthetic polymers such as acrylonitrile butadiene styrene (ABS) or styrene-butadiene rubber (SBR) which results in a cumulative mass concentration based on styrene concentration rather than a polymer-specific concentration. An overestimation due to the presence of natural polymers can in fact be overcome by using the trimer instead of the monomer; however, differentiation of its origin, either from PS, ABS or SBR, is not possible.

5.5 Thermal Extraction and Desorption-Gas Chromatography Mass Spectrometry (TED-GC-MS)

TED-GC-MS involves a combination of thermal extraction of samples with thermogravimetric analysis (TGA) on solid-phase adsorbers and subsequent analysis of the adsorbers with thermal desorption-gas chromatography mass spectrometry (TDS-GC-MS) [121, 122]. This combination is known as TED-GC-MS. In this approach, the entire sample is pyrolysed in the TGA at temperatures of up to 1,000°C. The synthetic polymer-specific degradation products, which have to be different from the degradation products of the environmental matrix, are adsorbed on a solid-phase adsorber. Decomposition of most synthetic polymers begins at 350°C,

therefore enabling the possibility to eliminate the components of the environmental matrix, which decompose at much lower temperatures and are not trapped on a solid-phase adsorber. These relevant degradation products of plastics are trapped out on a solid-phase adsorber such as polydimethylsiloxane (PDMS). The trapped decomposition products are desorbed by thermal desorption and transferred by an inert gas such as helium into a cooled injection system. Furthermore, only compounds that can be thermally desorbed at temperatures 200–300°C are evaporated for analysis with GC-MS. This acts as a filter as it prevents most long-chain polymer-specific contamination products from reaching the GC-MS [123]. Analysis of these products by GC-MS makes identification and quantification of samples like MPs from different environments possible. Synthetic polymer identity is confirmed based on the occurrence of mass fragments representative of different decomposition products specific to that polymer. Thus, TED-GC-MS can act as a fast tool for identification and quantification of MPs in environmental samples. However, up to date, the range of plastic types that were confirmed to be analysed by TED-GC-MS in environmental samples is very limited (e.g. PE, PP, PS, PA 6 and PET, [121]).

Limitations
Large-scale testing and implementation of this approach for the analysis of MPs from different environmental samples is yet to be realized. However, samples such as in the Pyr-GC-MS approach are not available for downstream analysis due to destruction. It also allows analysis of only 20 mg of dry sample during one TED-GC-MS run; thus, several sequential runs will be needed to analyse many samples. Nevertheless, it is suggested that up to 100 mg of a sample can be analysed in a time span of 2–3 h. MP particle size, shape, colour and related information are not relevant in this approach due to complete homogenization of the sample. First studies have shown a detection limit of around 0.5 up to 1 wt% in a 20 mg sample which seems to be relatively high. Furthermore, calibration for an exhaustive collection of relevant synthetic polymers is yet to be realized and proven.

5.6 Scanning Electron Microscopy Coupled with Energy Dispersive X-Ray Spectroscopy (SEM-EDX)

Scanning electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM-EDX) can be used to obtain high-resolution data of the morphology and qualitative information about the chemical composition of samples such as MPs. SEM provides high-magnification, high-resolution images of the surface of the investigated samples [124]. SEM microscopes apply various detectors which generate images based on emission and detection of secondary electrons. Furthermore, the backscattered electron detector provides information on topography and material contrast based on atomic number (Z). SEM can be coupled to EDX where element-specific radiation is used for chemical characterization of the surface near volume. This approach provides elemental analysis on areas as small as nanometres in

diameter. The electron beam incident on the sample produces X-rays that are characteristic of the elements found on the sample. The intensities yield quantitative information on the element composition and distribution. Hence, the approach can be used to obtain detailed information on the elemental composition of MPs, with information also on the inorganic additives present in the sample. Furthermore, intense signals from some elements such as nitrogen can be attributed to the presence of substances such as biomass on MPs. In general, SEM-EDX is a promising approach to obtain high-quality images of MPs in comparison to optical microscopy and to deduce their elemental composition subsequently. This information can help in the analysis of synthetic polymer-based materials and nonplastic materials. It can be used as a complementary technique to fluorescence microscopy for tracking MP localization in biota.

Limitations

SEM-EDX is not suitable for discrimination of different plastic polymers in environmental samples as EDX information is only based on elemental composition. In addition, it is a very expensive technique which requires tremendous time, effort and expertise for sample preparation and analysis. Moreover, samples must be sputtered with conducting materials such as Au, AuPd or carbon on nonconducting MP samples. Thereby, samples cannot be used for downstream analysis. However, working with low kV beam energy such as 0.5–1.5 kV may negate the need for sputtering of samples. This may allow speeding up of sample preparation and making the analysis much easier to perform.

5.7 Quantitative Nuclear Magnetic Resonance Spectroscopy (^{1}H-qNMR)

Quantitative nuclear magnetic resonance (^{1}H-qNMR) is a simple, rapid and nondestructive technique that allows simultaneous characterization and quantification of a multitude of molecules. It has been extensively used in various fields of research and development such as polymers, metabolites, pharmaceuticals, forensic sciences, environmental applications, etc. [125]. ^{1}H-qNMR provides access to a wealth of in-depth information about the structure and dynamics of complex molecules. This approach probes changes in the magnetic field experienced by the nuclei of each atom in a molecule when exposed to an external magnetic field. Nuclei with an odd mass or atomic number have a nuclear spin, this includes ^{1}H and ^{13}C, and the spins of their nuclei are sufficiently different to be probed by NMR measurements. Modern instruments are sensitive to local magnetic variations as small as one part in a billion, from which information about the molecular structure can be determined, and even the complex molecules like proteins can be effectively analysed with high accuracy [125]. Information from ^{1}H-qNMR is based on the proportional relationship of integrated signal area and number of resonant nuclei. Different types of determination methods are possible such as relative determination, absolute

determination by use of internal and external standards, standard addition and calibration curve method. ^{1}H-qNMR can be applied for the size-independent qualitative and quantitative characterization and determination of MP particles from different matrices. Preliminary studies used for the analysis of MPs such as PE, PET and PS have shown that the calibration curve method is best suited for MP analysis, since the exact composition of all analytes other than polymers in an environmental sample need not be determined and this method is a preferred choice [125]. Therefore, ^{1}H-qNMR is a precise quantification method which can be applied to achieve a quantification accuracy of >98%. It is an effective and fast approach (about 1 min per sample measurement). The calibration curve method allows for a high-throughput analysis.

Limitations

There is the possibility of significant issues arising from environmental components such as microorganisms and other bioorganic components. The errors arising from these components are signal overlays that are not correctable, and systematic errors are complicated to be detected. Thereby, an effective sample preparation step is necessary to remove all these components before analysis. A major drawback of this approach is the need for dissolution of analytes in a suitable deuterated solvent leading to a loss of size information of the MP particles. Thereby, it is of utmost importance that a suitable solvent is identified for the dissolution of different types of synthetic polymers, and this is challenging due to the inherent chemical and physical properties of polymers in comparison to other organic substances. Furthermore, the solvent signal may overlay or coincide within the signal range of the respective synthetic polymers which can make the analysis highly complicated. ^{1}H-qNMR is a promising approach for the size-independent analysis of MPs; nevertheless, several parameters remain to be optimized in the context of analysis of MPs and are in a very preliminary stage of being applied for this purpose.

6   Conclusion

The chapter summarizes the currently applied methods of sampling, preparation and analysis of environmental MPs. Furthermore, advantages, disadvantages and limitations of these methods are highlighted and discussed in detail.

In general, MPs are heterogeneously distributed in the environment. Thus, replicate samples are needed for valid and reliable data on location and time-dependent MP abundances. Sampling should (ideally) generate data for modelling studies to understand the sources and fate of environmental MPs. The selection of sampling equipment must be given critical importance to cover a broad size range of MPs and to ensure an adequate sample volume for representative counts. Likewise, a stringently regulated, ethically complied and harmonized protocol must be implemented for the sampling of biota.

For sample preparation, the diversity of environmental matrices requires the use of adaptable and combinable processing techniques. Here, validation and recovery experiments as well as laboratory controls are mandatory to prevent underestimations or overestimations of MP abundances (e.g., particle loss, fragmentation, contamination). As the applied methods affect the detectable range of MPs (e.g. polymer type, size), only a thorough documentation allows for cross-study comparisons.

Identification of the plethora of environmental MPs is challenging as it encompasses diverse sizes, shapes and colours of aged polymers. Considering all these parameters, it is not possible to recommend a specific analytical method as the most suitable and efficient for MP identification and quantification. Moreover, every method has its unique advantages and disadvantages. For instance, visual identification is biased, and vibrational spectroscopy techniques such as FTIR and Raman spectroscopy strongly depend on the quality of the sample and the available references (e.g., spectra). Thus, a combination of several analytical approaches may be favourable in order to capture and characterize the diversity of environmental MPs.

Overall, there is currently a lack of harmonized and validated sampling and analytical methodologies due to non-standardized research associated with the analysis of MPs. Furthermore, studies report microplastic concentrations related to different units, size classes, synthetic polymer types, forms and shapes. This hampers comparability between studies from laboratories across the world. Thereby, it is of importance to harmonize the methodologies and to report critical parameters and limitations (e.g. mesh size, sample volume, MP size distribution). Standardization of the methodologies can eventually lead to addressing of key issues such as environmental risk assessment of MPs.

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