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Critical Assessment of Analytical Methods for the Harmonized and Cost-Efficient Analysis of Microplastics

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Abstract

Microplastics are of major concerns for society and is currently in the focus of legislators and administrations. A small number of measures to reduce or remove primary sources of microplastics to the environment are currently coming into effect. At the moment, they have not yet tackled important topics such as food safety. However, recent developments such as the 2018 bill in California are requesting the analysis of microplastics in drinking water by standardized operational protocols. Administrations and analytical labs are facing an emerging field of methods for sampling, extraction, and analysis of microplastics, which complicate the establishment of standardized operational protocols. In this review, the state of the currently applied identification and quantification tools for microplastics are evaluated providing a harmonized guideline for future standardized operational protocols to cover these types of bills. The main focus is on the naked eye detection, general optical microscopy, the application of dye staining, flow cytometry, Fourier transform infrared spectroscopy (FT-Ir) and microscopy, Raman spectroscopy and microscopy, thermal degradation by pyrolysis–gas chromatography–mass spectrometry (py-GC-MS) as well as thermo-extraction and desorption gas chromatography–mass spectrometry (TED-GC-MS). Additional techniques are highlighted as well as the combined application of the analytical techniques suggested. An outlook is given on the emerging aspect of nanoplastic analysis. In all cases, the methods were screened for limitations, field work abilities and, if possible, estimated costs and summarized into a recommendation for a workflow covering the demands of society, legislation, and administration in cost efficient but still detailed manner.

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Introduction

The ubiquitous pollution of the environment with small plastic particles (<5 mm) called microplastics (MP)[1–4] has raised concerns within societies and government. In the near future, detection and quantification need to be addressed, for example in drinking water in California, in the United States of America[5,6] until 1 July 2021. Such bills require fast and reliable count and polymer type assessments with short processing times. Currently, the analytical procedures available are still being developed and refined (see Lusher et al.[7] and Brander et al.,[8] in this special issue).

In the scope of these efforts, analytical identification methods are examined regarding their potential to fulfill the demands for legislation and analytical labs in a harmonized manner. This review critically assesses optical identification methods (naked eye identification, visual microscopy, fluorescence microscopy, and flow cytometry) and chemical identification/quantification methods (Fourier transform Infrared (FT-IR) and Raman spectroscopy, as well as pyrolysis–gas chromatography–mass spectrometry (py-GC-MS) and thermo-extraction and desorption gas chromatography–mass spectrometry (TED-GC-MS)) together with selected chosen further techniques. A guideline to provide the necessary harmonization in the given time frame is determined. This includes methods ranging from low cost towards higher analytical demands to measure MP in an effective way by field laboratories and governmental institutions while maximizing information for risk assessment.

In the following sections, each technique is shortly described and screened by experts of the field with focus on the following questions: limit of detection (LOD; particle size or mass), approximate costs, expenditure of time, and fieldwork capability.

Optical Identification Methods

Naked Eye

Naked eye identification for MP is one of the easiest and cheapest techniques to collect data on plastic pollution. Here, samples are collected from an environmental system and may be sieved or separated by density first. Then plastics are manually selected from the sample by eye and quantified. However, naked eye identification may not be appropriate for all scientific questions.

Important considerations for sample collection include the following: (i) Samples from the environment need to be gathered in a way that maximizes the amount of visible plastic in them (Fig. 1b). (ii) Large particles are in low abundance, so samples should be highly aggregated (in streams on the order of multiple cubic meters of water (Fig. 1a). (iii) The range of particle sizes that can be accurately quantified using this technique is determined by the size range between the aperture and mesh size (Fig. 1c).

To apply the naked eye sorting technique, the sample needs to be preprocessed, sorted, and quantified. The sample does not necessarily need an extraction step by matrix digestion or density separation, though in the case of very large samples they are recommended.[9,10] A team can usually sort any visible plastics from the matrix by hand and it is advised to use forceps to remove the smallest sizes of plastics.[10] To sort the sample, three containers are needed: one container for the field sample, another container for the nonplastic materials that have been thoroughly inspected, and the third container for the found plastic materials.

Removing the nonplastic materials from the field samples eases the sorting and helps to reduce the size of the combined matrix. Sediment samples can be shaken to rise plastics to the surface. After the particles are extracted, they can be weighed,[11] photographed,[10] measured for their dimensions, and assessed for their shape, color, and texture characteristics (covered in depth in Cowger et al.,[12] within this special issue). The naked eye sorting technique can also be easily deployed in the field, using a field scale and/or photogrammetry. Yet the accuracy of field scales often does not allow precise measurement for small MP, which can weigh fractions of grams or less. Harmoniously, an image of the particles can be taken in presence of a measuring scale and the particle dimensions can be measured using an image processing software to quantify the particle projected surface area.[13] In total, the necessary equipment for field sampling and lab sorting costs around US$200 for buckets, camera, and sieves while a scale (∼US $500) will be the most expensive part.

Limits of Detection. The minimum particle size observed in the presented preliminary study was 1 mm. However, the study can only accurately determine particles larger than the mesh size of the net (5 mm). This is apparent in the decrease in particle counts observed below 5 mm (Fig. 1c). A 1 mm threshold for naked eye was proposed by Zhang et al.[14] and we agree with this limit as long as the mesh size is less than or equal to 1 mm. Similarly, the maximum particle size captured by the Gray Lab was constrained by the net aperture (500 mm) (Fig. 1c). Whenever a mesh and aperture are applied to sort or collect a sample, it should be made clear that only particles larger than the mesh size and smaller than the aperture are targeted (Figs. 1a and 1c).

Limitations. For assessing risk of larger plastics, which have been found to harm sea turtles and whales,[15] this method may be better than traditional microplastic survey methods. However, for application in laboratories or risk assessment like food safety, this technique reaches its limits quite quickly as it only can determine particles to a minimum of 1 mm. The naked eye method should not be used without chemical identification for the smallest size class (1–2 mm) because there are large error rates for visual identification of particles smaller than ∼2 mm in size but lower error rates above ∼2 mm.[16]
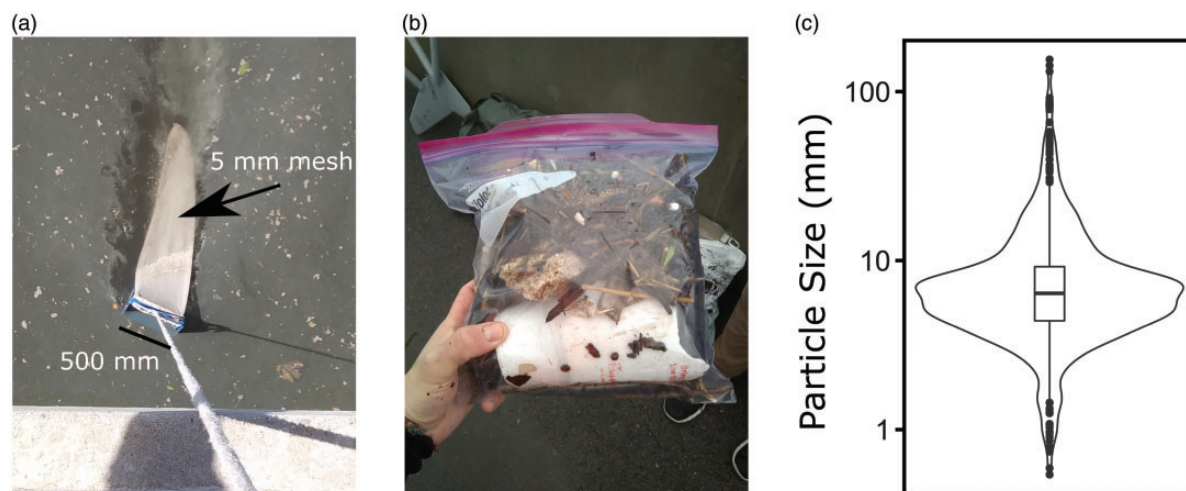
Figure 1. (a) This sample was collected using a 5 mm mesh with a 500 mm aperture net lowered from a bridge in Southern California. (b) Given the abundance of visible plastic, this sample is a good candidate for naked eye identification. (c) Violin plot (kernel density function) with centered boxplot of particle size observed by the Gray Lab during the full sampling season using this technique. Particle size is a normalized length computed using the square root of the particle's projected surface area.
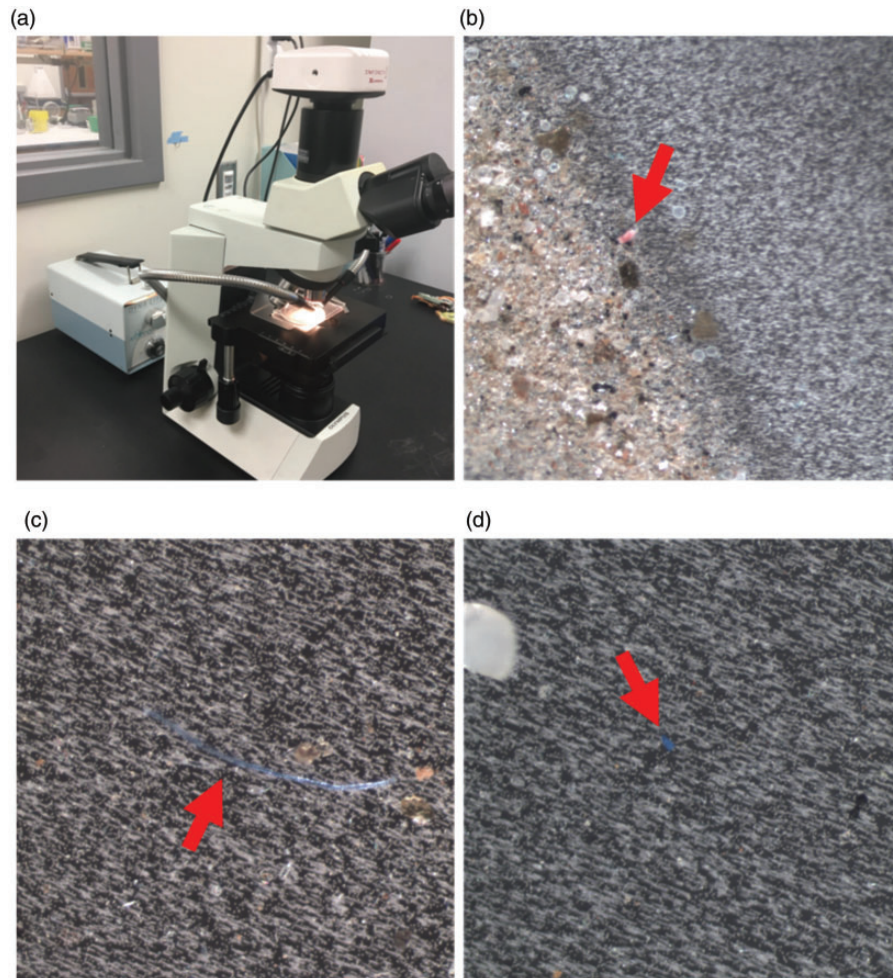
General Light Microscopy

The use of light microscopy to visually examine plastic particles is one of the most commonly used identification methods in MP studies. It has been utilized in almost every type of environmental sample studied in the literature including water,[17–22] sediment,[23,24] soil,[25,26] marine and freshwater organisms including fish,[27–29] commonly ingested food items such as table salt,[30] atmospheric deposition including street dust,[31,32] and wastewater treatment plant influent, effluent, or sludge.[25,33] These techniques have been employed in studies completed across the globe and adapted for numerous laboratories.

Light microscopes (optical microscopes, binocular microscopes)[22,23,29,31,33] and dissecting microscopes (stereo microscopes) are commonly used for this technique.[17–21,24,26,28,30,32,34] Optimal visualization of plastic particles uses a microscope with an external light source (see Fig. 2) in order to retain the 3D shape and color of suspected plastic particles to aid in visual identification.

Light microscopy is often paired with an extraction protocol in order to isolate plastic particles and eliminate false positives. For larger particles ($\geq$500 µm), studies[35] suggest separating the sample into fractions in which the larger particles are directly visually identified, while the fraction containing the smaller sizes (<500 µm) undergoes further sample extraction. Additionally, almost all studies that rely on, or apply, light microscopy outline a basic set of guidelines for visual MP identification. Visual guidelines include bright and unnaturally colored particles that are homogeneously colored, fragments with sharp geometrical shapes, shiny surfaces, and featureless fibers with a consistent width. Physical and tactile guidelines include the particle holding its shape or stretched when poked, melting at high

temperatures, and resistance to easy breakage. Here, the so-called poke test or hot needle test is used to confirm potential plastic particles; however, this is limited to large microplastics, likely above 100 µm or 500 µm.[36–38] Many studies either outline the guidelines used for particle selection within their methods or cite a previous study whose guidelines they were followed.[17–19,21–25,30,31] It is imperative that all future studies utilizing light microscopy have reported guidelines for the visual identification of MP particles (Cowger et al.[39]). Once the MP particles have been identified, most studies categorize the suspected particles by size classes, MP shape, and/or particle color. Most studies categorize MP particles into six common types, including fragments, pellets, fibers and line, films, foams, and beads. The used size classes vary widely between studies due to the detection limit of the study and the different sieves used to size fractionate the samples.

The benefit of light microscopy in MP detection studies is that it is relatively cheap, since most laboratories are equipped with an appropriate microscope. These microscopes cost ~US $500–2000 and can be combined with a microscope camera (~US$200–1000) to assess the particles manually or with the help of different types of software such as Histolab[20,32] or ImageJ,[25,30] some of which may add additional costs but can help reduce the expenditure of time for particle counting. Further, glass fiber (GF) or polycarbonate (PC) filters can be used which cost around US$1–2 per filter. This is cost effective compared to other methods requiring costly filter types like aluminum oxide or metal-coated PC filters. All other necessary devices like glass Petri dishes (~US$10 per dish) for storage, filtration units, wash bottles, and forceps (~US$150 in total) are low cost as well. Individual filters can be processed within an hour working time depending on the

Figure 2. Visual examination of microplastics using a compound microscope. (a) Light microscope with external light source and camera attachment. (b–d) Microplastic fragments and fibers between 63 and 500 μm in size as visualized at 40 × total magnification. Microplastic from the urbanized waterways in Long Beach, California, as seen in Wiggin and Holland.[22]

filter size, sample type, size class of interest, and density of MP and other nonplastic materials. This is a relatively quick method compared to commonly used chemical analytical techniques, where polymer type identification of one particle alone can range from a few seconds to nearly an hour.[40,41] Because of the efficiency of this method, it can be used as a fast pre-screening tool for chemical analytical techniques. Moreover, this method has good fieldwork capability as boats and larger research vessels can accommodate microscopes on board and field microscopes are available to directly screen samples on site.

Limit of Detection. Current limits range from ≥5002 μm,[28] ≥250 μm,[19] ≥100 μm,[20] and ≥50 μm.[26,32] Recently, MP particles were visually identified down to 7 μm42 and 3 μm22 with a strict exclusion protocol to avoid false positives[22] or validation by spectroscopic analysis.[42] However, most studies have a lower detection limit of >100 μm and reach the upper size limit of MP at 5 mm in size.[17,18,20,28,30] The lower detection limit of light

microscopy is connected to sampling and processing constraints (see Dye Staining section below), to the difficulty in following commonly used visual guidelines for small particles (<100 μm), and to the magnification of the used microscope. As such, with decreasing size, especially below 20 μm, the appearance or debris features (organic structures or shiny surface) are harder to determine from other items present in environmental debris (personal observation).

Limitations. In many cases, light microscopy can be used without complex extraction methods, and researchers can be quickly and easily trained to visually identify MP. However, the number of MP in a sample is commonly biased due to the difficulty of identifying non-obvious plastic particles from similar looking organic particles. This particularly applies to black, white, brown, and clear particles, which are encouraged to be eliminated because they do not represent bright or synthetically colored debris as outlined in selection criteria above thus leading to

uncertainty.[17–19,21–25,30,31] Additionally, light microscopy cannot identify the polymer type in order to validate suspected MP. Currently, this can only be reliably achieved through chemical analytical techniques, such as FT-IR and Raman spectroscopy.[17–19,23,29,33,43–49] Although this validation step allows for more confidence in the identified MP particles, many past studies that manually presort particles using light microscopy fail to eliminate the researcher bias towards large and brightly colored MP. However, recent studies are adopting particle mapping software in order to further reduce this bias (see the respective sections below).

Dye Staining

Commonly employed MP detection techniques such as spectroscopic analysis (e.g., FT-IR or Raman, discussed later in this review) can often require researchers to manually preselect particles for analysis due to the technical limitations of available instruments. This makes the process costly in terms of working time and the results are potentially affected by researcher bias. These issues are pronounced in the detection of smaller sized MP ($<100 \mu m$) that may not be distinguished with a light microscope and are hard to manually select for further analysis.[41] Several solutions are currently available to aid with these limitations like particle finder mechanisms[40,48,49] or imaging systems,[50–53] but increase the cost for the spectroscopic instruments. As an alternative, the use of MP dyes or stains act as a cost and time effective method that has the potential to reduce researcher bias and include smaller MP[54] in count data.

The hydrophobic fluorescent dye Nile red (NR, CAS-7285-67-3) is the most used stain in MP studies. It was originally used as a lipid stain for animal cells,[55] microorganisms, algae, chitin, wood lignin, and natural and semi-synthetic fibers (e.g., cotton and rayon).[22,56] Therefore, it is essential that NR in environmental MP studies follows validated digestion protocols for organic materials. NR staining was first suggested for use in MP detection by Andrady[57] and was subsequently validated for its ability to stain various MP polymer types. It now has been used in the detection of MPs extracted from environmental water,[22,56,58] atmospheric deposition samples,[54] beach sand,[56,58] drinking water,[59] aquatic sediment,[60,61] and marine species tissue.[62,63]

Using the NR procedure, polymers of interest or MPs extracted from environmental samples are filtered onto a membrane, incubated with a NR solution, the solution removed through filtration, followed by washing steps, or by evaporation. It should be noted that it is currently unclear whether letting solvents evaporate during the NR staining process (rather than filtration with filter washes) leads to unforeseen background noise due to NR residues on a chosen filter or residue on nonplastic particles.

Regardless, once stained the filter is visualized through fluorescent or other imaging techniques and finally analyzed through manual counting or automated particle recognition of stained particles. Filters can be assessed in a short period of time as they can be photographed and counted either manually (0.5–1 h per filter) or by automated systems (>10 min per filter).

Application in the field is limited compared to simple light microscopy, due to the need for extra equipment and hazardous chemicals. In addition, the staining method ($\sim$US\$9 per filter) increases the costs over general light microscopy due the purchase of the dye and additional solvents in combination with instrument costs depending on the microscope and type of illumination used. Costs of illumination equipment range from under US\$2k for a single wavelength device (e.g., Crime-Lite Foster+Freeman or Orion Lite, Evident; wavelengths between 450 and 510 nm) up to $\sim$US\$50k–150k if using a scientific fluorescent microscope that is not already available at a facility.

Limit of Detection. With this technique, detection limits from $\geq 300 \mu m$,[60,63,64] $\geq 20 \mu m$,[56] $\geq 6.5 \mu m$[59] and $\geq 3 \mu m$[22] have been reported. have been reported. Detection limits are rather caused by differences in sampling, processing, or detection protocols than by limits of NR. For example, Mason et al.[59] assessed MP in bottled water using NR and assessed MP numbers using an automated counting method able to detect particles down to 6.5 μm. Fischer et al.[60] sampled lake water using a manta net with a 300 μm mesh size, and Wiggin and Holland[22] collected whole oceanic and estuarine water samples and filtered onto 3 μm PC filters before staining. Overall, as MP size of interest is decreased, the time needed and the cost associated with one sample due to the clogging of filters and potential occlusion of smaller MP are increased. Thus, samples may need to be separated on to several filters. In addition, there appears to be an increase in potential contamination sources for MP in the smaller size classes, which will depend on a given processing facility,[22] thus increasing processing time. As the field of MP detection and MP regulatory frameworks grow, size classes detected will need to be standardized, especially as concerns over the smaller MP ($<100 \mu m$) increase due to their dominance in environmental samples.[65]

Limitations. Considerable research has been done to validate methods used before staining, for staining with NR and post staining visualization protocols. Before staining, validated digestion protocols are mandatory to reduce the detection of false positives (e.g., cotton). Digestion protocols use 15% to 35% hydrogen peroxide (H_2O_2) for various incubation periods (7–24 h),[24,56,58] sodium hypochlorite (6–14%; used at 0.15:1 v/v ratio),[54] iron(II) sulfate ($FeSO_4$) with 30% H_2O_2,[61] or Corolase7089 enzyme[62] or potassium hydroxide ($\sim$168 g/L working solution) with sodium hypochlorite (6–14%)[63] or for tissue digestion. To avoid degradation, H_2O_2 concentrations may need to be
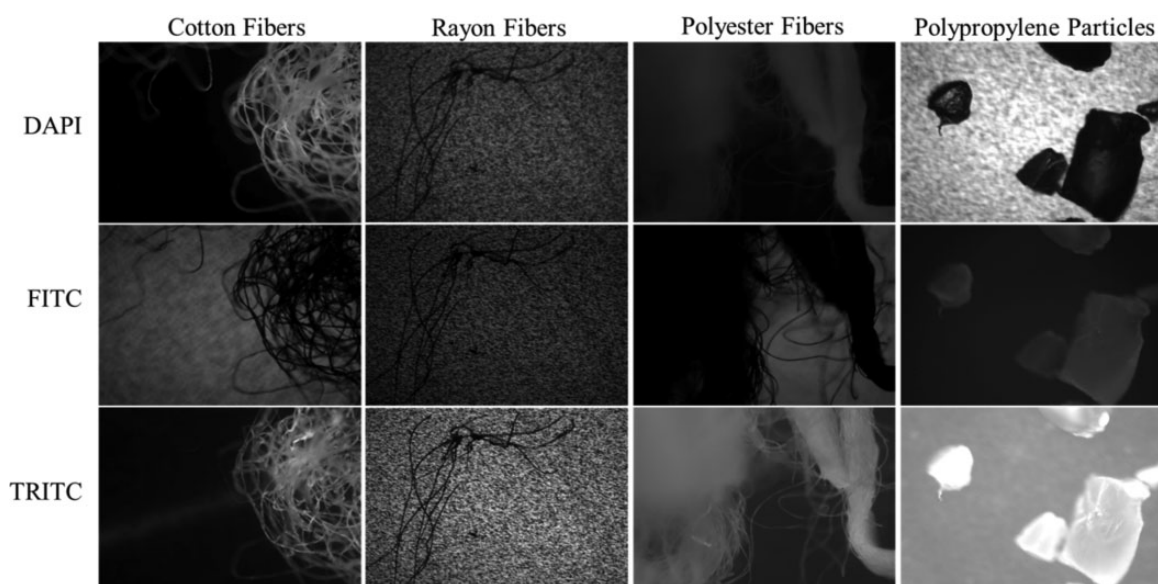
the light microscope alone, when suspected plastic items are assessed using FT-IR or Raman spectroscopy subsequent to detection.[21,66]

Other dyes have been considered for use in MP detection. However, NR is superior due to its ability to adsorb to plastics and its elevated fluorescent intensity relative to MP staining with Oil Red EGN, Eosin B, Rose Bengal, and Hostasol Yellow 3G.[66] Research has suggested that Rose Bengal displays a marked advantage over NR, because it may allow the color of MP still to be assessed, which may be lost when using the fluorescent dye NR. However, these findings are inconsistent in the literature, where some work shows that Rose Bengal can alter the color of PS and PVC.[67]

Flow Cytometry

Flow cytometry was originally utilized for counting and sizing cells to monitor growth, degradation, or aggregation processes. The sample is diluted by a sheath fluid and transported into a flow cell. Here, the cells or particles scatter light from a laser beam and are counted based on changes in the optical signal in a forward or side scattering angle. A variety of lasers and detectors may be combined in one instrument to analyze selectively stained samples. It is possible to differentiate sample components or regions in cells with specific dyes. This way, their size, quantity, and distribution are accessible, especially when combined with a strong camera for imaging. Typical size ranges of analyzed objects are between 0.5 and 40 μm, with 10^4 counts per milliliter.

Only a few studies have analyzed environmental samples containing MP using this technique. One study examined the effect of PS MP beads with defined sizes on algae photosynthesis and growth by monitoring cell growth and PS concentrations with flow cytometry.[68] In a different application, size and fluorescence intensity of aggregates formed through the interaction of PS beads and exopolymeric substances were investigated.[69]

Another study reported the identification of a small cluster of particles in a flow cytometry plot of stream biofilms from Rhine waters.[70] An advanced flow cytometer with separation function (MoFlo) then collected the particle fraction containing the cluster particles. Scanning electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM-EDS) was applied for imaging the collected particles and determining the elemental composition of the material, giving further indication, but not proof, of polymer identity.

Staining of MP with NR before flow cytometry has been proposed but not developed into a commonly applied method.[56–58,66] Basic flow cytometers range from ~US$50k upwards, depending on the choice of optical components. Measuring times depend on sample concentration, with single runs for particle counting approximately
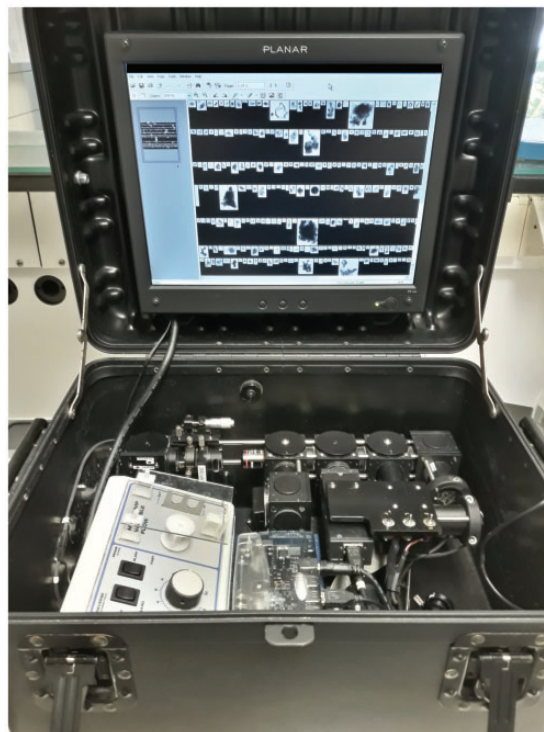
Figure 4. Flow imaging of a sample for particle size determination and counting to calculate filter coverages for spectroscopic analysis.[71–74]

lasting between 2 and 30 minutes. However, the method works best with few defined size populations and is not validated for analyzing polydisperse particle samples.

Another approach for analyzing particles and cells in the range of 2 μm to 1 mm in flowing solution is flow imaging. Here the diluted sample is monitored by a camera system combined with a microscope unit and every passing particle is photographed (Fig. 4). With the help of image analysis software, size parameters are available together with count numbers. Numbers of plastic particles released from household water kettles during boiling were counted this way.[75]

Flow imaging is more expensive than flow cytometry with prices starting at US$130k. However, its big advantages are that (i) it visualizes and counts single particles with options of later validation and correction of counts in cases where bubbles were detected, and (ii) there are mobile versions that can be moved into the field. Association with other particles or coiling of long fibers may hamper size determination of the single particle in both techniques, so that pre-filtration and purification may be necessary prior to analysis.

Combining flow cytometry and imaging with spectroscopic identification provides several benefits. The first two provide information on particle sizes, possible aggregation, and particle content of liquid samples.[75] In the case of imaging, even shapes are visible (Fig. 4). With particle

areas determined from the images, sample preparation can be optimized for later spectroscopic identification on filters. They are the basis for calculating the necessary volume of sample to obtain a monolayer of particles with an interparticle distance sufficient for spectroscopic analysis.[71–74]

Chemical Analysis Methods

The chemical identification of the basic polymers of MP is crucial for many types of studies like ecotoxicological risk assessments, since in addition to the effect of plastic particles interacting with biota, the chemical nature of the plastic particle may also be a threat. For this purpose, several analytical techniques are available. In general, two types of chemical analysis methods are applied for the identification of MP. (i) In spectroscopy, the sample is targeted with a beam of specialized light like infrared (IR) sources for FT-IR or lasers for Raman resulting in spectra which are further analyzed. (ii) For thermal degradation, the sample is heated at defined conditions giving characteristic decomposition products. These are usually further analyzed using GC-MS.

FT-IR Spectroscopy for Microplastic Analysis

The investigation of material and chemicals using FT-IR has been commonly applied in chemistry and material sciences for several decades and is a routine measurement for quality control in these fields. By IR light, the chemical bonds between the atoms within a molecule are excited causing a reduced transmission of light specific to the needed energy for this process. As every chemical contains different bonds and has a different chemical constitution, these spectra represent a fingerprint of the substance. Using a library search or other chemometric analyses, an unknown substance can be identified (Fig. 5). A detailed discussion on these data analysis methods was reviewed within this special issue.[12]

The spectra can be measured via two general principles, transmission and reflectance FT-IR. In short, transmission FT-IR analyzes the interaction of the IR beam after full penetration of the sample. This has the advantages that the full material spectrum is collected but is prone to total absorption, which either yields the loss of spectral details or blocks the complete beam. In reflectance, the IR beam hits the sample, is reflected, and collected again. This type of measurements mainly investigates the surface of the sample and cannot be hampered by total absorption, but the surface needs to be able to reflect the beam. If applied on reflective surface such as a gold-coated mirror or filter, a measurement in reflectance–absorbance can be performed which yields spectra similar to transmission.

Both types of measurement are available in most instruments, either as a component in the measurement chamber for single measurements or combined with microscopes allowing measurements of single particles and surfaces (μFT-IR). Microscopes can be combined with focal plane array (FPA) detectors[79] which allow the measurement of several thousand spectra within one measurement.

On surfaces, attenuated total reflection (ATR) can be applied. Here, a crystal is pressed onto the surface of the material and target by the IR beam. At the interface of the crystal, the beam is mainly reflected, but a small part is interacting with the tested material resulting in an IR spectrum. This technique is either available as handhelds,[80] for single particles or introduced into microscopes.

The current application of FT-IR in MP research was determined by a literature review conducted via Web of Science, completed on 1 May 2019, with the search terms "microplastic FT-IR, microplastics FT-IR, microplastic infrared, microplastics infrared, microplastic analysis review, microplastics analysis review" and additional terms for environmental sciences journals "microfiber FT-IR, microfiber infrared, microfiber review" resulted in 258 articles, of which 211 were research articles, 41 reviews, and six of other types. After careful investigation of the reported Materials and Methods and consideration of the focus of this review (environmental samples), 161 articles were chosen for further calculations on use of FT-IR for the analysis of MP. Further technical advancements published later to this date were also included into the text but excluded for the mentioned calculations to exclude a bias.

In the reviewed studies, FT-IR was performed either by measuring single particles,[16,54,59,81–150] via handheld FT-IR,[137,151] by a fiber optic,[152] by using μFT-IR,[18,29,35,42,51,78,100,153–212] by applying both techniques on separate instruments,[40,50,53,66,73,78,213–224] or referenced to other publications.[225] Larger single particles were ground with potassium bromide (KBr) for transmission measurements, targeted via diffuse reflection, or by ATR measurements. For μFT-IR, the particles were sorted onto IR transparent slides/windows, placed into compression cells, concentrated onto membrane filters, or placed onto reflective slides to measure either each single particle or perform a chemical mapping of the surface.

In the recent years, the number of studies using visual identification followed by chemical analysis has increased and it has been recently recommended by the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP)[226] to chemically identify any particles smaller 1 mm. For the investigated studies, a chemical identification was performed by 47% for all investigated/visually identified particles. Further 41% of these studies identified the polymer type for at least a fraction of the sample while 12% did not provide enough information. In addition, 7% combined their studies with method evaluation and validation.

Single Selected Particles. Visual presorting was mainly used for particles with sizes larger than 200–300 μm. Here, single particles were commonly preselected for chemical
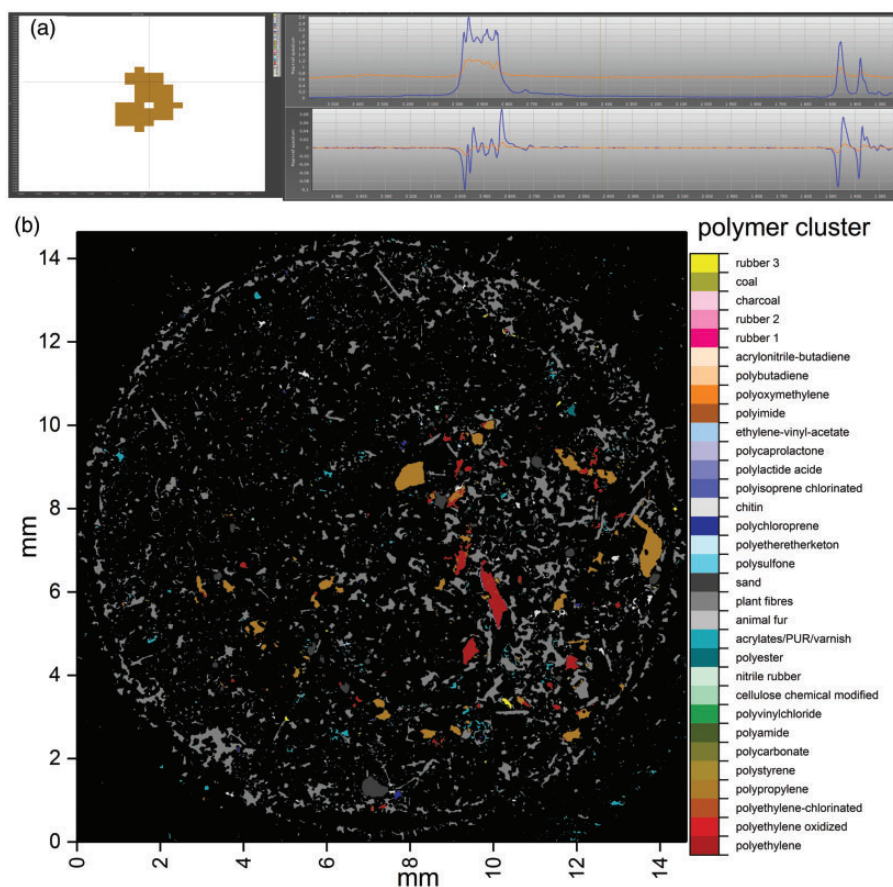
Figure 5. (a) FT-IR spectra of an identified particle using FT-IR imaging by use of the free software siMPle[52,76,77] found on the sample RefEnv2.[78] (b) False color image of the filter area of filter RefEnv2[78] after automated analysis and image analysis.[52] Reproduced from Primpke et al.[78] (CC BY 4.0).

evaluation. Depending on the study, a general separation between MP into a larger and lower size fraction is performed to reduce the expenditure of time for sample processing of the smaller size fraction.[35] Typically, such a size fractionation was performed at 500 μm.[35,40,71–74,161,203,213]

Attenuated total reflection FT-IR[16,29,54,59,83–85,87,88,91–93,95,99–149,154,157,158,162–165,215,219,220] was used by 58% of the overall studies, especially for larger particles, as it is most cost efficient, since no sample preparation (e.g., transmission) or difficult mathematical corrections (e.g., pure or diffuse reflection[196]) are required. Other than the initial cost of the ATR unit, only a cleaning solution for the crystal is necessary, while for transmission and reflection more consumables may be required.

Only reflection is fully nondestructive,[196] while ATR may harm or destroy particles due to the pressure applied and for common (KBr window)[150] transmission FT-IR, the particle needs to be destroyed to prepare suitable samples. Still, the working time per particle is high, as each particle has to be measured individually (approx. 2 to 3 minutes per particle). Measurements can be performed on bench top instruments available starting at US$25k from several

manufacturers. These come along with options to measure MP using ATR, transmission, or reflection FT-IR mode. Spectral analysis normally follows by library searches[227] via spectral correlation or other approaches.[228,229] (See also Cowger et al.,[12] in this special issue for more details.) Handheld FT-IR spectrometers[151,230] allow a direct measurement of plastics in the field, but are more expensive than the benchtop FT-IR systems. These techniques require the particles handled by the operator and are commonly limited to particle sizes of 300–500 μm.

μFT-IR Measurements of Selected Particles and Imaging Techniques. Using a microscopic coupled ATR,[29,154,157,158,162–165,215,219,220] reflection[53,167,188,196,199,200,205,224,231] and transmission FT-IR[51,155,172,178,180,182,186,190,191,197] can be applied on particles sorted onto slides,[29,187] compression cells,[29,173,181,198,215] windows,[209] meshes,[232] or various filter membranes (see below). By visual selection of the particles, a measurement can be performed with relative ease. Still, some limitation needs to be considered: Microscopy-based single-particle ATR has high measurement times and poses the risk of sample contamination

or loss. Transmission-based microscopy may shows total absorption for large or thick particles. Reflection-based microcopy needs good reflection properties and is less suitable for small or dark-colored particles.

Particles $< 500\,\mu m$ are often concentrated onto membrane filters, slides, and windows made of IR transparent or reflective materials. Filter materials were tested for the applicability.[51] It was found that aluminum oxide filters were the only ones suitable for transmission measurement with a broad range available for FT-IR measurements (for details see Löder et al.[51]). In recent studies, aluminum oxide filters[35,51,52,71,73,74,78,161,192,193,213,233,234] were widely used but also metal covered PC filters[40] (also suitable for Raman) and silicon membranes[159,185] were reported. Other studies work with slides,[53] or windows made of IR transparent materials.[76,203,235]

Independent of the filter materials, the particles need to be identified. Three options are available: preselecting the particles by chemical imaging of the entire filter surface,[51,53] pre-counting via (automated) particle finder mechanisms,[163] or visual identification on the filter surface.[177,199] For visual identification and particle finding algorithms, the particles need to be well separated, which is often challenging for environmental samples. Typically, just a small fraction of the extracted sample can be placed onto the filter/slide.[40] Otherwise groups of small particles may be misidentified as larger particles or particles are missed due to researcher bias. As mentioned above, the combination with staining techniques may enhance the identification rate and reduce researcher bias.

Using chemical imaging (FT-IR imaging), all particles can be addressed independently if they are in close proximity to each other but the number of spectra that need to be measured is significantly higher. Using mercury cadmium telluride (MCT) detectors is possible, but time-consuming for large filter areas.[50,167] FPA detectors were utilized for analysis of these large areas[51,53] and have been used in several high detailed studies of MP present in different ecosystems and waste management systems (e.g., Lorenz et al.,[72] Liu et al.,[76] and Vianello et al.[235]). Furthermore, automated analysis of the large data sets generated by FPA devices is available,[52,76,77,234] and freeware tools were recently released reducing the working time and costs for analysis.[77]

The time needed to scan an area of $14 \times 14\,mm$ by FT-IR imaging is currently 4 h with a pixel resolution of $\sim 5.5\,\mu m$ (128×128 FPA)[76] or $\sim 11\,\mu m$ (64×64 FPA).[236] Data analysis time by spectral correlation for 1.8 million spectra depends on the used software and ranges from 48 h (Bruker OPUS) down to 4 h (siMPle)[233,77] on the same computer. Subsequently, the resulting data can be further analyzed for particle and fiber numbers using automated analysis tools.[52,76–78,234] These tools are using extended

data analysis together with image analysis based on either the resulting combined hit quality for a polymer type[52,78] or based on the calculation of a weighted hit quality and reconstruction using threshold.[76] For the first variant, the image analysis was extended for the differentiation between particles and fibers.[234] Alternatively to spectral correlation, an approach was recently published using classifiers[237] instead of spectral correlation, but is currently limited to six polymers. One set of these tools[52,78] was already used in a harmonized manner on a large variety of different environmental matrices.[40,71–74,77,213,233,236]

The costs for particle finder systems start at approximately US\$100k–125k for a system with a typical LOD of 20–25 μm while FPA-based systems start at around US\$200–250k per system with a typical LOD of $\sim 10\,\mu m$. Depending on the type of detector used for analysis, liquid nitrogen supply may be necessary. For FPA systems, it is mandatory and increases the cost per measurement. The overall time demand for one sample depends on the system and method used, the targeted resolution (e.g., by using binning on high-resolution lenses)[51] and the data analysis. Of the filters available on the market, aluminum oxide filters were the cheapest option ($\sim$US\$5–20 per filter),[35,51,52,71,73,74,78,161,192,193,213,233,234] and metal-coated and silicon filters[40,159,185] are more expensive ($\sim$US\$20–50 per filter) while the price for FT-IR transparent windows[76,203,235] is driven by the chosen diameter ($\sim$US\$50–100 per window). The drawback of using aluminum oxide is the wavenumber limitation towards 3600–1250 cm^{-1} compared to other filter materials (3600–900 cm^{-1} for FPA, even lower for MCT) which yields more spectral information in the fingerprint region.[51] Still, it was found that a minimum of 32 polymer types can be spectral separated using aluminum oxide filters with relative ease.[78]

Limitations. Using FT-IR is a good compromise between time demand and level of detail needed to address MP concentrations in different sample types. Due to the often mandatory sample preparation for all spectroscopic tools to identify small MP, it is the most cost-efficient way to gain information about particle numbers, polymer types, and sizes simultaneously.[52,76,234] In addition, since FT-IR is a nondestructive method in most operation modes, it can be combined or followed up by the choice of the filter material with Raman analysis or other techniques,[40,158,185] if necessary. Due to the diffraction limit,[238] the LOD of FT-IR is currently discussed between 20 and 10 μm, while the determined size distribution was confirmed by comparison with Raman[40] down to 10 μm. Particles $< 10\,\mu m$ are challenging to measure[40,185] with FT-IR and need to be identified by additional or combined techniques. Further, measurements cannot be performed in presence of water as its spectrum will overlay the target spectra.

Raman Microspectroscopy for MP analysis

Raman spectroscopy is typically a nondestructive method, where a laser beam is focused on a particle surface and induces an inelastic scattering of photons (excite molecules to higher energy levels), which is known as Raman effect.[239] By library search of the resulting vibrational fingerprint, chemical identification of the unknown particle is possible. To increase spatial resolution, a Raman spectrometer can be coupled to a microscope, so-called Raman microspectroscopy (RM) and renders information on polymer type, particle number, size distribution, and morphology.

The applicability of RM for MP analysis in different environmental matrices has been shown in numerous scientific studies.[43–47,240,241] For the reliable analysis of MP particles in the environment, a method with clear particle identification (chemical information) and characterization (morphological parameters) on a single-particle level over the entire particle size range (from 1 µm up to 5 mm) is desired. RM fulfills these requirements and offers the possibility for the development of such a method.[40,242] Therefore, RM has been highly recommended, particularly for the analysis of particles <10 µm.[179,243] Furthermore, using handheld spectrometers Raman measurements can be performed in the field.[80]

The high number of organic (e.g., humic substances) and inorganic (e.g., clay minerals) contaminants in environmental samples can hamper the detection of single MPs. Fluorescence from dyes or other additives within the particle can interfere with Raman spectroscopy and prevent reliable identification of spectra. Additionally, MPs can be agglomerated or overlapped by natural particles, leading to over- or underestimation of particle size or number. Therefore, rigorous sample purification is strongly recommended (see Lusher et al.[7] within this special issue), but the extent or best practice is still a matter of debate.[242]

Analysis of MP can be performed via three approaches: (i) manual measurement of single particles, (ii) automated particle identification with "Particle Finding" algorithm, or (iii) point-by-point mapping with "Imaging Mode."

Identification of MP as Single Particles. When using Raman spectroscopy on a manual particle by particle basis, sample preparation is important for accurate identification. For larger particles (>300 µm), it is advised to adhere particles to a flat base material such as double-sided tape on transparency paper,[244] or an aluminum sheet to minimize background fluorescence.[245] Particles should be clearly characterized (e.g., circled and numbered) to aid particle identification using the Raman microscope. For smaller particles (<300 µm) that cannot be manually picked, particles should be analyzed directly from a filter to avoid contamination or particle loss. Measurement time per particle is dependent on the parameters chosen, the sample, the desired spectral quality (signal to noise ratio), and the Raman spectrometer (starting at US$50k) used. On average, the time taken to obtain spectra for a singular particle ranges from a few seconds[246] to several minutes.[247] Measurement time should be optimized to get a signal to noise ratio >3. Parameters that influence this are the spectral range (commonly 200–3200 cm^{-1}), excitation wavelength (acquisition time: 785 nm > 632 nm > 532 nm), type of objective (magnification and aperture), spectral grating (resolution), and number of accumulations. Analysis of all particles in a given sample can be timely; therefore, a more reasonable method is the use of automated analysis.[40,48]

Automated Particle Identification with "Particle Finding" Algorithm. The detection of MP particles < 20 µm with RM requires particle isolation by filtration onto a membrane. Filter substrate characteristics are of critical importance for successful MP analysis. For high-quality results, the filter material should offer good filtration characteristics, be chemically nonreactive, have a flat and homogeneous surface, and minimal spectral interferences or fluorescence in the range of the polymer bands.[159,245]

For RM information on filter material is still scarce. The favored filter materials are metal covered PC membranes (~US$20–50), e.g., gold coated[40,49,242] or aluminum coated.[245] Käppler et al.[159,185] suggested a specially fabricated silicon membrane (~US$10–30) as a possible filter material for FPA-FT-IR and RM analysis, which was limited in pore size to 10 µm. In previous studies, aluminum oxide (e.g., Anodisc) membranes (~US$5–20) or GF filters were recommended (for particles > 100 µm). Consequently, for a reliable and reproducible RM analysis, an appropriate filter material fulfilling the requirements of the measurement method (e.g., pore size, light, or black background) must be found.

A promising approach is the automated particle identification ("Particle Finding") (ii) using image analysis software as displayed in Figs. 6a to 6c.

First, in a selected filter area, all particles are detected using an image analyzing step based on particle finding with image processing criteria (e.g., black/white contrast). Subsequently, the detected (or manually selected) particles are automatically measured using RM.[40,48,49,242,248] Image processing can be done with different illumination modes (bright field or darkfield) to enhance the contrast between particles and filter, and with advanced features (e.g., montaged mosaic image with/without AutoFocus) particle detection can be improved. A great advantage is that particle number, size distribution, and morphological parameters (e.g., shape) will be documented for each particle. Schymanski et al.[49] automatically identified a maximum of 5000 particles larger than 5 µm performed at a rate of 56 min/mm².[242] Cabernard et al.[40] analyzed 5243 particles of 10–500 µm diameter with a total time of 44 h on 23.5 mm² and 10 550 particles of 1–10 µm in 29 h on 3.8 mm². Total time consumption for the entire automated measurement, spectral analysis, and data evaluation
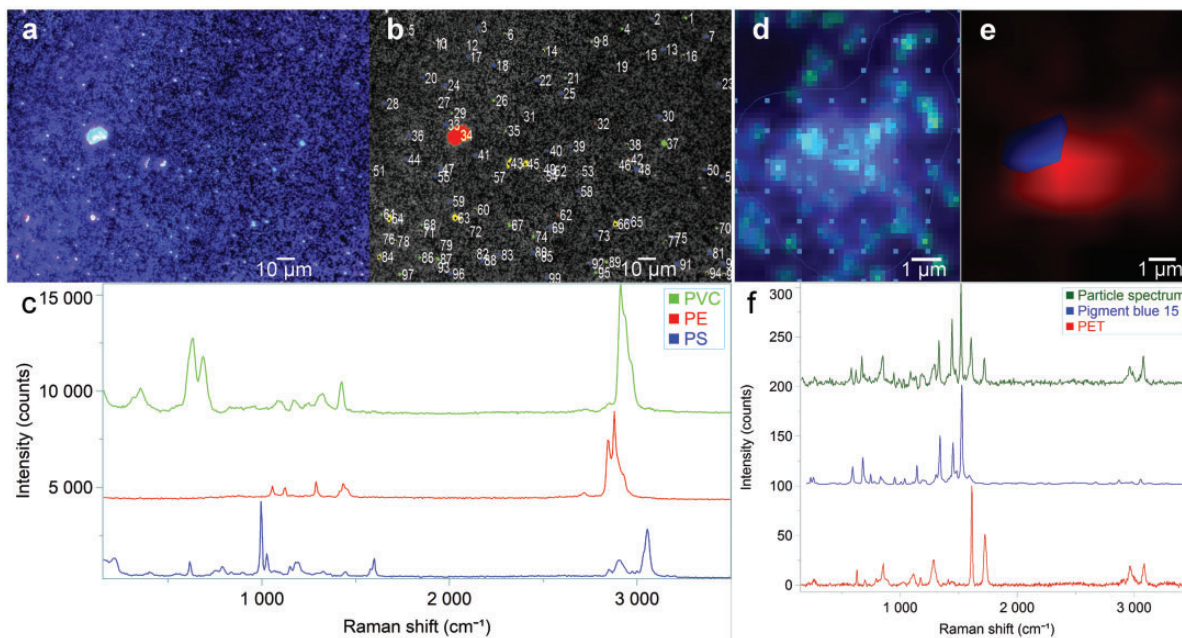
Figure 6. Analysis of MPs using Raman microspectroscopy. Application of automatic particle detection (a–c): Microscopic image of standard MPs on the filter surface in darkfield (a) and as a grayscale image analyzed with Particle Finder module (Horiba NanoGPS OxyO Scale) (b) along with the corresponding Raman spectra (c). Raman mapping on the surface of a PET particle whose Raman spectrum was interfered by a dye (d–f): Microscopic image of the particle with the raster used for mapping (d), result of the Raman mapping over the particle surface using the Raman spectrum of PET (red) and Pigment Blue 15 (blue) as references (e) with the corresponding Raman spectra (f). Adapted from the Ossmann et al.[245] with permission from Springer Nature and Ossmann et al.[248] with permission from Elsevier.

procedure was on average 43 ± 4 h per 18 mm^2 (for MPs of 10–500 μm).[40] This approach is very time-consuming (several days to weeks) for large particle numbers >1000, expected for the toxicologically relevant MP <10 μm.[40,49,247–250] Therefore, often small sub-areas of the filter membrane (0.1–30%) are analyzed depending on the total particle number.[40,49,248] The improvement of a fully automated RM analysis coupled to an automated database search is urgently needed and is currently being developed within the framework of different research projects.

Point-by-Point Mapping with "Imaging Mode." Using point-by-point mapping ("Raman Imaging") (iii), the laser is focused to a spot and Raman spectra are acquired step-by-step in a defined step size at various measuring points along a grid,[185,242] as exemplarily shown for just one particle in Figs. 6a to 6f. An electron-multiplying charge-coupled device detector (EM-CCD) can be used to require less acquisition time and to enhance the signal readout (signal-to-noise, or S/N ratio).[185,242] Käppler et al.[185,242] used point-by-point mapping on a Si filter membrane (10 μm pore size) with a step size of 10 μm taking an enormous measurement time of 38 h per 1 mm^2. A more time-consuming investigation of particles sized <10 μm by Raman imaging is also possible. New techniques are continuously developed to decrease the measurement time like the approach of Zada et al.[251] by stimulated Raman

scattering (SRS). SRS microscopy is based on the coherent interaction of two different laser beams to simultaneously absorb both pump and Stokes photons, which causes specific transitions in the molecules of the sample. The signal intensity of SRS is several orders of magnitude higher as of spontaneous Raman scattering and enables much faster detection and identification of microplastics, yet this is limited to particles in 12 μm resolution. Further approaches, which optimized the integration times down to 1 ms are also available. The analysis of macro areas (centimeter range) is still too time-consuming to analyze the entire filter (diameter of 25 mm or 10–12 mm), with measurement times of several days for only a small part of the filter (1–2%).

For automated RM (ii and iii) analysis, expensive instrumentation is required, starting at basic systems (Raman spectrometer + microscope + CCD detector) of around US$100k–200k up to US$400k equipped with an EM-CCD detector for fast imaging, different excitation wavelengths and illumination modes for particle finding.

Limits in Particle Size. The minimum lateral resolution for a diffraction-limited microscope in RM analysis is defined by diffraction limit[238] and can be calculated from the laser wavelength used and the numerical aperture of the microscope objective.[252] The resulting detection limit is 300–500 nm using standard objectives for single-particle

detection, enabling chemical and morphological characterization.[252] There are some techniques ("super-resolution microscopy") that achieve a higher resolution than possible by use of diffraction-limited optics.[242,253,254] However, these techniques generally lead to increasing costs and complexity.

From a theoretical point of view (see above), the lower size limit of 1 μm discussed for MP[4] seems attainable using RM and leaving room for improvement. Ossmann et al.[248] analyzed mineral waters for MPs down to 1 μm, but in all other studies analyzed particles were >5 μm in diameter.[40,49,185,247,248] The theoretical limit of detection (300 nm) has not yet been reached in recent studies using RM.[45,48,56,185,247,249,255–259] In practical application, the analysis of such particles is complex and partly limited for real samples due to filter type (e.g., an uneven surface or limits in pore size), sample complexity (e.g., natural matrix), and measurement parameters. These can hamper the lateral or depth resolution of particle focusing. These small MP particles are high in numbers but not in mass and potentially exhibit the largest environmental threat.[243,250,260]

Other Method Limitations. When using RM, it is easy to cause photodamage to particles with the laser while obtaining spectra, particularly for particles with a thin, delicate structure. If this occurs, the affected section of the particle is no longer acceptable for analysis, as its chemical structure will be altered. It is therefore best practice to initially use conservative laser intensities for delicate particles to avoid burning and preserve particle components. Laser intensity can be increased gradually until the spectra obtained are of acceptable quality (S/N).

Spectral quality can be limited by interference from the sample material itself, adhering material (fluids and biological films), or the atmosphere. Especially, signals from dyes and additives can often interfere with (cf. Figs. 6d to 6f) or overlay the polymer spectrum, hiding their characteristic peaks.[44,247] This limits particle identification from a specific polymer type to a more general categorization such as "anthropogenic" or "synthetic".

Raman microspectroscopy is an unambiguous and typically nondestructive method, with a spatial resolution down to 1 μm and enables a simultaneous determination of particle number, size distribution, and morphological parameters. Compared to FT-IR, analysis it shows a wider spectral coverage, higher sensitivity to nonpolar functional groups, lower water interference and narrower spectral bands.[242] Commonly, drawbacks of RM can usually be handled by pre-cleaning protocols and software optimization. However, sample analysis is extremely time-consuming (several days), thus often only a fraction of the sample is analyzed (0.1–30%). There is an urgent need for automation routines that enable faster and more reliable detecting especially of small MPs. Consequently, the speed of analysis continues to increase through sophisticated approaches and analysis tools developed recently using automatic routines for image stitching and focus stacking, algorithms for particle fragmentation, and classification in combination with a direct identification of the chemical composition per database search.[252,261,262] Another approach is the use of statistical models to calculate the minimum number of particles required for a statistically meaningful analysis.[252] These advances are expected to enable a higher sample throughput (one to two samples per day) and simultaneously provide high analytical accuracy for particle characterization. For particles > 10 μm, this is not recommended due to the increased amount of time needed to invest a smaller filter area compared to FT-IR imaging.[40] The use of both methods should be considered as complementary.[185]

The relocation and accurate recognition in micrometer range of the same area and MP particles in RM and FT-IR or in general in different instruments can be problematic. Special attention must be given to the individual handling and usability of different filter types and in general the particle fixation on the filter surface. However, new high-precision repositioning technologies that can be also applied to MP analysis independent of the instruments' manufacturer are already available (e.g., Horiba NanoGPS OxyO Scale).

Thermal Degradation Methods with Subsequent GC-MS Detection Analysis

The use of thermal degradation products for the determination of MP in environmental samples is emerging in recent years. It relies on pyrolysis products generated at defined temperatures under the exclusion of oxygen. After gas chromatographic (GC) separation, the so-called pyrograms act as fingerprint of the respective polymer. Coupled with MS, these pyrolysis products can be identified on molecular level. Based on specific pyrolysis products, polymer mass quantification is possible and enables simultaneous identification and quantification of different MP in complex environmental samples. These mass-related data have to be considered as bulk values of a given plastic type, e.g., PS, disregarding if it is a pure polymer or a share of a copolymer,[263] and are independent from any kind of particle appearance such as shape, size, density, texture, surface aberrations, color, brightness, opacity, or weathering. Further, pyrolytic methods allow the simultaneous characterization of (MP) polymers and additives. As reviewed by Tsuge et al.,[264] pyrolysis of polymers has a wide use in polymer characterization and led to broad applications in the analysis of polymeric materials.[265–269]

Already applied in a few early plastic-related environmental studies,[270–273] it became recently more popular for isolated MP particles[13,274–276] and regarding simultaneous identification of different polymers in complex mixtures.[263,277,278]

Here, two principles can be applied: (i) on-line py-GC-MS[263,279] and (ii) TED (thermo-extraction and desorption) GC-MS.[278,280–282] Both allow the general polymer

identification and quantification, but have different performances regarding requirements for quantification, explained later in the text.

Microplastic Analysis Using Pyrolysis–GC-MS

Polymer Identification of Isolated Particles. For polymer identification with py-GC-MS, a selected (plastic-) particle or a representative particle fraction of a few micrograms in total is transferred into a pyrolyzer target. Several types of pyrolyzers and respective targets are available, relevant, and restrictive for sample capacity. Filament pyrolyzers use open or semi-closed quartz tubes (system-dependent variable dimensions approximately Ø 0.2–1.3 cm and different length, e.g., Dekiff et al.,[274] Fries et al.,[275] Nuelle et al.[276]), placed in a platinum coil; Curie point (CP) pyrolyzers use semi-closed ferromagnetic targets (typical dimensions Ø 2 mm, 8 mm height, e.g., Fischer and Scholz-Böttcher[263]); whereas micro furnace (MF) pyrolyzers use stainless steel cups (typical dimensions approximately Ø 4 mm, 8 mm height, e.g., Käppler et al.,[158] and Fischer and Scholz-Böttcher[279]). In each case, the sample is heated to a defined temperature in an inert atmosphere, usually helium, which is also used as carrier gas for gas chromatographic separation.

Thermal decomposition kinetics and mechanisms,[263,283,284] the complexity of pyrolysis products, and the respective pyrograms differ highly among different polymer types. Here the range spans from highly complex (e.g., PE, PP, PET) over moderate (e.g., PS) to simple (e.g., PMMA).[263,264] In almost all cases, monomers and oligomers of the respective polymers are present, accompanied by pyrolytic reaction products determined by the chemical reactivity of the polymer. Dependent on pyrolysis temperature, thermal degradation results in unique and reproducible signature pattern characteristic for a given polymer. The use of pyrogram data bases and associated mass spectra from reference polymers or literature reference data bases (that include more than 165 common polymers[264]) as well as comprehensive data interpretation enable a reliable identification. Thermo-volatile, organic additives that are incorporated in the polymer matrix can be identified as well.[275,285–291] This can be done in a single run with the polymeric matrix or separately in a thermodesorption run at lower temperatures prior to pyrolysis ("double shot"). Additive identification requires extensive compound knowledge (inclusively their thermal behavior) as well as the availability of mass spectrometric chemical libraries (e.g., NIST library, currently >267 350 individual mass spectra). In principle, even the detection of adhered compounds like pollutants, if sufficiently concentrated, might be possible but was not reported so far.

For polymer identification of isolated particles, common thermal GC-MS methods are typically by far more time-consuming than FT-IR or Raman methods. Usually, well-resolved GC-MS runs for polymers take half an hour or even more. Alternatively temperature-ramped evolved gas (EGA)-MS, using very short deactivated silica capillary tubes (2.5 m, 0.15 mm i.d.) might be used for precise additive and polymer identification via totally combined mass spectra within half an hour or less.[264] Reference polymer databases are commercially available for this purpose like the library F-Search (Frontier Laboratories Ltd.), that is broadened continuously.

Single MP particles of sufficient mass (~1 µg), isolated from natural samples, can be reliably identified with pyrolysis-GC-MS.[158] The power of thermal degradation GC-MS methods regarding MP analysis is in the simultaneous identification and quantification of different polymers in complex samples.

Simultaneous Polymer Identification and Quantification in Complex Samples Identification. Since polymers differ in their respective pyrograms, single polymers can be identified in complex mixtures by characteristic and selective pyrolysis products of individual indicator compounds.

These indicator compounds enable a systematic and sequential identification of each polymer of interest in a given pyrogram. The relative intensity indicator compounds vary polymer dependent, and therefore, directly affect the detection sensitivity of the respective polymer. Ion chromatograms enhance the detection sensitivity and are often necessary to detect polymers in complex samples (Fig. 7). They represent the ion current over time extracted for a selected fragment ion of an indicator compound from mass spectrometric data.

Indicator ion selection is dependent of its intensity and polymer specificity, whereof polymer specificity is decisive. An example is the styrene monomer, which is the most prominent peak in the pyrogram of PS. Since styrene is generated by several anthropogenic polymers as well as from natural compounds (e.g., chitin) during pyrolysis, it is very unspecific. In contrast, the styrene trimer is less prominent in the pyrogram but has an exceedingly high specificity for PS and thus is much more suitable for a reliable identification (and subsequent quantification). For further details particularly regarding the quality criteria of choice for indicator compounds, see literature and associated supplements, e.g., Fischer and Scholz-Böttcher,[263] Tsuge et al,[264] Dumichen et al.,[278] and Fischer and Scholz-Böttcher.[279] Table 1 lists the typical indicator compounds and respective ions that are used for nine common polymers in thermal polymer analysis from complex mixtures according to published data.[263,278–281] These compounds represent more than 80% of the global plastic demand.[292] Relative intensities of individual peaks can vary from instrument to instrument and are directly related to pyrolysis temperature.[13]

Quantification. For MP quantification by thermal degradation, an appropriate removal of accompanying inorganic and organic matter is obligate to improve sensitivity
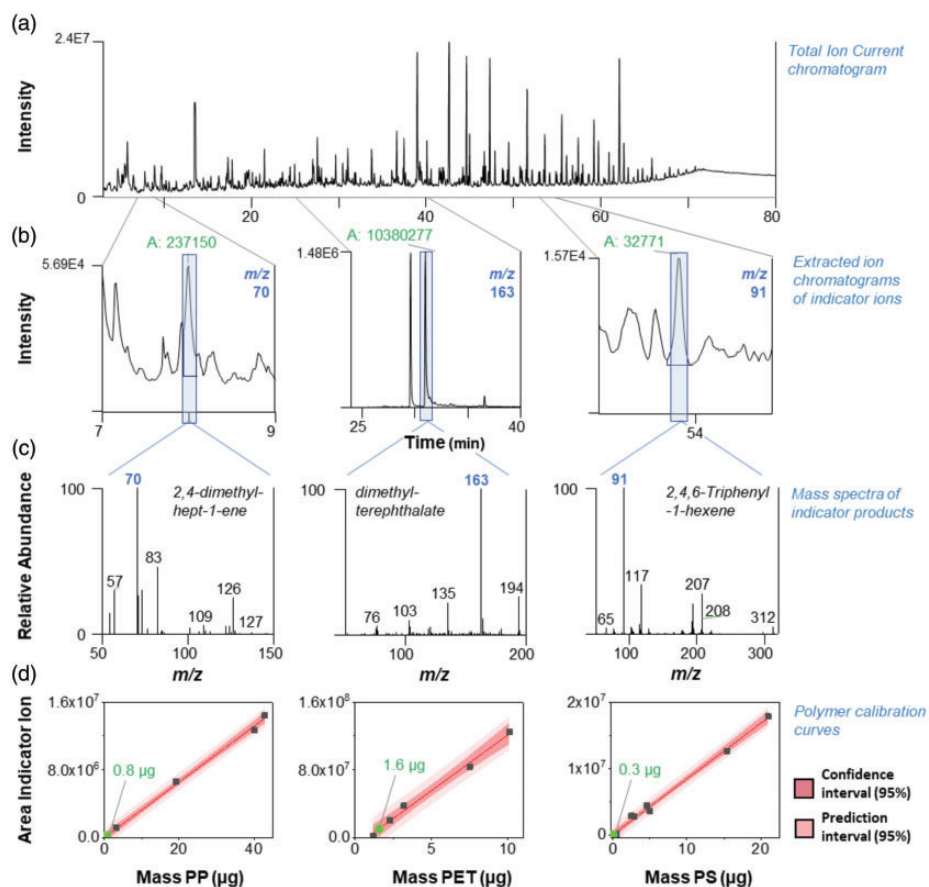
Figure 7. Example for the quantification procedure of three polymers (PP, PET, and PS) in a sea water filtrate (Fischer and Scholz-Böttcher, unpublished results).

and to avoid direct interferences of any other nonplastic-- derived pyrolysis products. Additionally, pyrolysis products of residual organic matter can induce surface interactions inside the pyrolysis system that need enhanced attention.[279]

Figure 7 gives an example for the simultaneous identification and quantification of PP, PET, and PS in a complex sea water sample (North Sea, German bight). Here 137 L were treated according to Fischer and Scholz-Böttcher[279] and measured with microfurnace py-GC-MS at 590 °C (conditions are stated in Fischer and Scholz-Böttcher[279]).

Figure 7a shows the section of interest from the resulting total ion chromatogram (pyrogram) and represents the complexity of an environmental sample. It is clearly indicated that diverse organic pyrolysis products are present. Characteristic pyrolysis products for PP are the 2,4-dimethyl-hept-1-ene and three isomers of 2,4,6,8-tetramethyl-1-undecene (Table I). 2,4-Dimethyl-hept-1-ene can be identified via its mass spectrum (Fig. 7c), and the selectively extracted as ion chromatogram of its indicator ion *m/z* 70. Integration of the respective signal results in an area of 237 150 units (Fig. 7b). This equals 0.8 µg PP, determined via external calibration (Fig. 7d). For PET and PS, quantification

was performed the same way using dimethylterephthalate (indicator ion *m/z* 163) and styrene trimer (5-hexene-1,3,5-triyltribenzene, indicator ion *m/z* 91), respectively.

The limits of quantifications (LOQ) for thermal methods are highly polymer dependent and reach lower µg levels, and even below; limits of detection (LOD) are even lower.[263,278,279,281] Of general relevance for detection sensitivity are the relative intensities of indicator products. Solubility or non-solubility (accordingly the sensitivity of the balance) of the polymers has a direct impact of the individual polymer calibration range. Finally, the quality of organic matrix removal determines the polymer quantification in terms of general background and possible interferences. To get an impression of the pyrolysis-GC-MS method potential, alternatively, the S/N for the lowest calibration points can be taken into consideration. For defined, polymer specific indicator ions the S/N varies between 478 (PP 0.3 µg) and 30 (PA6 0.5 µg). In case of PS that is easily solvable, 0.01 µg shows a S/N of 8. For further details, see Fischer and Scholz-Böttcher.[279]

The reduction of possible interfering matrix distinguishes between the online py-GC-MS and the TED-GC-MS quantification approach:

Table I. Characteristic indicator compounds and respective indicator ions for the simultaneous identification and quantification of different types of plastics using thermal decomposition methods.

Polymer	Abbreviation	Characteristic degradation product(s)	M (m/z)	Indicator ions (m/z)	Thermochemolysis product(s)	M (m/z)	Indicator ions (m/z)
Polyethylene	PE	Alkanes (e.g., C$_{20}$)	282	85	Alkanes (e.g., C$_{20}$)	282	85
		α-Alkenes (e.g., C$_{20}$)	280	83	α-Alkenes (e.g., C$_{20}$)	280	83
		α,ω-Alkenes (e.g., C$_{20}$)[a]	278	55, 82	α,ω-Alkenes (e.g., C$_{20}$)[a]	278	55, 82
Polypropylene	PP	2,4-Dimethylhept-1-ene	126	126, 70	2,4-Dimethylhept-1-ene	126	126, 70
		2,4,6,8-Tetramethyl-1-undecenes[b]	210	100, 69	2,4,6,8-Tetramethyl-1-undecenes[b]	210	100, 69
		2,4,6,8-Tetramethyl-1-undecenes[c]	210	100, 69	2,4,6,8-Tetramethyl-1-undecenes[c]	210	100, 69
		2,4,6,8-Tetramethyl-1-undecenes[d]	210	100, 69	2,4,6,8-Tetramethyl-1-undecenes[d]	210	100, 69
Polystyrene	PS	Styrene	104	104	Styrene	104	104
		3-Butene-1,3-diyldibenzene	208	91	3-Butene-1,3-diyldibenzene	208	91
		5-Hexene-1,3,5-triyltribenzene	312	91	5-Hexene-1,3,5-triyltribenzene	312	91
Polyvinyl chloride	PVC	Benzene	78	78	Benzene	78	78
		Chlorobenzene	112	112	Chlorobenzene	112	112
Poly(methyl methacrylate)	PMMA	Methylacrylate	86	55	Methylacrylate	86	55
		Methyl methacrylate	100	100, 69	Methyl methacrylate	100	100, 69
Polyamide	PA6	ε-Caprolactam	113	113	ε-Caprolactam	113	113
			127	127	N-methyl caprolactam	127	127
Polyethylene terephthalate	PET	Vinyl benzoate	148	105	Dimethyl terephthalate	194	163
		Ethyl benzoate	150	105			
		1,1-Biphenyl	154	105			
Polycarbonate	PC	Bisphenol-A	213	228	p-Methoxy-tert-butylbenzene	242	164, 149
					2,2-Bis(4'-methoxy-phenyl)propane	256	256, 241
MDI-polyurethane	MDI-PUR	Diphenylmethane diisocyanate	250	250	4,4'-Methylenbis(N-methylaniline)	226	226
					N,N-Dimethyl-4-(4-methylamino)benzylaniline	240	240
					4,4'-Methylenbis(N,N-dimethylaniline)	254	253, 254

M: molecular ion; m/z: mass to charge ratio.

[a] For quantification more than one homologues of α,ω-alkenes are used, e.g., C$_{16-26}$[263;279] or C$_{11-14}$.[282]

[b] Isotactic.

[c] Heterotactic.

[d] Syndiotactic.

Online py-GC-MS. Curie point (CP) or microfurnace pyrolyzers guarantee a high pyrolysis temperature precision and are therefore preferred towards filament pyrolyzers for quantification purposes. Online py-GC-MS requires an adequate, sample-adapted removal of accompanying natural inorganic and organic matter. This "matrix" removal is similar to spectroscopic methods and includes filtration cascades, density separation as well as enzymatic or chemical oxidation processes, and their optional combinations. Subsequently, preconcentrated samples are transferred into a pyrolysis target and ready to be measured with py-GC-MS under defined, reproducible conditions. Residual organic matrix can be tolerated to a certain extent. Online pyrolysis has the advantage to enable on-line pyrolytic derivatization as an additional tool. Thermochemolysis, e.g., by addition of tetramethyl ammonium hydroxide (TMAH) solution, is such a reaction that induces ester- and ether-cleavage followed by methylation.[293] The direct pyrolytic products of PET show high diversity and different polarity. This leads to limited sensitivity and poor chromatography. Thermochemolysis of PET results in almost one single pyrolysis product with remarkably enhanced detection sensitivity. Same holds true for PC.[263] The new indicator products generated by thermochemolysis for affected polymers are given in Table I. So far, quantification of PE, PP, PS, PET, PVC, PMMA, PC, PA-6, and methylene-diphenyl-diisocyanate (MDI)–PUR was successfully performed.[263,279] In MP quantification with py-GC-MS, pre-concentration steps determine the final sample volume and initial sample volume can be adapted to the expected content of MP and respective calibration range.

TED-GC-MS. In TED-GC-MS, pyrolysis is conducted with a thermogravimetric analyzer (TGA). This analyzer enables the pyrolysis of a sample under controlled and temperature-ramped conditions. The TGA is coupled to a thermo adsorption–desorption unit, containing a solid phase adsorber. The temperature range of the trapped gases can be selected in advance, e.g., 25–650 °C, representative for all volatile pyrolysis products, or 350–600 °C to cut out a large share of pyrolysis product generated by more thermo-labile organic matrix compounds, since most common polymers have degradation temperatures above 350 °C. The unit is mounted to a GC-MS system where the pyrolysis products are measured after quantitative thermo-desorption. TED-GC-MS was successfully established for PE, PP, and PS quantification[278,280,281] as well as for identification of tire wear and natural rubber particles.[282] The special assembly of TED-GC-MS with a sample capacity up to 100 mg enables a direct measurement of selected MP polymers in a given environmental sample, provided their respective concentration exceeds 0.5–1 wt%. However, sample-dependent organic matrix still can severely hamper quantification and an adsorption cut out below 350 °C leads to losses of more thermolabile polymers like PVC. Here, like in online pyrolysis, MP pre-concentration per filtration and other

sample pretreatment steps enhance detection and quantification sensitivity.

Benefits of Using Pyrolysis GC-MS. Currently, mass spectrometry is one of the most applied techniques in analytical chemistry for most (in)organic contaminants and can be considered a gold standard for many applications. Therefore, many laboratories already have and routinely use the various specialized GC-MS systems and can cost efficiently add the pyrolysis component to the existing GC-MS systems. However, a pyrolysis unit is not only another injection system, but provides its own well thought out running and maintenance procedures. Complex environmental samples accompanied with (residual) organic matrix might introduce interferences that need a prudent principle of operation. Therefore, a separated system is preferred for py-GC-MS applications. Depending on the targeted sample (single particles versus complex sample matrix), py-GC-MS requires practically no sample preparation except for the cutting of a tiny piece of sample for analysis for single plastics identification. However, if studies are restricted on this type of analysis, FT-IR, Raman, or EGA-MS systems are preferred regarding time efficiency and budget unless there is no further interest in additives and other chemicals.

If spatial and temporal MP analysis of more complex, or even several types of environmental samples is in the focus of interest, thermal decomposition GC-MS methods are those of choice. The generated data can further be used for mass balances and modelling. If the expected load exceeds 0.1% w/w of single plastic types, respectively, TED-GC-MS might be an appropriate method; at these concentrations, a reliable detection of selected polymers is possible from sample amounts down to 20 mg without any further sample treatment or with a thermal pre-treatment to reduce the organic matrix "noise". Nevertheless, for (much) lower (micro-) plastic concentrations as expected for most environmental samples, e.g., biota, sediments, river-, coastal-, and wastewater, an adequate and often comprehensive MP pre-concentration and/or pre-treatment is required. For drinking water and open sea waters, filtration might be sufficient for MP analysis with any thermal decomposition method. However, pyrolysis generates often unpredictable pyrolysis products. Any residual organic matrix compounds cause partly nonvolatile compounds that might interfere with those of interest. These aspects and viable solutions are discussed in other studies.[263,279]

Although py-GC-MS and TED-GC-MS are destructive analytical methods that hampers any re-measurements, the resulting pyrograms can be re-analyzed retrospectively for further indicator ions of new polymers. When internal standards are used,[279] even semi-quantitative data of these new polymers might be calculated.

Autosamplers are common equipment for py-GC-MS/TED-GC-MS systems and enable analysis of samples

sequences that should include an appropriate number of calibration standards, samples, and procedural blanks. Occasional blank cups should be run to be aware of possible memory effects. Strict pyrolyzer and GC-MS maintenance intervals are obligatory and avoid obscured results. Even though, mass spectrometry offers selected ion monitoring (SIM) mode processing to enhance the detection sensitivity (that provides potential regarding LOQs and LODs), its application should be regarded with caution in py-GC-MS practice. Working in MS-full scan mode combined with chromatographic retention time assures compound identification; additionally, the presence of further polymer specific degeneration products ensures polymer identification. All these details hamper an efficient cost analysis. While systems start at similar costs as high developed Raman and FT-IR systems (approximately US$200k–300k), the individual costs per measurement are difficult to calculate as they depend on the quality assurance and quality control (QA/QC) procedures and system used in the laboratories.

Since thermal degradation methods provide quantitative masses of single polymer types, independent of any particle appearance, they are not competitive but complementary techniques to FT-IR and Raman methods that result quantitative particle size related counts. Ideally, both techniques are conducted consecutive (nondestructive before destructive) to receive as much comprehensive information and data quality as possible.

Additional Techniques

The techniques described above are currently recommended for monitoring by GESAMP.[226] Still, there are further techniques suitable for the analysis of MP, which are already in use or have been tested in literature. These techniques will be described in appropriate detail in this section discussing their application and potential for MP analysis.

Hyperspectral Imaging. Remote sensing from airplanes and satellites utilizing hyperspectral imaging (HSI) allows for fast and, relatively speaking, inexpensive analysis of large areas to monitor the environment.[294,295] This technology has been transferred to other applications including microscopy and spectroscopy. When applied to microscopy, it could help in identifying MP[296–300] or nanoplastics (NP). When applied to spectroscopy, it could help in identifying chemistry and structure of, e.g., organic compounds, biological materials, minerals, semiconductor and photovoltaic materials, polymers, and plastics.[301–304]

An HSI system records a hypercube (e.g., Fig. 2 in Lyon et al.[301]), which combines spatial and spectral information in one data set. The spectral information is then analyzed to extract chemical information (or other material characteristics such as crystal phases, alloy compositions, and elemental compositions) or enhance spatial contrast. In the case of MP, hyperspectral imaging microscopy can be used to

distinguish plastics in a variety of environments including in tissue,[305] on filter membranes,[296] and in soil.[298]

In a mode of hyperspectral microscopic imaging, the sample is illuminated using a broadband light source. The resulting light (in transmission or reflection) is collected and imaged on the slit of a spectrograph. An automated stage is used to sweep the sample along one axis, while spectral data are collected along the perpendicular axis (commonly termed "push broom" scanning). The resulting hypercube can be used to discriminate plastics from other types of particulate, and multivariate analysis can help in further analyzing the spectral signatures.

An HSI system (spectrograph and detector) can be exceedingly small, so it may be easily added to other microspectroscopy systems (e.g., Raman), creating multimodal spectroscopy systems without compromising the performance of either technique. This allows application of co-localized microspectroscopy and data fusion. Frequently, HSI is used to rapidly screen and discriminate for micro- or nanoparticles followed by a more definite chemical identification using, for example, Raman spectroscopy. HSI systems typically cost around US$40k to US$120k, while the LOD needs still to be investigated.

Scanning Electron Microscopy. The application of scanning electron microscopy (SEM) is known since the 1930s. It allows the investigation of size, shape, crystallography, and other physical and chemical parameters of surfaces and particles down to a few hundred nanometers in size.[306] Via a focused electron beam directed onto the target surface, the formation of backscattered electrons (BSE) and secondary electrons (SE) is caused, which are collected by special detectors. The resulting count will be represented in a grayscale image of the electron signal.[306] The instrument normally operates in high vacuum ($<10^{-4}$ Pa) with insolating samples coated by a metal or carbon to provide electric discharge. Untreated samples can be investigated using variable pressure SEM (VPSEM), which have a chamber pressure of 1 Pa to 2000 Pa and use atmospheric gas or water vapor.[29,306]

Both types are currently used in MP research, either for surface studies, particle investigation,[101,103,110,174,189,206,208,219,223] weathering,[142] as well as the influence of artificial weathering or biodegradation[169,307] and material identification[29,93,98,143,154,167,205,308] with the help of an energy dispersive X-ray spectrometer (EDS). EDS is an extra module connected to the setup and considered a combined technique, which measures the X-ray radiation emitted from the surface during beam–surface interaction.[306] This X-ray beam contains the emission characteristics of the targeted elements, which yield a fingerprint spectrum. Subsequently, this spectrum is compared against a database. Relative quantification is possible when the instrument is calibrated for the specific elements. Light elements such as H, C, N, and O are more difficult to determine accurately

than metals, and for nanosized particles, adsorbed humidity or surface oxidation may additionally affect the elemental ratios. During scanning, amorphous carbon may be deposited onto the sample from residual organic contamination in the chamber or within the sample, which causes charging and may hamper analysis.

The cost of such instruments ranges from US$120k for benchtop models up to US$175k to 800$k for better systems depending on the desired application. To allow the identification using EDS, additional instrumental cost ranging from US$40k to US$80k is necessary. While SEM can measure down to the nanometer range, this is often accompanied with increasing measurement times and an appropriate sample preparation while the identification using EDS may be hampered.

Size Exclusion Chromatography. For polymer science, one of the most applied techniques to determine the molecular weight distribution is size exclusion chromatography.[309] It is based on high-performance liquid chromatography (HPLC) and uses a porous column material for separation. These materials consist in most cases of crosslinked polymers and different pore sizes, which can be combined to a larger setup consisting of a series of columns. In principal, the dissolved polymer chains can diffuse in and out of the pores. Depending on their size, the number of available pores gets limited as large chains can only enter large pores, but short chains enter nearly every available pore. The set of columns defines the upper and lower exclusion limit, as depending on the largest available pore size and the smallest molecule separable. Only sizes between these borders can be successfully characterized. Moreover, each polymer has its own separation behavior due to its hydrodynamic volume in the solvent, and therefore, a calibration of the system with narrowly distributed polymers is mandatory. The separation is followed by a set of detectors, which is in general a refractive index (RI) detector to determine the concentration of the chains. For further analysis, IR, ultraviolet–visible (UV–Vis), and fluorescence detectors can be added to monitor functional groups. To obtain direct information on molar mass, multi-angle laser light-scattering (MALLS)-detectors and online viscometers are available but the price of the instrument is also increased. High-temperature SEC, which is used for polyolefins, uses often online viscometers accompanied by an IR detector instead of a RI detector.

For MP, this technique is often used in addition to screen the polymers for the presence of short polymer chains from weathering,[114,115,142] to understand the mechanisms behind particle fragmentation. Biver et al.[310] tested SEC for the application to identify PS and PE using fluorescence detection while Elert et al.[179] used it for quantification of dissolved polymers against a calibration.

The cost shows a high variety of cost depending on the applied eluent, temperature, and detector systems and can range from US$50k to US$300k or even higher with a permanent demand of high-quality eluent for analysis. The LODs for MP are currently limited and dependent on the calibration (0.02 mg/ml for PS, 0.54 mg/mL for PE;[310] 0.5 mg/mL for PS, 1.0 mg/mL for PET[179]) and used detector systems. Second, SEC measures mainly the polymer chain length distribution rather than perform a chemical identification, which may hamper the analysis of complex samples. This might be overcome by the use of 2D separation techniques or coupling with MS,[311] which are even more expensive.

Nuclear Magnetic Resonance. The nuclear magnetic resonance (NMR) is currently one of the standard analytical tools in chemistry.[312] This technique uses the nuclear spin I of an atom which can take values of 0, ½, 1, 3/2, etc. Within an external magnetic field, which is commonly generated by a superconductive magnet, this spin can yield $2I + 1$ energy levels compared to the absence of a magnetic field. Depending on the investigated nucleus, these energy levels can be exited using radio waves. The performance of NMR-spectrometers is stated by the Lamor frequency of the hydrogen atom, the simplest and most measured nucleus for NMR. This frequency is depending on the strength of the magnetic field (e.g., 4.7 T for 200 MHz). By Fourier Transformation methods, a range of frequencies is measured and the relative shift to an internal standard determined in parts per million (ppm). For hydrogen, ranges from −1 to 12 ppm are possible and the experiments need careful planning due to the complexity of this method.[312] This method is currently available in many chemical analytical labs and can also be used for the investigation of polymers.[313]

There are only few publications using 1H-NMR within their study on or for effects by MP.[115,135,314] Only Peez et al.[314] reported on the application for the identification and quantification of MP via this method with a LOD of 30 µg/mL for PE, PET, and PS. While these instruments are available in most chemical labs, they have quite high cost ranging from simple benchtop systems (~US$100k), which have a rather low resolution over US$200k up to US$1000k for high-performance instruments. Additionally, deuterated solvents are necessary to allow a measurement with these techniques. High-performance machines further need a supply by liquid nitrogen and helium to achieve the high magnetic field strengths.

Inductively Coupled Plasma MS. The inductively coupled plasma (ICP) combined with mass spectrometry is a specialized case for the analysis of MP as its main focus is the analysis of trace metals. Within the formed plasma, the sample is ionized and broken down to atomic level allowing the measurement of elements with a higher sensitivity compared to atomic emission spectrometry.

For MP, this technique is mainly used to investigate heavy metal contaminations on MP from the environment due to their potential role as transport vector for this and all kind of contaminants.[93,205,247,315–317]

Application of Combined Techniques for MP Analysis

The use of combined and correlative methods has been proven to be essential for the accurate identification of MP in different matrices as exemplary shown in Fig. 8.[29,228,308,318]

Commonly employed techniques cover millimeter down to nanometer sizes and include optical microscopy with stereo zoom, SEM–BSEM with EDS, py-GC-MS as well as FT-IR, and RM. Being a simple and fast method, optical microscopy was used for coarse evaluation of the extraction procedure and prescreening of plastics to decrease work effort. Despite its relatively low magnification, optical microscopy was employed to distinguish between fibers, plastic, and nonplastic particles based on their color, shape, and surface texture as well as to study their sizes, fragmentation, and adhered species.[29,165,308] However, some nonplastic particles or biological species can optically resemble the morphology and size of plastic materials resulting in possible misidentification, requiring more accurate MP identification methods.[29,308,318–320] For example, microbeads, manufactured fibers, or PE particles in marine samples were correctly labeled as diatoms, broken spines, or shell fragments using high-resolution surface
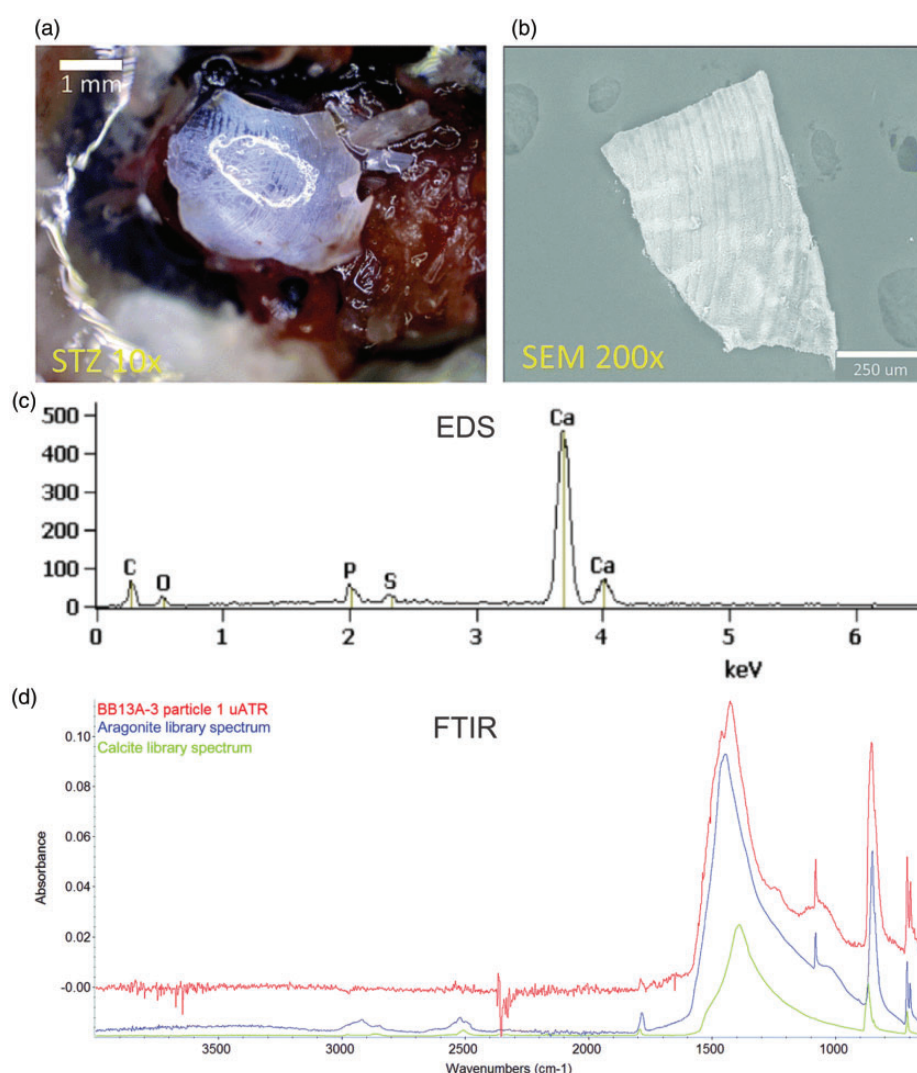
Figure 8. Example of combined techniques used to distinguish between non-plastic and plastic particles. (a) Stereozoom optical image of a myctophid gut with a broken shell fragment resembling a polyethylene particle. (b) Scanning electron microscopy (SEM) image showing parallel striations characteristic to calcium-based structures. (c) Energy dispersive X-ray spectroscopy (EDS) spectrum indicates the presence of Ca and the negligible signal of C. (d) FT-IR spectra confirm unambiguously aragonite and calcite. These measurements were conducted on different particles of the same type. Adapted from Wagner et al.[29] with permission from The Royal Society of Chemistry and Wang et al.[309] with permission from Elsevier.

morphology by SEM combined with elemental analysis by EDS based on their Si, Sr and S, and Ca signals, respectively.[29,308]

Techniques such as SEM, BSEM, EDS were also employed to examine the surface of MPs for cracks, geometrical regularity, adhered pigment particles, biofilms, and micro-organisms, regardless of high costs for the instrument and time-consuming investigations.[29,223,308] The presence and density of cracks indicate plastic degradation that can occur through polymer aging as well as mechanical and oxidative weathering depending on the environmental local conditions.[142,174] Particles and fibers with irregular edges are mostly considered degradation fragments resulting from the mechanical breakage of larger plastics.[154,321] Composite pigment–plastic particles are likely manufactured materials. The adhered surface species can mainly be separated based on brighter BSEM contrasts and distinct EDS spectra.[29,308] Similar to optical microscopy, false positives are possible, since only the PVC and polytetrafluoroethylene (PTFE) particles were confirmed from the chlorine and fluorine elements, respectively, by EDS, while other MP types show carbon-dominant EDS spectra.[154,308,318]

Thermodegradation methods like py-GC-MS can be used to simultaneously identify the type of polymer and organic additives, without a signal overlap from inorganic contaminations. However, this mainly works when distinct polymers exhibit separately different pyrolysis products and the sample mass to overcome the detection limit for a particular polymer type.[158,275] Therefore, FT-IR and Raman microspectroscopic techniques have to be applied to unambiguously detect the plastics, supporting or disproving the results by optical and electron microscopy, as well as py-GC-MS.

Moreover, the number of MPs found by Raman in identical samples is higher (up to two times) as compared to FT-IR, since the particle concentration increases substantially with decreasing MP size, e.g., through environmental fragmentation. On the other hand, FT-IR imaging needs less time in total (up to four times) than Raman for the same scanned filter area due to the large number of particles present.[40] However, the reduction of non-MP particles prior to analysis by means of efficient pre-treatments, Raman might be faster than FT-IR imaging for a small number of particles. Despite these differences, FT-IR and Raman are currently the most accurate and reliable methods for MP identification, being supported by extended spectral libraries and automated particle localization, despite expensive instruments and laborious work.[40,242] In this context, it is worth considering of hyphenated SEM-EDS-Raman systems that have already been applied to other environmental pollutants. Such instruments will enable successive visual and spectroscopic measurements of the same plastic objects, while excluding any changes due to electron beam and laser exposures.[322,323]

Nanoplastics Outlook

Numerous studies report trends in MP abundances towards particle sizes in the small micron regime.[22,40,52,72,73,233] There are first indications of NP (1–999 nm[4]) formation from MP through digestion by krill,[324] as well as a multitude of studies suggesting adverse effects of NP on diverse organisms from marine species[325] to humans.[326] Thus, the question of reliable identification and quantification of NP becomes more urgent every day.

While a range of techniques is available and in use for characterizing and identifying inorganic nanomaterials from environmental samples,[327] there are less options when it comes to plastics due to low or no crystallinity of the materials and the limited elements incorporated, which are light in molar mass and ubiquitous in the surroundings (C, H, N, O).

The following sections summarize methods with reported application in the characterization of nanoscale polymers and especially environmental samples.

Detection Using Single Methods. The least expensive (<US\$120k) and fastest (<5 min/sample) techniques to detect nanoparticles in solution use laser scattering to determine size and concentration of the particles in solution. Dynamic light scattering (DLS) examines the change in laser intensity as particles move past the beam by diffusion. Due to differences in scattering behavior between particles of different sizes, larger particles mask small ones in polydisperse samples, so that no accurate size distribution is obtained. Static light scattering (SLS) returns the molecular weight and concentration-dependent intensity of scattering at fixed angles. Results from both methods can be enhanced for polydisperse samples by measuring at multiple angles (MA(D)LS), as the scattering angle from a particle is related to its size. The first article reporting NP in surface water from the North Atlantic Subtropical Gyre took DLS results as an indication of nanoparticles in the filtered and concentrated sample.[277] The authors point out that the particles were polydisperse and that the low concentration prevented an average size analysis. A typical lower detection limit for DLS is 10^8 particles/mL.

Another laser-based method is nanoparticle tracking analysis (NTA). In a flow cell, the Brownian motion of single particles passing a laser beam is filmed through a microscope unit and converted into particle size by image analysis algorithms. NTA recorded NP formation from plastic products in aqueous solution under UV-irradiation.[328,329] Costs are approximately US\$60k upwards depending on chosen lasers and accessories. NTA provides concentrations and size distributions even for polydisperse samples in the range of 10–2000 nm at concentrations of at least 10^7–10^8 particles/mL. In context with engineered nanoparticles, there has been some debate and suggestions for strict protocols to guarantee the reproducibility of results, as more parameters have to be optimized than in the case of DLS.[330–333]

New developments in flow imaging promise a lower detection limit of 300 nm with single-particle detection. The equipment has a prize of >US$125k and so far, there is no report on its application for small MP and NP.

Single-Particle Spectroscopy

Several new promising technologies making single NP spectroscopy accessible have yet to prove their full potential in the analysis of unknown environmental nanofractions.

Recently, Raman tweezers were applied to capture and spectroscopically identify single particles in the range of 50–20 000 nm directly in distilled and seawater.[334] Here, light traps the particles and Raman spectroscopy is possible with two irradiation wavelengths (633 and 785 nm). Apart from polymer reference materials, the authors tested PE particles synthesized in the presence of a "biosurfactant" and were still able to identify the material. Quantification is envisaged but will require the storage and analysis of vast amounts of collected images and data to be representative.

Scanning electron microscopy–Raman (see also combined methods) has a resolution of approximately 200–300 nm and is capable of sizing and identification of single particles. With this combination of methods, flame retardants and a larger PE fragment were identified in environmental samples.[323] The most severe difficulties that may occur when combining these methods in NP analysis are carbon deposition on the sample during SEM, challenges in relocating the same particle when switching, and a need for vacuum conditions in the SEM chamber.[322,335] The latter two depend on the specific equipment and set-up.

Scanning Probe Microscopy Coupled to Spectroscopy. AFM-IR or (photothermal induced resonance), nano-FT-IR, and AFM-TERS are techniques that couple atomic force microscopy (AFM) with spectroscopy to break the diffraction limit.[336] All three methods find application in the characterization of polymer thin films and composites.[337] In the first case, an IR beam is focused onto the sample, either from above in reflection or from below, to cause rapid local heating and expansion at wavenumbers that excite molecular vibrations in the polymer. The probe is in contact with the sample surface and registers the local expansion during tuning of the wavelength of the laser irradiation.[338] The expansion is correlated with the absorption coefficient at the respective wavenumber and plotted as an IR spectrum. Polymer wires of 100 nm in diameter on a flat and IR-transparent ZnSe substrate,[339] as well as polymer particles of 170 nm inside cells were detected by point spectroscopy and monochromatic imaging.[340]

Nano-FT-IR is based on scattering type near-field optical microscopy (s-SNOM). Here, the IR-beam is focused on the tip and a local antenna effect creates a nanoscaled focus with the dimension of the tip. The tip taps along the surface while scanning, leading to periodic changes in near field interactions occurring between tip and sample. When combined with a broadband laser source and an asymmetric Michelson interferometer, these changes can be detected and mathematically converted into IR spectra. Polymers are analyzed with a local resolution of 20 nm, allowing for spectroscopy on single polymer particles with dimensions <50 nm.[341,342] Successful polymer identification was demonstrated in conjunction with commercial or siMPle analysis software.[343]

Atomic force microscopy–tip-enhanced Raman spectroscopy (TERS) is a Raman pendant to nano-FT-IR when conducted in reflection mode on an opaque sample substrate.[344] Bottom and top illumination configurations are available as well. The resolution is also dominated by the tip radius, reaching ~ 20 nm in transmission through a polymer blend film.[345] Currently, there is no report on small polymer particles detected this way.

The aforementioned single-particle techniques come with certain requirements for sample preparation. Particles have to be deposited on appropriate filter substrates that do not hamper spectroscopic identification with their own signal, are flat, but at the same time allow proper sample fixation. In some SEM-Raman, set-ups particles must be relocated when moving from one technique to the other, which becomes more difficult with smaller particle sizes. Sample purity is another critical factor.[322,335] Additionally, carbon deposition during the SEM analysis may similarly hamper identification due to fluorescence. In probe-based techniques, contamination of the tip by residues and mechanical wear are critical, which may present a significant factor regarding measurement time and costs, depending on the characteristics of the samples. TERS probes are even more delicate.[344]

With prizes well in the six-digit region, these techniques are comparatively expensive, and measurements can be time-consuming, but offer information of not only identity but also the possibility to examine associated components and local distributions of polymers.

Values for environmental concentrations of NP are currently not available. In order to obtain valid results, a combination of pre-concentration and several detection methods will most likely be the best solution.

In a study be Ter Halle et al.[277] on aqueous samples from the North Atlantic Subtropical Gyre, even a hundredfold up concentration by ultrafiltration did not yield a concentration high enough for unambiguous detection by DLS. Several approaches to concentrate and separate the nanofraction from environmental samples were tested with a focus on inorganic or other colloidal and recently NP components, as reviewed in Tiede et al.,[346] Lespes and Gigault,[347] and Schwaferts et al.[259] Here, we mention three recent examples for different strategies: (i) Fractionated filtration with membrane and syringe filters was applied to size-separate polymer particles in facial scrubs. The fractions were examined in bulk by analytical methods including dynamic light scattering (DLS), SEM, X-

Table II. Cost estimation for various analytical techniques ranging from optical methods towards chemical analysis.

Methods	Unit	Naked eye	Optical microscopy	Nile red staining	Flow cytometry	Flow imaging	FT-IR qualitative	Particle based μFT-IR	μFT-IR imaging	Raman qualitative	Particle based μRaman	py-GC-MS qualitative	Quantitative py-GC-MS	TED-GC-MS	HIS	SEM-EDX
LOD		1 mm	100 μm	3–20 μm	500 nm	2 μm	>300 μm	25 μm	10 μm	>300 μm	1 μm	~1 μg IP	<<1 μg PD	<1 μg PD	NA	nm
Instrument costs[a]	$k	<1	2–3	2–50	>50	>130	25–50	100–125	200–250	50–100	200–400	>150	>215	>250	40–120	>100
Special consumables				Dye and solvent	Cleaning solutions	Cleaning solutions		Liquid nitrogen	Liquid nitrogen			GC-columns and filaments	GC-columns and filaments	GC-columns and filaments		Sample coating
Field applicability		Good	Good	No	No	Possible	Handheld	No	No	Handheld	No	No	No	No	Device dependent	No
Limitations		NoID	NoID, NoM, PA/SA	NoID, NoM, PA/SA	NoID, NoM	NoID, NoM		TA, NoM	TA, NoM		PA/SA, NoM		NoN, NoS	NoN, NoS	LID	LID
Automated data evaluation		No	No	No[d]	No	No	No	Yes	Yes	Yes[e]	Yes	No	No[f]	No[f]	No	No
Measurement time[b]	min	1	60	35	30	30	1	360	240	2	2580–>10 000	35–120	120	120	5	120
Data Analysis time[b]	min	NA			5	5	1	60	360	1	1	5–10	60[g]	60[g]	5	60
Working time[b]	min	1	60	35	48	48	2	120	60	3	60–580	5	30 (qual.)[g] 72 (quant.)[g]	30 (qual.) 72 (quant.)[g]	10	180
Typical fractions per sample		50 P	7 F	7 F	3 REP	3 REP	50 P	1 F	1 F	50 P	1 F	50 P	1–5 CQ	1–5 CQ	1 F	1 F
Instrument availability for analysis[c]	d	261	261	261	237	249	250	250–261	250–261	250–261	250–261	250	250	250	NA	NA
Average working time per sample	min	PND	420	245	144	144	PND	120	60	PND	60	PND	72–216	72–216	10	180
Field of application		MD, MO	MD, MO, R	MD, MO, R	MD, MO, R	MD, MO, R	MD, MO, R, RA, RE	MD, MO, R, RA, RE	MD, MO, R, RA, RE	MD, MO, R, RA, RE	MD, MO, R, RA, RE	MD, MO, RE	MD, MO, R, RE	MD, MO, R, RE	MO, RE	MD, RA, RE

[a]Raw estimates which may strongly vary dependent on the country.
[b]Calculated for one filter/particle per analysis.
[c]Working days (normal work hours/days, maximal 261 if a 2 days weekend applies) exclusive instrument maintenance time.
[d]Image analysis possible.
[e]For Raman microscopes.
[f]Autosamplers are available.
[g]Calculated based on a micro-furnace system with an average sequence size (6 standards, 10 samples).

CQ: pyrolysis cubs or quartz tubes; F: filters; IP: isolated particle; LID: limited chemical identification; MO: monitoring; MD: modeling; NoID: no chemical identification; NoM: no mass determination; NoN: no particle number determination; NA: no information available; NoS: no particle sizes determination; R: routine; RA: risk assessment; RE: research; PA/SA: partial analysis/subsampling analysis on filter; P: particle; PND: particle number dependent; REP: replicates; TA: total absorption.

Identification and Quantification of Microplastics
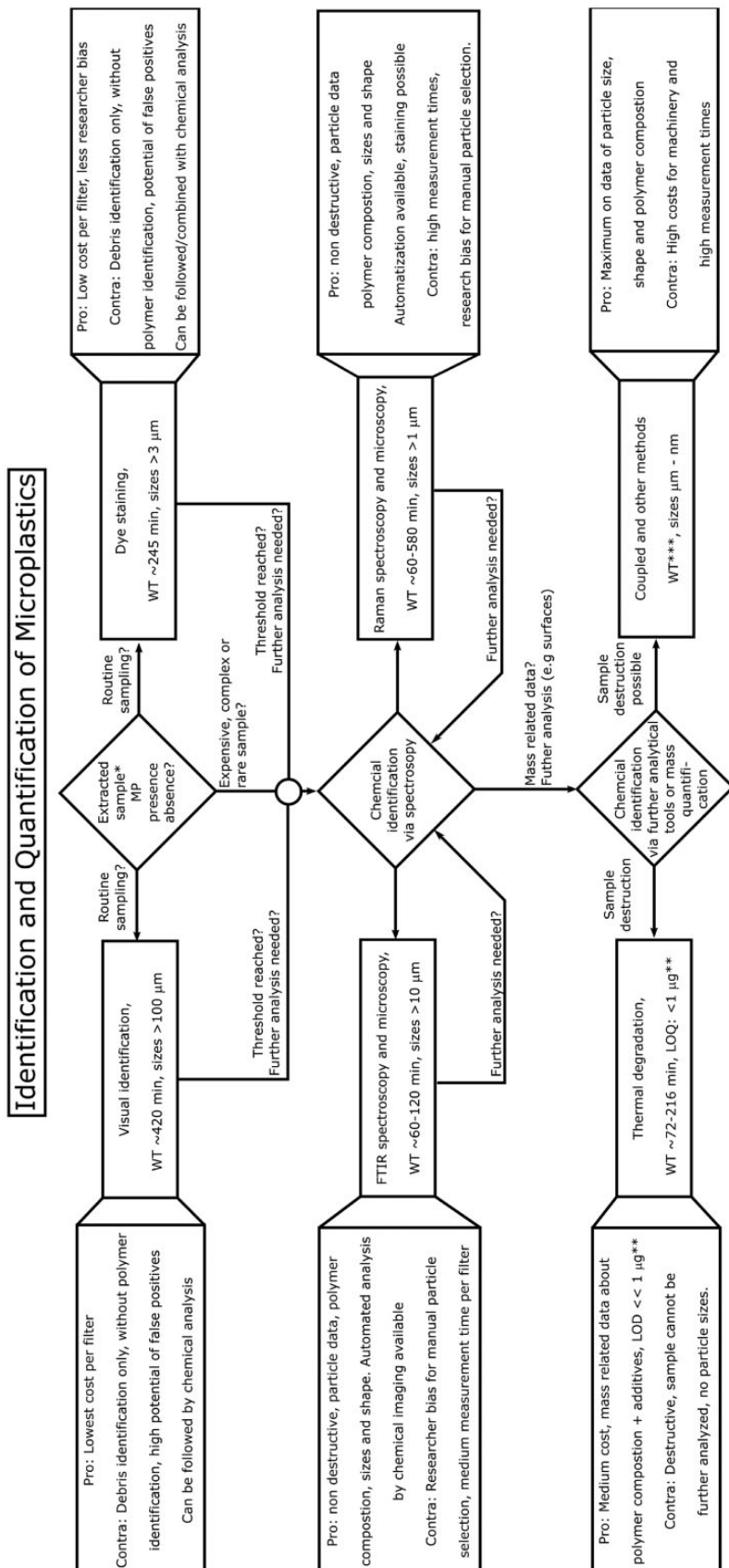
Figure 9. Flowchart decision profile for study planning on microplastics via available identification techniques. WT: Working time to measure a full sample with this technique. *All analytical techniques require a sample extraction which add additional costs. **Depends on the measured polymer type. ***Working time was difficult to estimate based on the data available.

ray photoelectron spectroscopy (XPS), and FT-IR.[88] (ii) A first study on extraction efficiencies of fluorescent PS beads from soil and biosolids by flotation with $ZnCl_2$ solution indicates a reduction in efficiency with smaller particle size and a further decrease by matrix digestion with H_2O_2.[348] Ultraviolet–visible spectroscopy was applied to determine the concentration. This method is comparatively cheap (<US$120k), but its accuracy relies on the colloidal stability of the examined particles in solution and it does not provide information on the size distribution. (iii) To analyze polymer particles in drinking water, a sequence of concentration by crossflow filtration, asymmetrical flow field-flow fractionation (AF4) with UV-spectroscopic and MALLS detection was proposed.[192] AF4 with MALLS was combined with a prior enzymatic digestion to recover and detect standard PS particles mixed with homogenized fish.[349] With commercial PE beads utilized under similar conditions, the difficulty in finding universal procedures and appropriate reference materials became apparent. The particles had to be stabilized in solution with a detergent, which caused a significant background in the light scattering signal of eluate from the separated sample.

A special challenge for quantitative assessment of small MP and NP is quality assurance of extraction processes and analytical techniques. Commercially available reference materials such as PS beads that are frequently applied in ecotoxicological studies are far away from what is expected in environmental samples in terms of size, shape, and surface functionalization. First approaches to produce materials with rougher shapes and oxidized or bio-functionalized surfaces rely on top-down laser ablation, UV light degradation, or milling of larger plastic fragments such as MP from environmental samples[350–352] or bottom-up chemical synthesis by emulsion polymerization or re-precipitation from aqueous suspension.[353–355] Labeling such particles with metals such as Pd and Ir[354] or rare earth elements[356] allows their detection by further methods such as two-photon excitation, time-resolved (TPE-TR) optical imaging, and single-particle ICP-MS.

For advancing towards detection and monitoring of nanoplastics, there are several important points to address. First comes contamination prevention. While many MP contaminations are easily identified visually or in the analysis, monitoring and preventing NP contamination is an even more time and cost-intensive task. On top of this, particle emission from filter membranes and the equipment require careful selection and testing before application: even ultrapure water produced in the lab may contain concentrations of nanoparticles close to environmental conditions, depending on the age of the filter with its 220 nm pores. Glass syringes and bottles can lose significant numbers of particles (own experiments). Costs of non-emitting filter materials such as Si membranes with 1 μm pore size currently range at approximately US$30–50 per filter. Flow filtration systems have to be designed with a minimum or no content of plastics that could contaminate the sample, which additionally increase material costs and design efforts.

Another aspect concerns the development of strategies to generate statistically meaningful data. Measurement times are long especially for single-particle methods (up to minutes per particles) so that only subsamples can be analyzed. Additionally, large amounts of generated data and their management will be similar issues as in MP analysis and may therefore profit from developments in this field.

Conclusion

The analysis of MP is challenging. Guidelines[226,357–359] and citations herein, as well as reviews[243,318,360–364] describing sampling and sample extraction are available from other expert groups. Most of these focus on detection limits and sample extraction efficiencies together with related problems but, if at all, only partly on potential costs. For MP analysis, these costs combine sampling (instruments, personnel, and vessel costs), sample extraction and identification (each: instruments, consumables, personnel, and infrastructure). Here, besides all other important information, we attempted to include estimated costs for instrumentation and consumables among different identification methods to get a more complete picture. All costs given were rough estimates only, conducted by the authors and may differ strongly depending on country and manufacturer. Further, we included the working time in minutes (see Table II), as these costs are linked to a high variety of parameters (e.g., country, infrastructure, sample complexity).

Each method shows advantages and disadvantages, as illustrated comprehensively. In the end, overall costs might be a crucial factor to be considered. Using the naked eye, a cheap method is suitable for characterization of large particles (>1 mm) and applicable in the field. Light microscopy, on the other hand, can be used for smaller particles but does not provide chemical identification, even if supported by staining with fluorescent dyes. In addition, to analyze a full sample many hours of microscopy and extraction need to be conducted and several filters may have to be investigated. This increases the working time rapidly and limits the applicability especially for commercial laboratories compared to other techniques. Due to its low initial costs, it is optimal for monitoring and routine measurements.

Conversely, flow cytometry and flow imaging provide both a fast measurement of particle or aggregates numbers and sizes and lesser working time compared to optical imaging. Liquid samples can be measured directly, without additional extraction steps.

However, reliable polymer identification requires higher up-front costs for instruments and can lead to longer measurement times. The available chemical analysis tools complement each other, but none is a golden standard for analyzing all particle types and matrices. Raman methods

have the highest measurement times combined with high working times (lab dependent) and yield increased costs, since instruments are in use for a long time per sample. Still, Raman is the optimal method for microplastics <10 μm. For particles > 10 μm, FT-IR spectroscopy has lower measurement times per sample in total, allowing several measurements per day. While the analysis of single particles is rather time intensive, particle finder routines allow an accelerated measurement. By applying imaging techniques combined with an automated analysis approach, low work times can be achieved. Our contrasting juxtaposition illustrates that thermal degradation methods have similar working times compared to FT-IR, but several analyses have to be performed, if all size class ranges should be covered enhancing costs and working time. Second, these types of sample cannot be re-measured in the case of any instrumental/sample issue in contrast to the spectroscopic methods. In a long-term perspective, FT-IR and thermal degradation methods with high investment costs become cheaper relative to optical, working time-intensive methods. Thus, they both are suitable for monitoring, routine risk assessment, routine, and research applications.

Additional techniques such as NMR, HIS, or SEC can also be used for the investigation of microplastics, but at current state of knowledge only very inaccurate cost assumptions for NMR and HIS were possible. For SEC, the potential is unclear, as research so far is limited, but coupling with MS might be promising. From the cost perspective, SEM is an attractive alternative, but metal coating of the samples and limitations regarding light atoms are current drawbacks. Nevertheless, it is superior to the analysis of particle surfaces and therefore extremely valuable for research if applied with a second method for identification, e.g., Raman.

Currently, first legislations like the California senate bills[5,6] demand a standard operational protocol to be developed within a short time frame (less than two years). Thus, method development needs a precise focus on the relevant leading questions with the goal of a regulation. This leads to a focus on combined or harmonized use of available methods in a sensible way for the determination of threshold values to protect humans and the environment. In Fig. 9, a flowchart is presented, which addresses this point.

Analysis of MP is ideally addressed via a hierarchical structure, as the techniques are complementary and can be conducted from nondestructive to destructive methods. Here it is important that samples are measured down to the smallest defined size class, independent of the method, since smaller particles are considered more hazardous. In the scope of the above-mentioned regulations, one should start with visual identification using microscopes, staining, or flowcytometry to screen samples for particles suspected to be plastics. If their numbers within this routine screening exceeds a threshold value, detailed measurements via chemical analysis methods become necessary.

This threshold needs to be set by risk assessment for environmental or human health status, depending on the targeted regulation or monitoring program. Whatever the threshold, some validation of visual and NR observations to ensure plastic identity via, e.g., spectroscopic techniques will be obligatory.

Optical identification can be followed by FT-IR or Raman spectroscopy analysis when samples pass the threshold value. Both are typically nondestructive or harmful to the sample depending on the measurement type, while giving information on particle size, composition, and particle count. MP count is important for risk assessment and should be quantified at these steps. MP mass related data, surface properties or data from other destructive methods can be determined in the final step if needed.

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References

1. J. Frias, R. Nash. "Microplastics: Finding a Consensus on the Definition". Mar. Pollut. Bull. 2019. 138: 145–147.

2. C.M. Rochman, C. Brookson, J. Bikker, et al. "Rethinking Microplastics as a Diverse Contaminant Suite". Environ. Toxicol. Chem. 2019. 38(4): 703–711.

3. J.L. Conkle, C.D. Baez Del Valle, J.W. Turner. "Are We Underestimating Microplastic Contamination in Aquatic Environments?". Environ. Manage. 2018. 61(1): 1–8.

4. N.B. Hartmann, T. Huffer, R.C. Thompson, et al. "Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris". Environ. Sci. Technol. 2019. 53(3): 1039–1047.

5. State of California. "SB-1422 California Safe Drinking Water Act: Microplastics". 2017–2018. https://leginfo.legislature.ca.gov/faces/billTextClient.xhtml?bill_id=201720180SB1422 [accessed 2 April 2020].

6. State of California. "SB-1263 Ocean Protection Council: Statewide Microplastics Strategy". 2017–2018. https://leginfo.legislature.ca.gov/faces/billTextClient.xhtml?bill_id=201720180SB1263 [accessed 2 April 2020].

7. A.L. Lusher, K. Munno, L. Hermabessiere, et al. "Isolation and Extraction of Microplastics from Environmental Samples: An Evaluation of Practical Approaches and Recommendations for Further Harmonization". Appl. Spectrosc. 2020. 74(9): 1049–1065.

8. S. Brander, V. Renick, M. Foley, et al. "Sampling and Quality Assurance and Quality Control: A Guide for Scientists Investigating the Occurrence of Microplastics Across Different Matrices". Appl. Spectrosc. 2020. 74(9): 1099–1125.

9. M. Fisner, S. Taniguchi, F. Moreira, et al. "Polycyclic Aromatic Hydrocarbons (PAHs) in Plastic Pellets: Variability in the Concentration and Composition at Different Sediment Depths in a Sandy Beach". Mar. Pollut. Bull. 2013. 70(1-2): 219–226.

10. J. Reisser, J. Shaw, C. Wilcox, et al. "Marine Plastic Pollution in Waters Around Australia: Characteristics, Concentrations, Pathways". PLoS One. 2013. 8(11): E80466.

11. M.D. Robards, J.F. Piatt, K.D. Wohl. "Increasing Frequency of Plastic Particles Ingested by Seabirds in the Subarctic North Pacific". Mar. Pollut. Bull. 1995. 30(2): 151–157.

12. W. Cowger, A. Gray, S.H. Christiansen, et al. "Critical Review of Processing and Classification Techniques for Images and Spectra in Microplastic Research". Appl. Spectrosc. 2020. 74(9): 989–1010.

13. L. Hermabessiere, C. Himber, B. Boricaud, et al. "Optimization, Performance, Application of a Pyrolysis-GC-MS Method for the Identification of Microplastics". Anal. Bioanal. Chem. 2018. 410(25): 6663–6676.

14. K. Zhang, W. Gong, J. Lv, et al. "Accumulation of Floating Microplastics Behind the Three Gorges Dam". Environ. Pollut. 2015. 204: 117–123.

15. C. Wilcox, M. Puckridge, Q.A. Schuyler, et al. "A Quantitative Analysis Linking Sea Turtle Mortality and Plastic Debris Ingestion". Sci. Rep. 2018. 8(1): 12536.

16. F. Kroon, C. Motti, S. Talbot, et al. "A Workflow for Improving Estimates of Microplastic Contamination in Marine Waters: A Case Study from North-Western Australia". Environ. Pollut. 2018. 238: 26–38.

17. C.A. Choy, B.H. Robison, T.O. Gagne, et al. "The Vertical Distribution and Biological Transport of Marine Microplastics Across the Epipelagic and Mesopelagic Water Column". Sci. Rep. 2019. 9(1): 7843.

18. R. Di Mauro, M.J. Kupchik, M.C. Benfield. "Abundant Plankton-Sized Microplastic Particles in Shelf Waters of the Northern Gulf of Mexico". Environ. Pollut. 2017. 230: 798–809.

19. A.L. Lusher, A. Burke, I. O'Connor, et al. "Microplastic Pollution in the Northeast Atlantic Ocean: Validated and Opportunistic Sampling". Mar. Pollut. Bull. 2014. 88(1-2): 325–333.

20. R. Dris, J. Gasperi, V. Rocher, et al. "Microplastic Contamination in an Urban Area: A Case Study in Greater Paris". Environ. Chem. 2015. 12(5): 592–599.

21. R. Sutton, S.A. Mason, S.K. Stanek, et al. "Microplastic Contamination in the San Francisco Bay, California, USA". Mar. Pollut. Bull. 2016. 109(1): 230–235.

22. K.J. Wiggin, E.B. Holland. "Validation and Application of Cost and Time Effective Methods for the Detection of 3–500 mm Sized Microplastics in the Urban Marine and Estuarine Environments Surrounding Long Beach, California". Mar. Pollut. Bull. 2019. 143: 152–162.

23. M. Claessens, S. De Meester, L. Van Landuyt, et al. "Occurrence and Distribution of Microplastics in Marine Sediments Along the Belgian Coast". Mar. Pollut. Bull. 2011. 62(10): 2199–2204.

24. K.A. Willis, R. Eriksen, C. Wilcox, et al. "Microplastic Distribution at Different Sediment Depths in an Urban Estuary". Front. Mar. Sci. 2017. 4: 1–8. DOI: 10.3389/fmars.2017.00419.

25. F. Corradini, P. Meza, R. Eguiluz, et al. "Evidence of Microplastic Accumulation in Agricultural Soils from Sewage Sludge Disposal". Sci. Total Environ. 2019. 671: 411–420.

26. G.S. Zhang, Y.F. Liu. "The Distribution of Microplastics in Soil Aggregate Fractions in Southwestern China". Sci. Total Environ. 2018. 642: 12–20.

27. S. Abbasi, N. Soltani, B. Keshavarzi, et al. "Microplastics in Different Tissues of Fish and Prawn from the Musa Estuary, Persian Gulf". Chemosphere. 2018. 205: 80–87.

28. C.M. Rochman, A. Tahir, S.L. Williams, et al. "Anthropogenic Debris in Seafood: Plastic Debris and Fibers from Textiles in Fish and Bivalves Sold for Human Consumption". Sci. Rep. 2015. 5: 14340.

29. J. Wagner, Z.M. Wang, S. Ghosal, et al. "Novel Method for the Extraction and Identification of Microplastics in Ocean Trawl and Fish Gut Matrices". Anal. Methods. 2017. 9(9): 1479–1490.

30. A. Karami, A. Golieskardi, C. Keong Choo, et al. "The Presence of Microplastics in Commercial Salts from Different Countries". Sci. Rep. 2017. 7: 46173.

31. S. Abbasi, B. Keshavarzi, F. Moore, et al. "Investigation of Microrubbers, Microplastics and Heavy Metals in Street Dust: A Study in Bushehr City, Iran". Environ. Earth Sci. 2017. 76(23): 798.

32. R. Dris, J. Gasperi, C. Mirande, et al. "A First Overview of Textile Fibers Including Microplastics, in Indoor and Outdoor Environments". Environ. Pollut. 2017. 221: 453–458.

33. S.A. Carr, J. Liu, A.G. Tesoro. "Transport and Fate of Microplastic Particles in Wastewater Treatment Plants". Water Res. 2016. 91: 174–182.

34. F. Corradini, H. Bartholomeus, E. Huerta Lwanga, et al. "Predicting Soil Microplastic Concentration Using Vis-NIR Spectroscopy". Sci. Total Environ. 2019. 650(Pt. 1): 922–932.

35. M.G.J. Loder, H.K. Imhof, M. Ladehoff, et al. "Enzymatic Purification of Microplastics in Environmental Samples". Environ. Sci. Technol. 2017. 51(24): 14283–14292.

36. B. De Witte, L. Devriese, K. Bekaert, et al. "Quality Assessment of the Blue Mussel (Mytilus edulis): Comparison Between Commercial and Wild Types". Mar. Pollut. Bull. 2014. 85(1): 146–155.

37. A.P.W. Barrows, C.A. Neumann, M.L. Berger, et al. "Grab vs. Neuston Tow Net: A Microplastic Sampling Performance Comparison and Possible Advances in the Field". Anal. Methods. 2017. 9(9): 1446–1453.

38. R.R. Hurley, J.C. Woodward, J.J. Rothwell. "Ingestion of Microplastics by Freshwater Tubifex Worms". Environ. Sci. Technol. 2017. 51(21): 12844–12851.

39. W. Cowger, C. Rochman, C. Thaysen, et al. "Reporting Guidelines to Increase the Reproducibility and Comparability of Research on Microplastics". Appl. Spectrosc. 2020. 74(9): 1066–1077.

40. L. Cabernard, L. Roscher, C. Lorenz, et al. "Comparison of Raman and Fourier Transform Infrared Spectroscopy for the Quantification of Microplastics in the Aquatic Environment". Environ. Sci. Technol. 2018. 52(22): 13279–13288.

41. W.J. Shim, S.H. Hong, S.E. Eo. "Identification Methods in Microplastic Analysis: A Review". Anal. Methods. 2017. 9(9): 1384–1391.

42. X. Sun, T. Liu, M. Zhu, et al. "Retention and Characteristics of Microplastics in Natural Zooplankton Taxa from the East China Sea". Sci. Total Environ. 2018. 640–641: 232–242.

43. F. Collard, B. Gilbert, G. Eppe, et al. "Detection of Anthropogenic Particles in Fish Stomachs: An Isolation Method Adapted to Identification by Raman Spectroscopy". Arch. Environ. Contam. Toxicol. 2015. 69(3): 331–339.

44. R. Lenz, K. Enders, C.A. Stedmon, et al. "A Critical Assessment of Visual Identification of Marine Microplastic Using Raman Spectroscopy for Analysis Improvement". Mar. Pollut. Bull. 2015. 100(1): 82–91.

45. L. Van Cauwenberghe, A. Vanreusel, J. Mees, et al. "Microplastic Pollution in Deep-Sea Sediments". Environ. Pollut. 2013. 182: 495–499.

46. S. Wolff, J. Kerpen, J. Prediger, et al. "Determination of the Microplastics Emission in the Effluent of a Municipal Waste Water Treatment Plant Using Raman Microspectroscopy". Water Res. X. 2019. 2: 100014.

47. L. Van Cauwenberghe, C.R. Janssen. "Microplastics in Bivalves Cultured for Human Consumption". Environ. Pollut. 2014. 193: 65–70.

48. L. Frère, I. Paul-Pont, J. Moreau, et al. "A Semi-Automated Raman Micro-Spectroscopy Method for Morphological and Chemical Characterizations of Microplastic Litter". Mar. Pollut. Bull. 2016. 113(1-2): 461–468.

49. D. Schymanski, C. Goldbeck, H.U. Humpf, et al. "Analysis of Microplastics in Water by Micro-Raman Spectroscopy: Release of Plastic Particles from Different Packaging into Mineral Water". Water Res. 2018. 129: 154–162.

50. J.P. Harrison, J.J. Ojeda, M.E. Romero-Gonzalez. "The Applicability of Reflectance Micro-Fourier-Transform Infrared Spectroscopy for the Detection of Synthetic Microplastics in Marine Sediments". Sci. Total Environ. 2012. 416: 455–463.

51. M.G.J. Löder, M. Kuczera, S. Mintenig, et al. "Focal Plane Array Detector-Based Micro-Fourier-Transform Infrared Imaging for the Analysis of Microplastics in Environmental Samples". Environ. Chem. 2015. 12(5): 563–581.

52. S. Primpke, C. Lorenz, R. Rascher-Friesenhausen, et al. "An Automated Approach for Microplastics Analysis Using Focal Plane Array (FPA) FT-IR Microscopy and Image Analysis". Anal. Methods. 2017. 9(9): 1499–1511.

53. A.S. Tagg, M. Sapp, J.P. Harrison, et al. "Identification and Quantification of Microplastics in Wastewater Using Focal Plane Array-Based Reflectance Micro-FT-IR Imaging". Anal. Chem. 2015. 87(12): 6032–6040.

54. M. Klein, E.K. Fischer. "Microplastic Abundance in Atmospheric Deposition Within the Metropolitan Area of Hamburg, Germany". Sci. Total Environ. 2019. 685: 96–103.

55. K.E. Cooksey, J.B. Guckert, S.A. Williams, et al. "Fluorometric-Determination of the Neutral Lipid-Content of Microalgal Cells Using Nile Red". J. Microbiol. Methods. 1987. 6(6): 333–345.

56. G. Erni-Cassola, M.I. Gibson, R.C. Thompson, et al. "Lost, but Found with Nile Red: A Novel Method for Detecting and Quantifying Small Microplastics (1 mm to 20 μm) in Environmental Samples". Environ. Sci. Technol. 2017. 51(23): 13641–13648.

57. A.L. Andrady. "Microplastics in the Marine Environment". Mar. Pollut. Bull. 2011. 62(8): 1596–1605.

58. W.J. Shim, Y.K. Song, S.H. Hong, et al. "Identification and Quantification of Microplastics Using Nile Red Staining". Mar. Pollut. Bull. 2016. 113(1-2): 469–476.

59. S.A. Mason, V.G. Welch, J. Neratko. "Synthetic Polymer Contamination in Bottled Water". Front. Chem. 2018. 6: 407.

60. E.K. Fischer, L. Paglialonga, E. Czech, et al. "Microplastic Pollution in Lakes and Lake Shoreline Sediments: A Case Study on Lake Bolsena and Lake Chiusi (Central Italy)". Environ. Pollut. 2016. 213: 648–657.

61. Z. Wang, B. Su, X. Xu, et al. "Preferential Accumulation of Small (<300 μm) Microplastics in the Sediments of a Coastal Plain River Network in Eastern China". Water Res. 2018. 144: 393–401.

62. A.I. Catarino, V. Macchia, W.G. Sanderson, et al. "Low Levels of Microplastics (MP) in Wild Mussels Indicate that MP Ingestion by Humans is Minimal Compared to Exposure via Household Fibres Fallout During a Meal". Environ. Pollut. 2018. 237: 675–684.

63. E.K. Fischer. "Distribution of Microplastics in Marine Species of the Wadden Sea Along the Coastline of Schleswig-Holstein, Germany". Final Report University Hamburg. 2019. https://www.researchgate.net/publication/330712669_Distribution_of_microplastics_in_marine_species_of_the_Wadden_Sea_along_the_coastline_of_Schleswig-Holstein_Germany [accessed 24 April 2020].

64. M. Tamminga, E. Hengstmann, E.K. Fischer. "Nile Red Staining as a Subsidiary Method for Microplastic Quantification: A Comparison of Three Solvents and Factors Influencing Application Reliability". J. Earth Sci. Environ. Studies. 2017. 2(2): 165–172.

65. G.A. Covernton, C.M. Pearce, H.J. Gurney-Smith, et al. "Size and Shape Matter: A Preliminary Analysis of Microplastic Sampling Technique in Seawater Studies with Implications for Ecological Risk Assessment". Sci. Total Environ. 2019. 667: 124–132.

66. T. Maes, R. Jessop, N. Wellner, et al. "A Rapid-Screening Approach to Detect and Quantify Microplastics Based on Fluorescent Tagging with Nile Red". Sci. Rep. 2017. 7: 44501.

67. M. Lares, M.C. Ncibi, M. Sillanpaa, et al. "Intercomparison Study on Commonly Used Methods to Determine Microplastics in Wastewater and Sludge Samples". Environ. Sci. Pollut. Res. Int. 2019. 26(12): 12109–12122.

68. S.B. Sjollema, P. Redondo-Hasselerharm, H.A. Leslie, et al. "Do Plastic Particles Affect Microalgal Photosynthesis and Growth?". Aquat. Toxicol. 2016. 170: 259–261.

69. S. Summers, T. Henry, T. Gutierrez. "Agglomeration of Nano- and Microplastic Particles in Seawater by Autochthonous and De Novo-Produced Sources of Exopolymeric Substances". Mar. Pollut. Bull. 2018. 130: 258–267.

70. L. Sgier, R. Freimann, A. Zupanic, et al. "Flow Cytometry Combined with viSNE for the Analysis of Microbial Biofilms and Detection of Microplastics". Nat. Commun. 2016. 7: 11587.

71. T. Mani, S. Primpke, C. Lorenz, et al. "Microplastic Pollution in Benthic Midstream Sediments of the Rhine River". Environ. Sci. Technol. 2019. 53(10): 6053–6062.

72. C. Lorenz, L. Roscher, M.S. Meyer, et al. "Spatial Distribution of Microplastics in Sediments and Surface Waters of the Southern North Sea". Environ. Pollut. 2019. 252(Pt B): 1719–1729.

73. M. Bergmann, V. Wirzberger, T. Krumpen, et al. "High Quantities of Microplastic in Arctic Deep-Sea Sediments from the HAUSGARTEN Observatory". Environ. Sci. Technol. 2017. 51(19): 11000–11010.

74. M. Haave, C. Lorenz, S. Primpke, et al. "Different Stories Told by Small and Large Microplastics in Sediment–First Report of Microplastic Concentrations in an Urban Recipient in Norway". Mar. Pollut. Bull. 2019. 141: 501–513.

75. M.T. Sturm, S. Kluczka, A. Wilde, et al. "Determination of Particles Produced During Boiling in Differenz [sic] Plastic and Glass Kettles via Comparative Dynamic Image Analysis Using Flowcam". Anal. News2019. https://info.fluidimaging.com/hubfs/Documents/Articles/Determination%20of%20particles%20produced%20during%20boiling%20in%20different%20plastic%20and%20glass%20kettles%20via%20comparative%20dynamic%20image%20analysis%20using%20FlowCam.pdf [accessed 2 April 2020].

76. F. Liu, A. Vianello, J. Vollertsen. "Retention of Microplastics in Sediments of Urban and Highway Stormwater Retention Ponds". Environ. Pollut. 2019. 255(Pt 2): 113335.

77. S. Primpke, R.K. Cross, S.M. Mintenig, et al. "Toward the Systematic Identification of Microplastics in the Environment: Evaluation of a New Independent Software Tool (Simple) for Spectroscopic Analysis". Appl. Spectrosc. 2020. 74(9): 1127–1138.

78. S. Primpke, M. Wirth, C. Lorenz, et al. "Reference Database Design for the Automated Analysis of Microplastic Samples Based on Fourier Transform Infrared (FT-IR) Spectroscopy". Anal. Bioanal. Chem. 2018. 410(21): 5131–5141.

79. C.J. Hirschmugl, K.M. Gough. "Fourier Transform Infrared Spectrochemical Imaging: Review of Design and Applications with a Focal Plane Array and Multiple Beam Synchrotron Radiation Source". Appl. Spectrosc. 2012. 66(5): 475–491.

80. R.A. Crocombe. "Portable Spectroscopy". Appl. Spectrosc. 2018. 72(12): 1701–1751.

81. I. Acosta-Coley, J. Olivero-Verbel. "Microplastic Resin Pellets on an Urban Tropical Beach in Colombia". Environ. Monit. Assess. 2015. 187(7): 435.

82. E. Besseling, E.M. Foekema, J.A. Van Franeker, et al. "Microplastic in a Macro Filter Feeder: Humpback Whale *Megaptera novaeangliae*". Mar. Pollut. Bull. 2015. 95(1): 248–252.

83. J. Brandon, M. Goldstein, M.D. Ohman. "Long-Term Aging and Degradation of Microplastic Particles: Comparing in Situ Oceanic and Experimental Weathering Patterns". Mar. Pollut. Bull. 2016. 110(1): 299–308.

84. I.L.N. Brate, D.P. Eidsvoll, C.C. Steindal, et al. "Plastic Ingestion by Atlantic Cod (*Gadus morhua*) from the Norwegian Coast". Mar. Pollut. Bull. 2016. 112(1-2): 105–110.

85. A.G.M. Caron, C.R. Thomas, K.L.E. Berry, et al. "Ingestion of Microplastic Debris by Green Sea Turtles (*Chelonia mydas*) in the Great Barrier Reef: Validation of a Sequential Extraction Protocol". Mar. Pollut. Bull. 2018. 127: 743–751.

86. A. Gallagher, A. Rees, R. Rowe, et al. "Microplastics in the Solent Estuarine Complex, UK: An Initial Assessment". Mar. Pollut. Bull. 2016. 102(2): 243–249.

87. J.E. Halstead, J.A. Smith, E.A. Carter, et al. "Assessment Tools for Microplastics and Natural Fibres Ingested by Fish in an Urbanised Estuary". Environ. Pollut. 2018. 234: 552–561.

88. L.M. Hernandez, N. Yousefi, N. Tufenkji. "Are There Nanoplastics in Your Personal Care Products?". Environ. Sci. Technol. Lett. 2017. 4(7): 280–285.

89. V. Iannilli, A. Di Gennaro, F. Lecce, et al. "Microplastics in *Talitrus saltator* (Crustacea, Amphipoda): New Evidence of Ingestion from Natural Contexts". Environ. Sci. Pollut. Res. Int. 2018. 25(28): 28725–28729.

90. N. Kalogerakis, K. Karkanorachaki, G.C. Kalogerakis, et al. "Microplastics Generation: Onset of Fragmentation of Polyethylene Films in Marine Environment Mesocosms". Front. Mar. Sci. 2017. 4.

91. I.V. Kirstein, S. Kirmizi, A. Wichels, et al. "Dangerous Hitchhikers? Evidence for Potentially Pathogenic Vibrio Spp. on Microplastic Particles". Mar. Environ. Res. 2016. 120: 1–8.

92. S. Klein, E. Worch, T.P. Knepper. "Occurrence and Spatial Distribution of Microplastics in River Shore Sediments of the Rhine-Main Area in Germany". Environ. Sci. Technol. 2015. 49(10): 6070–6076.

93. S. Kuhn, A. Van Oyen, A.M. Booth, et al. "Marine Microplastic: Preparation of Relevant Test Materials for Laboratory Assessment of Ecosystem Impacts". Chemosphere. 2018. 213: 103–113.

94. K. Lei, F. Qiao, Q. Liu, et al. "Microplastics Releasing from Personal Care and Cosmetic Products in China". Mar. Pollut. Bull. 2017. 123(1-2): 122–126.

95. A.R. Mcgoran, P.R. Cowie, P.F. Clark, et al. "Ingestion of Plastic by Fish: A Comparison of Thames Estuary and Firth of Clyde Populations". Mar. Pollut. Bull. 2018. 137: 12–23.

96. A. Naji, Z. Esmaili, F.R. Khan. "Plastic Debris and Microplastics Along the Beaches of the Strait of Hormuz, Persian Gulf". Mar. Pollut. Bull. 2017. 114(2): 1057–1062.

97. A. Naji, Z. Esmaili, S.A. Mason, et al. "The Occurrence of Microplastic Contamination in Littoral Sediments of the Persian Gulf, Iran". Environ. Sci. Pollut. Res. Int. 2017. 24(25): 20459–20468.

98. A. Naji, M. Nuri, A.D. Vethaak. "Microplastics Contamination in Molluscs from the Northern Part of the Persian Gulf". Environ. Pollut. 2018. 235: 113–120.

99. M.O. Rodrigues, N. Abrantes, F.J.M. Goncalves, et al. "Spatial and Temporal Distribution of Microplastics in Water and Sediments of

a Freshwater System (Antua River, Portugal)". Sci. Total Environ. 2018. 633: 1549–1559.

100. G. Tang, M. Liu, Q. Zhou, et al. "Microplastics and Polycyclic Aromatic Hydrocarbons (PAHs) in Xiamen Coastal Areas: Implications for Anthropogenic Impacts". Sci. Total Environ. 2018. 634: 811–820.

101. J. Wang, M.X. Wang, S.G. Ru, et al. "High Levels of Microplastic Pollution in the Sediments and Benthic Organisms of the South Yellow Sea, China". Sci. Total Environ. 2019. 651: 1661–1669.

102. G. Alvarez, A. Barros, A. Velando. "The Use of European Shag Pellets as Indicators of Microplastic Fibers in the Marine Environment". Mar. Pollut. Bull. 2018. 137: 444–448.

103. M.C. Ariza-Tarazona, J.F. Villarreal-Chiu, V. Barbieri, et al. "New Strategy for Microplastic Degradation: Green Photocatalysis Using a Protein-Based Porous N-TiO$_2$ Semiconductor". Ceram. Int. 2019. 45(7): 9618–9624.

104. G. Bordos, B. Urbanyi, A. Micsinai, et al. "Identification of Microplastics in Fish Ponds and Natural Freshwater Environments of the Carpathian Basin, Europe". Chemosphere. 2019. 216: 110–116.

105. F.J. Biginagwa, B.S. Mayoma, Y. Shashoua, et al. "First Evidence of Microplastics in the African Great Lakes: Recovery from Lake Victoria Nile Perch and Nile Tilapia". J. Great Lakes Res. 2016. 42(1): 146–149.

106. A.B. Castillo, I. Al-Maslamani, J.P. Obbard. "Prevalence of Microplastics in the Marine Waters of Qatar". Mar. Pollut. Bull. 2016. 111(1-2): 260–267.

107. C.C. Cheang, Y. Ma, L. Fok. "Occurrence and Composition of Microplastics in the Seabed Sediments of the Coral Communities in Proximity of a Metropolitan Area". Int. J. Environ. Res. Pub. Health. 2018. 15(10): E2270.

108. N. Digka, C. Tsangaris, M. Torre, et al. "Microplastics in Mussels and Fish from the Northern Ionian Sea". Mar. Pollut. Bull. 2018. 135: 30–40.

109. B. Gewert, M. Ogonowski, A. Barth, et al. "Abundance and Composition of Near Surface Microplastics and Plastic Debris in the Stockholm Archipelago, Baltic Sea". Mar. Pollut. Bull. 2017. 120(1-2): 292–302.

110. A.D. Gray, H. Wertz, R.R. Leads, et al. "Microplastic in Two South Carolina Estuaries: Occurrence, Distribution, Composition". Mar. Pollut. Bull. 2018. 128: 223–233.

111. E. Hendrickson, E.C. Minor, K. Schreiner. "Microplastic Abundance and Composition in Western Lake Superior as Determined via Microscopy, Pyr-GC-MS, FT-IR". Environ. Sci. Technol. 2018. 52(4): 1787–1796.

112. E. Hermsen, R. Pompe, E. Besseling, et al. "Detection of Low Numbers of Microplastics in North Sea Fish Using Strict Quality Assurance Criteria". Mar. Pollut. Bull. 2017. 122(1-2): 253–258.

113. H.K. Imhof, R. Sigl, E. Brauer, et al. "Spatial and Temporal Variation of Macro-, Meso-, and Microplastic Abundance on a Remote Coral Island of the Maldives, Indian Ocean". Mar. Pollut. Bull. 2017. 116(1-2): 340–347.

114. M.R. Jung, G.H. Balazs, T.M. Work, et al. "Polymer Identification of Plastic Debris Ingested by Pelagic-Phase Sea Turtles in the Central Pacific". Environ. Sci. Technol. 2018. 52(20): 11535–11544.

115. M.R. Jung, F.D. Horgen, S.V. Orski, et al. "Validation of ATR FT-IR to Identify Polymers of Plastic Marine Debris, Including Those Ingested by Marine Organisms". Mar. Pollut. Bull. 2018. 127: 704–716.

116. R. Karthik, R.S. Robin, R. Purvaja, et al. "Microplastics Along the Beaches of Southeast Coast of India". Sci. Total Environ. 2018. 645: 1388–1399.

117. W. Khalik, Y.S. Ibrahim, S. Tuan Anuar, et al. "Microplastics Analysis in Malaysian Marine Waters: A Field Study of Kuala Nerus and Kuantan". Mar. Pollut. Bull. 2018. 135: 451–457.

118. V.M. Leon, I. Garcia-Aguera, V. Molto, et al. "PAHs, Pesticides, Personal Care Products and Plastic Additives in Plastic Debris from Spanish Mediterranean Beaches". Sci. Total Environ. 2019. 670: 672–684.

119. J. Li, H. Zhang, K. Zhang, et al. "Characterization, Source, Retention of Microplastic in Sandy Beaches and Mangrove Wetlands of the Qinzhou Bay, China". Mar. Pollut. Bull. 2018. 136: 401–406.

120. T. Mani, P. Blarer, F.R. Storck, et al. "Repeated Detection of Polystyrene Microbeads in the Lower Rhine River". Environ. Pollut. 2019. 245: 634–641.

121. Y. Matsuguma, H. Takada, H. Kumata, et al. "Microplastics in Sediment Cores from Asia and Africa as Indicators of Temporal Trends in Plastic Pollution". Arch. Environ. Contam. Toxicol. 2017. 73(2): 230–239.

122. M. Mecozzi, M. Pietroletti, Y.B. Monakhova. "FT-IR Spectroscopy Supported by Statistical Techniques for the Structural Characterization of Plastic Debris in the Marine Environment: Application to Monitoring Studies". Mar. Pollut. Bull. 2016. 106(1-2): 155–161.

123. M. Mistri, V. Infantini, M. Scoponi, et al. "Small Plastic Debris in Sediments from the Central Adriatic Sea: Types, Occurrence and Distribution". Mar. Pollut. Bull. 2017. 124(1): 435–440.

124. S. Morgana, L. Ghigliotti, N. Estevez-Calvar, et al. "Microplastics in the Arctic: A Case Study with Sub-Surface Water and Fish Samples Off Northeast Greenland". Environ. Pollut. 2018. 242(Pt B): 1078–1086.

125. C. Munari, V. Infantini, M. Scoponi, et al. "Microplastics in the Sediments of Terra Nova Bay (Ross Sea, Antarctica)". Mar. Pollut. Bull. 2017. 122(1-2): 161–165.

126. K. Munno, P.A. Helm, D.A. Jackson, et al. "Impacts of Temperature and Selected Chemical Digestion Methods on Microplastic Particles". Environ. Toxicol. Chem. 2018. 37(1): 91–98.

127. L. Pantoja Munoz, A. Gonzalez Baez, D. McKinney, et al. "Characterisation of 'Flushable' and 'Non-Flushable' Commercial Wet Wipes Using MicroRaman FTIR Spectroscopy, and Fluorescence Microscopy: To Flush or Not to Flush?". Environ. Sci. Pollut. Res. Int. 2018. 25(20): 20268–20279.

128. S.E. Nelms, T.S. Galloway, B.J. Godley, et al. "Investigating Microplastic Trophic Transfer in Marine Top Predators". Environ. Pollut. 2018. 238: 999–1007.

129. N.H. Nor, J.P. Obbard. "Microplastics in Singapore's Coastal Mangrove Ecosystems". Mar. Pollut. Bull. 2014. 79(1-2): 278–283.

130. G.P. Olivatto, M.C.T. Martins, C.C. Montagner, et al. "Microplastic Contamination in Surface Waters in Guanabara Bay, Rio De Janeiro, Brazil". Mar. Pollut. Bull. 2019. 139: 157–162.

131. S. Piehl, A. Leibner, M.G.J. Loder, et al. "Identification and Quantification of Macro- and Microplastics on an Agricultural Farmland". Sci. Rep. 2018. 8(1): 17950.

132. T.J. Pinon-Colin, R. Rodriguez-Jimenez, M.A. Pastrana-Corral, et al. "Microplastics on Sandy Beaches of the Baja California Peninsula, Mexico". Mar. Pollut. Bull. 2018. 131(Pt A): 63–71.

133. G. Renner, T.C. Schmidt, J. Schram. "A New Chemometric Approach for Automatic Identification of Microplastics from Environmental Compartments Based on FT-IR Spectroscopy". Anal. Chem. 2017. 89(22): 12045–12053.

134. M.O. Rodrigues, A.M.M. Goncalves, F.J.M. Goncalves, et al. "Effectiveness of a Methodology of Microplastics Isolation for Environmental Monitoring in Freshwater Systems". Ecol. Indic. 2018. 89: 488–495.

135. A. Rodriguez-Seijo, J.P. Da Costa, T. Rocha-Santos, et al. "Oxidative Stress, Energy Metabolism and Molecular Responses of Earthworms (*Eisenia fetida*) Exposed to Low-Density Polyethylene Microplastics". Environ. Sci. Pollut. Res. Int. 2018. 25(33): 33599–33610.

136. D. Rose, M. Webber. "Characterization of Microplastics in the Surface Waters of Kingston Harbour". Sci. Total Environ. 2019. 664: 753–760.

137. S. Savoca, G. Capillo, M. Mancuso, et al. "Microplastics Occurrence in the Tyrrhenian Waters and in the Gastrointestinal Tract of Two Congener Species of Seabreams". Environ. Toxicol. Pharmacol. 2019. 67: 35–41.

138. G. Smiroldo, A. Balestrieri, E. Pini, et al. "Anthropogenically Altered Trophic Webs: Alien Catfish and Microplastics in the Diet of Eurasian Otters". Mammal Res. 2019. 64(2): 165–174.

139. A.D. Syakti, R. Bouhroum, N.V. Hidayati, et al. "Beach Macro-Litter Monitoring and Floating Microplastic in a Coastal Area of Indonesia". Mar. Pollut. Bull. 2017. 122(1-2): 217–225.

140. A.D. Syakti, N.V. Hidayati, Y.V. Jaya, et al. "Simultaneous Grading of Microplastic Size Sampling in the Small Islands of Bintan Water, Indonesia". Mar. Pollut. Bull. 2018. 137: 593–600.

141. K. Tanaka, H. Takada. "Microplastic Fragments and Microbeads in Digestive Tracts of Planktivorous Fish from Urban Coastal Waters". Sci. Rep. 2016. 6: 34351.

142. A. Ter Halle, L. Ladirat, M. Martignac, et al. "To what Extent are Microplastics from the Open Ocean Weathered?". Environ. Pollut. 2017. 227: 167–174.

143. M. Tiwari, T.D. Rathod, P.Y. Ajmal, et al. "Distribution and Characterization of Microplastics in Beach Sand from Three Different Indian Coastal Environments". Mar. Pollut. Bull. 2019. 140: 262–273.

144. Y.Y. Tsang, C.W. Mak, C. Liebich, et al. "Microplastic Pollution in the Marine Waters and Sediments of Hong Kong". Mar. Pollut. Bull. 2017. 115(1-2): 20–28.

145. A. Turner, C. Wallerstein, R. Arnold. "Identification, Origin and Characteristics of Bio-Bead Microplastics from Beaches in Western Europe". Sci. Total Environ. 2019. 664: 938–947.

146. S. Veerasingam, M. Mugilarasan, R. Venkatachalapathy, et al. "Influence of 2015 Flood on the Distribution and Occurrence of Microplastic Pellets Along the Chennai Coast, India". Mar. Pollut. Bull. 2016. 109(1): 196–204.

147. S. Veerasingam, M. Saha, V. Suneel, et al. "Characteristics, Seasonal Distribution and Surface Degradation Features of Microplastic Pellets Along the Goa Coast, India". Chemosphere. 2016. 159: 496–505.

148. A. Vidyasakar, K. Neelavannan, S. Krishnakumar, et al. "Macrodebris and Microplastic Distribution in the Beaches of Rameswaram Coral Island, Gulf of Mannar, Southeast Coast of India: A First Report". Mar. Pollut. Bull. 2018. 137: 610–616.

149. N. Weithmann, J.N. Moller, M.G.J. Loder, et al. "Organic Fertilizer as a Vehicle for the Entry of Microplastic into the Environment". Sci. Adv. 2018. 4(4): Eaap8060.

150. Q.Q. Wu, H.C. Tao, M.H. Wong. "Feeding and Metabolism Effects of Three Common Microplastics on Tenebrio Molitor L". Environ. Geochem. Health. 2019. 41(1): 17–26.

151. S. Abidli, J.C. Antunes, J.L. Ferreira, et al. "Microplastics in Sediments from the Littoral Zone of the North Tunisian Coast (Mediterranean Sea)". Estuar. Coast. Shelf Sci. 2018. 205: 1–9.

152. A. Paul, L. Wander, R. Becker, et al. "High-Throughput NIR Spectroscopic (NIRS) Detection of Microplastics in Soil". Environ. Sci. Pollut. Res. Int. 2019. 26(8): 7364–7374.

153. M.C. Andrade, K.O. Winemiller, P.S. Barbosa, et al. "First Account of Plastic Pollution Impacting Freshwater Fishes in the Amazon: Ingestion of Plastic Debris by Piranhas and Other Serrasalmids with Diverse Feeding Habits". Environ. Pollut. 2019. 244: 766–773.

154. J.F. Ding, J.X. Li, C.J. Sun, et al. "Detection of Microplastics in Local Marine Organisms Using a Multi-Technology System". Anal. Methods. 2019. 11(1): 78–87.

155. J.F. Ding, J.X. Li, C.J. Sun, et al. "Separation and Identification of Microplastics in Digestive System of Bivalves". Chin. J. Anal. Chem. 2018. 46(5): 690–697.

156. E.M. Crichton, M. Noel, E.A. Gies, et al. "A Novel, Density-Independent and FT-IR-Compatible Approach for the Rapid Extraction of Microplastics from Aquatic Sediments". Anal. Methods. 2017. 9(9): 1419–1428.

157. G. De Lucia, A. Vianello, A. Camedda, et al. "Sea Water Contamination in the Vicinity of the Italian Minor Islands Caused by Microplastic Pollution". Water. 2018. 10(8): 1108.

158. A. Käppler, M. Fischer, B.M. Scholz-Bottcher, et al. "Comparison of Mu-ATR-FT-IR Spectroscopy and Py-GCMS as Identification Tools for Microplastic Particles and Fibers Isolated from River Sediments". Anal. Bioanal. Chem. 2018. 410(21): 5313–5327.

159. A. Käppler, F. Windrich, M.G.J. Loder, et al. "Identification of Microplastics by FT-IR and Raman Microscopy: A Novel Silicon Filter Substrate Opens the Important Spectral Range Below 1300 cm^{-1} for FT-IR Transmission Measurements". Anal. Bioanal. Chem. 2015. 407(22): 6791–6801.

160. S. Magni, A. Binelli, L. Pittura, et al. "The Fate of Microplastics in an Italian Wastewater Treatment Plant". Sci. Total Environ. 2019. 652: 602–610.

161. S.M. Mintenig, I. Int-Veen, M.G.J. Loder, et al. "Identification of Microplastic in Effluents of Waste Water Treatment Plants Using Focal Plane Array-Based Micro-Fourier-Transform Infrared Imaging". Water Res. 2017. 108: 365–372.

162. P. Muhlschlegel, A. Hauk, U. Walter, et al. "Lack of Evidence for Microplastic Contamination in Honey". Food Addit. Contam. Part A. 2017. 34(11): 1982–1989.

163. A. Palatinus, M. Kovac Virsek, U. Robic, et al. "Marine Litter in the Croatian Part of the Middle Adriatic Sea: Simultaneous Assessment of Floating and Seabed Macro and Micro Litter Abundance and Composition". Mar. Pollut. Bull. 2019. 139: 427–439.

164. T. Pegado, K. Schmid, K.O. Winemiller, et al. "First Evidence of Microplastic Ingestion by Fishes from the Amazon River Estuary". Mar. Pollut. Bull. 2018. 133: 814–821.

165. Y.K. Song, S.H. Hong, M. Jang, et al. "A Comparison of Microscopic and Spectroscopic Identification Methods for Analysis of Microplastics in Environmental Samples". Mar. Pollut. Bull. 2015. 93(1-2): 202–209.

166. L. Su, H. Deng, B.W. Li, et al. "The Occurrence of Microplastic in Specific Organs in Commercially Caught Fishes from Coast and Estuary Area of East China". J. Hazard. Mater. 2019. 365: 716–724.

167. A. Vianello, A. Boldrin, P. Guerriero, et al. "Microplastic Particles in Sediments of Lagoon of Venice, Italy: First Observations on Occurrence, Spatial Patterns and Identification". Estuar. Coast. Shelf Sci. 2013. 130: 54–61.

168. C. Alomar, A. Sureda, X. Capo, et al. "Microplastic Ingestion by Mullus Surmuletus Linnaeus, 1758 Fish and Its Potential for Causing Oxidative Stress". Environ. Res. 2017. 159: 135–142.

169. H.S. Auta, C.U. Emenike, S.H. Fauziah. "Screening of Bacillus Strains Isolated from Mangrove Ecosystems in Peninsular Malaysia for Microplastic Degradation". Environ. Pollut. 2017. 231(Pt 2): 1552–1559.

170. F.M. Baalkhuyur, E.A. Bin Dohaish, M.E.A. Elhalwagy, et al. "Microplastic in the Gastrointestinal Tract of Fishes Along the Saudi Arabian Red Sea Coast". Mar. Pollut. Bull. 2018. 131(Pt A): 407–415.

171. F. Bessa, P. Barria, J.M. Neto, et al. "Occurrence of Microplastics in Commercial Fish from a Natural Estuarine Environment". Mar. Pollut. Bull. 2018. 128: 575–584.

172. M.A. Browne, T.S. Galloway, R.C. Thompson. "Spatial Patterns of Plastic Debris Along Estuarine Shorelines". Environ. Sci. Technol. 2010. 44(9): 3404–3409.

173. H. Cai, F. Du, L. Li, et al. "A Practical Approach Based on FT-IR Spectroscopy for Identification of Semi-Synthetic and Natural Celluloses in Microplastic Investigation". Sci. Total Environ. 2019. 669: 692–701.

174. L. Cai, J. Wang, J. Peng, et al. "Characteristic of Microplastics in the Atmospheric Fallout from Dongguan City, China: Preliminary Research and First Evidence". Environ. Sci. Pollut. Res. Int. 2017. 24(32): 24928–24935.

175. R.O. Castro, M.L. Silva, M.R.C. Marques, et al. "Evaluation of Microplastics in Jurujuba Cove, Niteroi #RJ, |Brazil, an Area of Mussels Farming". Mar. Pollut. Bull. 2016. 110(1): 555–558.

176. A.I. Catarino, R. Thompson, W. Sanderson, et al. "Development and Optimization of a Standard Method for Extraction of Microplastics in Mussels by Enzyme Digestion of Soft Tissues". Environ. Toxicol. Chem. 2017. 36(4): 947–951.

177. A. Cincinelli, C. Scopetani, D. Chelazzi, et al. "Microplastic in the Surface Waters of the Ross Sea (Antarctica): Occurrence, Distribution and Characterization by FT-IR". Chemosphere. 2017. 175: 391–400.

178. M. Compa, A. Ventero, M. Iglesias, et al. "Ingestion of Microplastics and Natural Fibres in *Sardina pilchardus* (Walbaum, 1792) and *Engraulis encrasicolus* (Linnaeus, 1758) Along the Spanish Mediterranean Coast". Mar. Pollut. Bull. 2018. 128: 89–96.

179. A.M. Elert, R. Becker, E. Duemichen, et al. "Comparison of Different Methods for MP Detection: What Can We Learn from Them, Why Asking the Right Question Before Measurements Matters?". Environ. Pollut. 2017. 231(Pt 2): 1256–1264.

180. J.P. Frias, J. Gago, V. Otero, et al. "Microplastics in Coastal Sediments from Southern Portuguese Shelf Waters". Mar. Environ. Res. 2016. 114: 24–30.

181. J.P. Frias, V. Otero, P. Sobral. "Evidence of Microplastics in Samples of Zooplankton from Portuguese Coastal Waters". Mar. Environ. Res. 2014. 95: 89–95.

182. J.P. Frias, P. Sobral, A.M. Ferreira. "Organic Pollutants in Microplastics from Two Beaches of the Portuguese Coast". Mar. Pollut. Bull. 2010. 60(11): 1988–1992.

183. E.A. Gies, J.L. Lenoble, M. Noel, et al. "Retention of Microplastics in a Major Secondary Wastewater Treatment Plant in Vancouver, Canada". Mar. Pollut. Bull. 2018. 133: 553–561.

184. B. Graca, K. Szewc, D. Zakrzewska, et al. "Sources and Fate of Microplastics in Marine and Beach Sediments of the Southern Baltic Sea: A Preliminary Study". Environ. Sci. Pollut. Res. Int. 2017. 24(8): 7650–7661.

185. A. Käppler, D. Fischer, S. Oberbeckmann, et al. "Analysis of Environmental Microplastics by Vibrational Microspectroscopy: FT-IR, Raman or Both?". Anal. Bioanal. Chem. 2016. 408(29): 8377–8391.

186. S. Kuhn, F.L. Schaafsma, B. Van Werven, et al. "Plastic Ingestion by Juvenile Polar Cod (*Boreogadus saida*) in the Arctic Ocean". Polar Biol. 2018. 41(6): 1269–1278.

187. A. Kunz, B.A. Walther, L. Lowemark, et al. "Distribution and Quantity of Microplastic on Sandy Beaches Along the Northern Coast of Taiwan". Mar. Pollut. Bull. 2016. 111(1-2): 126–135.

188. M. Lares, M.C. Ncibi, M. Sillanpaa, et al. "Occurrence, Identification, and Removal of Microplastic Particles and Fibers in Conventional Activated Sludge Process and Advanced MBR Technology". Water Res. 2018. 133: 236–246.

189. X. Li, L. Chen, Q. Mei, et al. "Microplastics in Sewage Sludge from the Wastewater Treatment Plants in China". Water Res. 2018. 142: 75–85.

190. M. Liu, S. Lu, Y. Song, et al. "Microplastic and Mesoplastic Pollution in Farmland Soils in Suburbs of Shanghai, China". Environ. Pollut. 2018. 242(Pt A): 855–862.

191. P.M. Lourenco, C. Serra-Goncalves, J.L. Ferreira, et al. "Plastic and Other Microfibers in Sediments, Macroinvertebrates and Shorebirds from Three Intertidal Wetlands of Southern Europe and West Africa". Environ. Pollut. 2017. 231(Pt 1): 123–133.

192. S.M. Mintenig, P.S. Bauerlein, A.A. Koelmans, et al. "Closing the Gap Between Small and Smaller: Towards a Framework to Analyse Nano- and Microplastics in Aqueous Environmental Samples". Environ. Sci.: Nano. 2018. 5(7): 1640–1649.

193. S.M. Mintenig, M.G.J. Loder, S. Primpke, et al. "Low Numbers of Microplastics Detected in Drinking Water from Ground Water Sources". Sci. Total Environ. 2019. 648: 631–635.

194. M. Mohsen, Q. Wang, L. Zhang, et al. "Microplastic Ingestion by the Farmed Sea Cucumber *Apostichopus japonicus* in China". Environ. Pollut. 2019. 245: 1071–1078.

195. J. Mu, L. Qu, F. Jin, et al. "Abundance and Distribution of Microplastics in the Surface Sediments from the Northern Bering and Chukchi Seas". Environ. Pollut. 2019. 245: 122–130.

196. K.L. Ng, J.P. Obbard. "Prevalence of Microplastics in Singapore's Coastal Marine Environment". Mar. Pollut. Bull. 2006. 52(7): 761–767.

197. G. Peng, B. Zhu, D. Yang, et al. "Microplastics in Sediments of the Changjiang Estuary, China". Environ. Pollut. 2017. 225: 283–290.

198. C.K. Pham, Y. Rodriguez, A. Dauphin, et al. "Plastic Ingestion in Oceanic-Stage Loggerhead Sea Turtles (*Caretta caretta*) Off the North Atlantic Subtropical Gyre". Mar. Pollut. Bull. 2017. 121(1-2): 222–229.

199. N.N. Phuong, L. Poirier, F. Lagarde, et al. "Microplastic Abundance and Characteristics in French Atlantic Coastal Sediments Using a New Extraction Method". Environ. Pollut. 2018. 243(Pt A): 228–237.

200. M. Poulain, M.J. Mercier, L. Brach, et al. "Small Microplastics as a Main Contributor to Plastic Mass Balance in the North Atlantic Subtropical Gyre". Environ. Sci. Technol. 2019. 53(3): 1157–1164.

201. K. Pozo, V. Gomez, M. Torres, et al. "Presence and Characterization of Microplastics in Fish of Commercial Importance from the Biobio Region in Central Chile". Mar. Pollut. Bull. 2019. 140: 315–319.

202. Q. Qiu, J. Peng, X. Yu, et al. "Occurrence of Microplastics in the Coastal Marine Environment: First Observation on Sediment of China". Mar. Pollut. Bull. 2015. 98(1-2): 274–280.

203. M. Simon, N. Van Alst, J. Vollertsen. "Quantification of Microplastic Mass and Removal Rates at Wastewater Treatment Plants Applying Focal Plane Array (FPA)-Based Fourier Transform Infrared (FT-IR) Imaging". Water Res. 2018. 142: 1–9.

204. J. Teng, Q. Wang, W. Ran, et al. "Microplastic in Cultured Oysters from Different Coastal Areas of China". Sci. Total Environ. 2019. 653: 1282–1292.

205. J. Wang, J. Peng, Z. Tan, et al. "Microplastics in the Surface Sediments from the Beijiang River Littoral Zone: Composition, Abundance, Surface Textures and Interaction with Heavy Metals". Chemosphere. 2017. 171: 248–258.

206. A.M. Wieczorek, L. Morrison, P.L. Croot, et al. "Frequency of Microplastics in Mesopelagic Fishes from the Northwest Atlantic". Front. Mar. Sci. 2018. 5: 39.

207. X. Yu, S. Ladewig, S. Bao, et al. "Occurrence and Distribution of Microplastics at Selected Coastal Sites Along the Southeastern United States". Sci. Total Environ. 2018. 613–614: 298–305.

208. M. Zbyszewski, P.L. Corcoran, A. Hockin. "Comparison of the Distribution and Degradation of Plastic Debris Along Shorelines of the Great Lakes, North America". J. Great Lakes Res. 2014. 40(2): 288–299.

209. C.F. Zhang, H.H. Zhou, Y.Z. Cui, et al. "Microplastics in Offshore Sediment in the Yellow Sea and East China Sea, China". Environ. Pollut. 2019. 244: 827–833.

210. W. Zhang, S. Zhang, J. Wang, et al. "Microplastic Pollution in the Surface Waters of the Bohai Sea, China". Environ. Pollut. 2017. 231(Pt 1): 541–548.

211. J. Zhao, W. Ran, J. Teng, et al. "Microplastic Pollution in Sediments from the Bohai Sea and the Yellow Sea, China". Sci. Total Environ. 2018. 640-641: 637–645.

212. L. Zhu, H. Bai, B. Chen, et al. "Microplastic Pollution in North Yellow Sea, China: Observations on Occurrence, Distribution and Identification". Sci. Total Environ. 2018. 636: 20–29.

213. S. Primpke, H. Imhof, S. Piehl, et al. "Environmental Chemistry Microplastic in the Environment". Chem. Unserer Zeit. 2017. 51(6): 402–412.

214. M. Cai, H. He, M. Liu, et al. "Lost but Can't Be Neglected: Huge Quantities of Small Microplastics Hide in the South China Sea". Sci. Total Environ. 2018. 633: 1206–1216.

215. I.R. Comnea-Stancu, K. Wieland, G. Ramer, et al. "On the Identification of Rayon/Viscose as a Major Fraction of Microplastics in the Marine Environment: Discrimination Between Natural and Manmade Cellulosic Fibers Using Fourier Transform Infrared Spectroscopy". Appl. Spectrosc. 2017. 71(5): 939–950.

216. E.M. Duncan, A.C. Broderick, W.J. Fuller, et al. "Microplastic Ingestion Ubiquitous in Marine Turtles". Glob. Chang. Biol. 2019. 25(2): 744–752.

217. S. Eo, S.H. Hong, Y.K. Song, et al. "Abundance, Composition, Distribution of Microplastics Larger Than 20mum in Sand Beaches of South Korea". Environ. Pollut. 2018. 238: 894–902.

218. R.Z. Miller, A.J.R. Watts, B.O. Winslow, et al. "Mountains to the Sea: River Study of Plastic and Non-Plastic Microfiber Pollution in the Northeast USA". Mar. Pollut. Bull. 2017. 124(1): 245–251.

219. K.A. Murrell, C.C. Ghetu, F.L. Dorman. "The Combination of Spectroscopy, Microscopy, Profilometry Methods for the Physical and Chemical Characterization of Environmentally Relevant Microplastics". Anal. Methods. 2018. 10(40): 4909–4916.

220. G. Peng, P. Xu, B. Zhu, et al. "Microplastics in Freshwater River Sediments in Shanghai, China: A Case Study of Risk Assessment in Mega-Cities". Environ. Pollut. 2018. 234: 448–456.

221. C.D. Rummel, M.G.J. Loder, N.F. Fricke, et al. "Plastic Ingestion by Pelagic and Demersal Fish from the North Sea and Baltic Sea". Mar. Pollut. Bull. 2016. 102(1): 134–141.

222. F. Saliu, S. Montano, M.G. Garavaglia, et al. "Microplastic and Charred Microplastic in the Faafu Atoll, Maldives". Mar. Pollut. Bull. 2018. 136: 464–471.

223. A. Ter Halle, L. Ladirat, X. Gendre, et al. "Understanding the Fragmentation Pattern of Marine Plastic Debris". Environ. Sci. Technol. 2016. 50(11): 5668–5675.

224. D.K. Kanhai, K. Gardfeldt, O. Lyashevska, et al. "Microplastics in Sub-Surface Waters of the Arctic Central Basin". Mar. Pollut. Bull. 2018. 130: 8–18.

225. W. Lv, W. Zhou, S. Lu, et al. "Microplastic Pollution in Rice-Fish Co-Culture System: A Report of Three Farmland Stations in Shanghai, China". Sci. Total Environ. 2019. 652: 1209–1218.

226. Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP). "Guidelines for the Monitoring and Assessment of Plastic Litter in the Ocean" 2019. http://www.gesamp.org/publications/guidelines-for-the-monitoring-and-assessment-of-plastic-litter-in-the-ocean [accessed 2 April 2020].

227. G. Renner, A. Nellessen, A. Schwiers, et al. "Data Preprocessing and Evaluation Used in the Microplastics Identification Process: A Critical Review and Practical Guide". TRaC, Trends Anal. Chem. 2019. 111: 229–238.

228. G. Renner, T.C. Schmidt, J. Schram. "Analytical Methodologies for Monitoring Micro(Nano)Plastics: Which are Fit for Purpose?". Curr. Opin. Environ. Sci. Health. 2018. 1: 55–61.

229. M. Kedzierski, M. Falcou-Prefol, M.E. Kerros, et al. "A Machine Learning Algorithm for High Throughput Identification of FT-IR Spectra: Application on Microplastics Collected in the Mediterranean Sea". Chemosphere. 2019. 234: 242–251.

230. P.L. Tang, R. McCumskay, M. Rogerson, et al. "Handheld FT-IR Spectroscopy for the Triage of Micro- and Meso-Sized Plastics in the Marine Environment Incorporating an Accelerated Weathering Study and an Aging Estimation". Spectroscopy. 2019. 34(2): 54–60.

231. N.N. Phuong, A. Zalouk-Vergnoux, A. Kamari, et al. "Quantification and Characterization of Microplastics in Blue Mussels (*Mytilus edulis*): Protocol Setup and Preliminary Data on the Contamination of the French Atlantic Coast". Environ. Sci. Pollut. Res. Int. 2018. 25(7): 6135–6144.

232. S. Ziajahromi, P.A. Neale, L. Rintoul, et al. "Wastewater Treatment Plants as a Pathway for Microplastics: Development of a New Approach to Sample Wastewater-Based Microplastics". Water Res. 2017. 112: 93–99.

233. I. Peeken, S. Primpke, B. Beyer, et al. "Arctic Sea Ice is an Important Temporal Sink and Means of Transport for Microplastic". Nat. Commun. 2018. 9(1): 1505.

234. S. Primpke, P.A. Dias, G. Gerdts. "Automated Identification and Quantification of Microfibres and Microplastics". Anal. Methods. 2019. 11(16): 2138–2147.

235. A. Vianello, R.L. Jensen, L. Liu, et al. "Simulating Human Exposure to Indoor Airborne Microplastics Using a Breathing Thermal Manikin". Sci. Rep. 2019. 9(1): 8670.

236. M. Bergmann, S. Mutzel, S. Primpke, et al. "White and Wonderful? Microplastics Prevail in Snow from the Alps to the Arctic". Sci. Adv. 2019. 5(8): Eaax1157.

237. B. Hufnagl, D. Steiner, E. Renner, et al. "A Methodology for the Fast Identification and Monitoring of Microplastics in Environmental Samples Using Random Decision Forest Classifiers". Anal. Methods. 2019. 11(17): 2277–2285.

238. E. Abbe. "Beiträge zur Theorie des Mikroskops und der Mikroskopischen Wahrnehmung". Arch. Mikrosk. Anat. 1873. 9(1): 413–418.

239. C.V. Raman, K.S. Krishnan. "A New Type of Secondary Radiation". Nature. 1928. 121(3048): 501–502.

240. K. Zhang, J. Su, X. Xiong, et al. "Microplastic Pollution of Lakeshore Sediments from Remote Lakes in Tibet Plateau, China". Environ. Pollut. 2016. 219: 450–455.

241. S.Y. Zhao, M. Danley, J.E. Ward, et al. "An Approach for Extraction, Characterization and Quantitation of Microplastic in Natural Marine Snow Using Raman Microscopy". Anal. Methods. 2017. 9(9): 1470–1478.

242. C.F. Araujo, M.M. Nolasco, A.M.P. Ribeiro, et al. "Identification of Microplastics Using Raman Spectroscopy: Latest Developments and Future Prospects". Water Res. 2018. 142: 426–440.

243. N.P. Ivleva, A.C. Wiesheu, R. Niessner. "Microplastic in Aquatic Ecosystems". Angew. Chem. Int. Ed. Engl. 2017. 56(7): 1720–1739.

244. X. Zhu. The Sources of Microplastics in San Francisco Bay, California, USA. [M.S. Thesis]. Toronto, Ontario, Canada: University of Toronto, 2019.

245. B.E. Ossmann, G. Sarau, S.W. Schmitt, et al. "Development of an Optimal Filter Substrate for the Identification of Small Microplastic Particles in Food by Micro-Raman Spectroscopy". Anal. Bioanal. Chem. 2017. 409(16): 4099–4109.

246. S. Sujathan, A.K. Kniggendorf, A. Kumar, et al. "Heat and Bleach: A Cost-Efficient Method for Extracting Microplastics from Return Activated Sludge". Arch. Environ. Contam. Toxicol. 2017. 73(4): 641–648.

247. H.K. Imhof, C. Laforsch, A.C. Wiesheu, et al. "Pigments and Plastic in Limnetic Ecosystems: A Qualitative and Quantitative Study on Microparticles of Different Size Classes". Water Res. 2016. 98: 64–74.

248. B.E. Ossmann, G. Sarau, H. Holtmannspotter, et al. "Small-Sized Microplastics and Pigmented Particles in Bottled Mineral Water". Water Res. 2018. 141: 307–316.

249. K. Enders, R. Lenz, C.A. Stedmon. "Abundance, Size and Polymer Composition of Marine Microplastics ≥10 mm in the Atlantic Ocean and Their Modelled Vertical Distribution". Mar. Pollut. Bull. 2015. 100(1): 70–81.

250. R. Triebskorn, T. Braunbeck, T. Grummt, et al. "Relevance of Nano- and Microplastics for Freshwater Ecosystems: A Critical Review". TRaC, Trends Anal. Chem. 2019. 110: 375–392.

251. L. Zada, H.A. Leslie, A.D. Vethaak, et al. "Fast Microplastics Identification with Stimulated Raman Scattering Microscopy". J. Raman Spectrosc. 2018. 49(7): 1136–1144.

252. P.M. Anger, E. Von Der Esch, T. Baumann, et al. "Raman Microspectroscopy as a Tool for Microplastic Particle Analysis". TRaC, Trends Anal. Chem. 2018. 109: 214–226.

253. L. Opilik, T. Schmid, R. Zenobi. "Modern Raman Imaging: Vibrational Spectroscopy on the Micrometer and Nanometer Scales". Annu. Rev. Anal. Chem. 2013. 6: 379–398.

254. P. Ribeiro-Claro, M.M. Nolasco, C. Araújo. "Characterization of Microplastics by Raman Spectroscopy". In: T.A.P. Rocha-Santos, A.C. Duarte, editors. Comprehensive Analytical Chemistry: Characterization and Analysis of Microplastics. Amsterdam; Oxford; Cambridge, Elsevier, 2017. Pp. 119–151.

255. A. Ballent, P.L. Corcoran, O. Madden, et al. "Sources and Sinks of Microplastics in Canadian Lake Ontario Nearshore, Tributary, and Beach Sediments". Mar. Pollut. Bull. 2016. 110(1): 383–395.

256. M. Di, J. Wang. "Microplastics in Surface Waters and Sediments of the Three Gorges Reservoir, China". Sci. Total Environ. 2018. 616–617: 1620–1627.

257. A.A. Horton, C. Svendsen, R.J. Williams, et al. "Large Microplastic Particles in Sediments of Tributaries of the River Thames, UK—Abundance, Sources and Methods for Effective Quantification". Mar. Pollut. Bull. 2017. 114(1): 218–226.

258. H.K. Imhof, N.P. Ivleva, J. Schmid, et al. "Contamination of Beach Sediments of a Subalpine Lake with Microplastic Particles". Curr. Biol. 2013. 23(19): R867–R868.

259. C. Schwaferts, R. Niessner, M. Elsner, et al. "Methods for the Analysis of Submicrometer- and Nanoplastic Particles in the Environment". TRaC, Trends Anal. Chem. 2019. 112: 52–65.

260. M. Carbery, W. O'Connor, T. Palanisami. "Trophic Transfer of Microplastics and Mixed Contaminants in the Marine Food Web and Implications for Human Health". Environ. Int. 2018. 115: 400–409.

261. P.M. Anger, L. Prechtl, M. Elsner, et al. "Implementation of an Open Source Algorithm for Particle Recognition and Morphological Characterisation for Microplastic Analysis by Means of Raman Microspectroscopy". Anal. Methods. 2019. 11(27): 3483–3489.

262. M. Böhmler, W. Ibach, D. Strom, et al. "Automated Microparticle Analysis with Raman Microscopy". Laborpraxis Worldwide. Wuerzburg: Vogel Communications Group Gmbh and Co. KG, 2019. https://www.lab-worldwide.com/automated-microparticle-analysis-with-raman-microscopy-a-869915/ (accessed 24 April 2020).

263. M. Fischer, B.M. Scholz-Bottcher. "Simultaneous Trace Identification and Quantification of Common Types of Microplastics in Environmental Samples by Pyrolysis-Gas Chromatography-Mass Spectrometry". Environ. Sci. Technol. 2017. 51(9): 5052–5060.

264. S. Tsuge, H. Ohtani, C. Watanabe. Pyrolysis-GC-MS Data Book of Synthetic Polymers: Pyrograms #Thermograms and MS of |Pyrolyzates. Amsterdam; Boston: Elsevier, 2011.

265. R. Calvert. "Applied Pyrolysis Handbook". Chromatographia. 2007. 66(9-10): 827.

266. P. Kusch. "Pyrolysis-Gas Chromatography/Mass Spectrometry of Polymeric Materials". In: M.A. Mohd, editor. Advanced Gas Chromatography–Progress in Agricultural, Biomedical and Industrial Applications. London, UK: Intechopen, 2012. Chap. 16, Pp. 343–362.

267. P. Kusch. "Identification of Synthetic Polymers and Copolymers by Analytical Pyrolysis-Gas Chromatography/Mass Spectrometry". J. Chem. Educ. 2014. 91(10): 1725–1728.

268. P. Kusch, V. Obst, D. Schroeder-Obst, et al. "Application of Pyrolysis–Gas Chromatography/Mass Spectrometry for the Identification of Polymeric Materials in Failure Analysis in the Automotive Industry". Eng. Failure Anal. 2013. 35: 114–124.

269. T.P. Wampler. "Pyrolysis Techniques in the Analysis of Polymers and Rubbers". In: Encyclopedia of Analytical Chemistry, 2006.

270. J.W. De Leeuw, E.W.B. De Leer, J.S.S. Damste, et al. "Screening of Anthropogenic Compounds in Polluted Sediments and Soils by Flash Evaporation/Pyrolysis Gas Chromatography-Mass Spectrometry". Anal. Chem. 2002. 58(8): 1852–1857.

271. D. Fabbri. "Use of Pyrolysis-Gas Chromatography/Mass Spectrometry to Study Environmental Pollution Caused by Synthetic Polymers: A Case Study: The Ravenna Lagoon". J. Anal. Appl. Pyrolysis. 2001. 58: 361–370.

272. D. Fabbri, C. Trombini, I. Vassura. "Analysis of Polystyrene in Polluted Sediments by Pyrolysis Gas Chromatography Mass Spectrometry". J. Chromatogr. Sci. 1998. 36(12): 600–604.

273. D. Fabbri, D. Tartari, C. Trombini. "Analysis of Poly(Vinyl Chloride) and Other Polymers in Sediments and Suspended Matter of a Coastal Lagoon by Pyrolysis-Gas Chromatography-Mass Spectrometry". Anal. Chim. Acta. 2000. 413(1-2): 3–11.

274. J.H. Dekiff, D. Remy, J. Klasmeier, et al. "Occurrence and Spatial Distribution of Microplastics in Sediments from Norderney". Environ. Pollut. 2014. 186: 248–256.

275. E. Fries, J.H. Dekiff, J. Willmeyer, et al. "Identification of Polymer Types and Additives in Marine Microplastic Particles Using Pyrolysis-GC-MS and Scanning Electron Microscopy". Environ. Sci. Process Impacts. 2013. 15(10): 1949–1956.

276. M.T. Nuelle, J.H. Dekiff, D. Remy, et al. "A New Analytical Approach for Monitoring Microplastics in Marine Sediments". Environ. Pollut. 2014. 184: 161–169.

277. A. Ter Halle, L. Jeanneau, M. Martignac, et al. "Nanoplastic in the North Atlantic Subtropical Gyre". Environ. Sci. Technol. 2017. 51(23): 13689–13697.

278. E. Dumichen, P. Eisentraut, C.G. Bannick, et al. "Fast Identification of Microplastics in Complex Environmental Samples by a Thermal Degradation Method". Chemosphere. 2017. 174: 572–584.

279. M. Fischer, B.M. Scholz-Bottcher. "Microplastics Analysis in Environmental Samples–Recent Pyrolysis-Gas Chromatography-Mass Spectrometry Method Improvements to Increase the Reliability of Mass-Related Data". Anal. Methods. 2019. 11(18): 2489–2497.

280. E. Dumichen, A.K. Barthel, U. Braun, et al. "Analysis of Polyethylene Microplastics in Environmental Samples, Using a Thermal Decomposition Method". Water Res. 2015. 85: 451–457.

281. E. Duemichen, P. Eisentraut, M. Celina, et al. "Automated Thermal Extraction-Desorption Gas Chromatography Mass Spectrometry: A Multifunctional Tool for Comprehensive Characterization of Polymers and Their Degradation Products". J. Chromatogr. A. 2019. 1592: 133–142.

282. P. Eisentraut, E. Dumichen, A.S. Ruhl, et al. "Two Birds with One Stone-Fast and Simultaneous Analysis of Microplastics: Microparticles Derived from Thermoplastics and Tire Wear". Environ. Sci. Technol. Lett. 2018. 5(10): 608–613.

283. C.L. Beyler, M.M. Hirschler. "Thermal Decomposition of Polymers". In: M.J. Hurley, D. Gottuk, J.R. Hall Jr., K. Harada, et al., editors. SFPE Handbook of Fire Protection Engineering. Quincy, Mass.: National Fire Protection Association; Boston: Society of Fire Protection Engineers, 2002. Chap. 7, Pp. 110–131.

284. A. Witkowski, A.A. Stec, T.R. Hull. "Thermal Decomposition of Polymeric Materials". In: M.J. Hurley, D. Gottuk, J.R. Hall, K. Harada, et al., editors. SFPE Handbook of Fire Protection Engineering. New York: Springer, 2016. Pp. 167–254.

285. M. Herrera, G. Matuschek, A. Kettrup. "Fast Identification of Polymer Additives by Pyrolysis-Gas Chromatography/Mass Spectrometry". J. Anal. Appl. Pyrolysis. 2003. 70(1): 35–42.

286. K.D. Jansson, C.P. Zawodny, T.P. Wampler. "Determination of Polymer Additives Using Analytical Pyrolysis". J. Anal. Appl. Pyrolysis. 2007. 79(1): 353–361.

287. R. Rial-Otero, M. Galesio, J.-L. Capelo, et al. "A Review of Synthetic Polymer Characterization by Pyrolysis–GC–MS". Chromatographia. 2009. 70(3): 339–348.

288. F.C.-Y. Wang. "Polymer Additive Analysis by Pyrolysis–Gas Chromatography: I. Plasticizers". J. Chromatogr. A. 2000. 883(1): 199–210.

289. F.C.-Y. Wang. "Polymer Additive Analysis by Pyrolysis–Gas Chromatography: IV. Antioxidants". J. Chromatogr. A. 2000. 891(2): 325–336.

290. F.C.-Y. Wang, W.C. Buzanowski. "Polymer Additive Analysis by Pyrolysis–Gas Chromatography: III. Lubricants". J. Chromatogr. A. 2000. 891(2): 313–324.

291. F.C.-Y. Wang. "Polymer Additive Analysis by Pyrolysis–Gas Chromatography: II. Flame Retardants". J. Chromatogr. A. 2000. 886(1): 225–235.

292. J.R. Jambeck, R. Geyer, C. Wilcox, et al. "Marine Pollution. Plastic Waste Inputs from Land into the Ocean". Science. 2015. 347(6223): 768–771.

293. J.M. Challinor. "Review: The Development and Applications of Thermally Assisted Hydrolysis and Methylation Reactions". J. Anal. Appl. Pyrolysis. 2001. 61(1-2): 3–34.

294. M. Ottaviani, J. Chowdhary, B. Cairns. "Remote Sensing of the Ocean Surface Refractive Index via Short-Wave Infrared Polarimetry". Remote Sens. Environ. 2019. 221: 14–23.

295. S.P. Garaba, H.M. Dierssen. "An Airborne Remote Sensing Case Study of Synthetic Hydrocarbon Detection Using Short Wave Infrared Absorption Features Identified from Marine-Harvested Macro- and Microplastics". Remote Sens. Environ. 2018. 205: 224–235.

296. T.M. Karlsson, H. Grahn, B. Van Bavel, et al. "Hyperspectral Imaging and Data Analysis for Detecting and Determining Plastic Contamination in Seawater Filtrates". J. Near Infrared Spectrosc. 2016. 24(2): 141–149.

297. J. Shan, J. Zhao, Y. Zhang, et al. "Simple and Rapid Detection of Microplastics in Seawater Using Hyperspectral Imaging Technology". Anal. Chim. Acta. 2019. 1050: 161–168.

298. J. Shan, J. Zhao, L. Liu, et al. "A Novel Way to Rapidly Monitor Microplastics in Soil by Hyperspectral Imaging Technology and Chemometrics". Environ. Pollut. 2018. 238: 121–129.

299. Y. Zhang, X. Wang, J. Shan, et al. "Hyperspectral Imaging Based Method for Rapid Detection of Microplastics in the Intestinal Tracts of Fish". Environ. Sci. Technol. 2019. 53(9): 5151–5158.

300. S. Serranti, R. Palmieri, G. Bonifazi, et al. "Characterization of Microplastic Litter from Oceans by an Innovative Approach Based on Hyperspectral Imaging". Waste Manag. 2018. 76: 117–125.

301. R.C. Lyon, D.S. Lester, E.N. Lewis, E. Lee, et al. "Near-Infrared Spectral Imaging for Quality Assurance of Pharmaceutical Products: Analysis of Tablets to Assess Powder Blend Homogeneity". AAPS Pharmscitech. 2002. 3(3): E17.

302. E.N. Lewis, P.J. Treado, R.C. Reeder, et al. "Fourier Transform Spectroscopic Imaging Using an Infrared Focal-Plane Array Detector". Anal. Chem. 1995. 67(19): 3377–3381.

303. G. Dal Sasso, I. Angelini, L. Maritan, et al. "Raman Hyperspectral Imaging as an Effective and Highly Informative Tool to Study the Diagenetic Alteration of Fossil Bones". Talanta. 2018. 179: 167–176.

304. D. Gursoy, T. Bicer, A. Lanzirotti, et al. "Hyperspectral Image Reconstruction for X-ray Fluorescence Tomography". Opt. Express. 2015. 23(7): 9014–9023.

305. K. Mattsson, E.V. Johnson, A. Malmendal, et al. "Brain Damage and Behavioural Disorders in Fish Induced by Plastic Nanoparticles Delivered Through the Food Chain". Sci. Rep. 2017. 7(1): 11452.

306. J.I. Goldstein, D.E. Newbury, J.R. Michael, et al. Scanning Electron Microscopy and X-ray Microanalysis. New York: Springer Nature, 2018.

307. H.S. Auta, C.U. Emenike, B. Jayanthi, et al. "Growth Kinetics and Biodeterioration of Polypropylene Microplastics by Bacillus Sp. and Rhodococcus Sp. Isolated from Mangrove Sediment". Mar. Pollut. Bull. 2018. 127: 15–21.

308. Z.M. Wang, J. Wagner, S. Ghosal, et al. "SEM/EDS and Optical Microscopy Analyses of Microplastics in Ocean Trawl and Fish Guts". Sci. Total Environ. 2017. 603-604: 616–626.

309. E.G. Malawar, L. Senak. "Introduction to Size Exclusion Chromatography". In: C.-S. Wu, editor. Handbook of Size Exclusion Chromatography and Related Techniques. New York; Basel: Marcel Dekker, Inc, 2004. Pp. 1–23.

310. T. Biver, S. Bianchi, M.R. Carosi, et al. "Selective Determination of Poly(Styrene) and Polyolefin Microplastics in Sandy Beach Sediments

by Gel Permeation Chromatography Coupled with Fluorescence Detection". Mar. Pollut. Bull. 2018. 136: 269–275.

311. G.F. Schirinzi, M. Llorca, R. Seró, et al. "Trace Analysis of Polystyrene Microplastics in Natural Waters". Chemosphere. 2019. 236: 124321.

312. T.D.W. Claridge. High-Resolution NMR Techniques in Organic Chemistry. Amsterdam; Oxford; Cambridge: Elsevier Ltd., 2016.

313. F.A. Bovey, P.A. Mirau. NMR of Polymers. San Diego; London; Boston; New York; Sydney; Tokyo; Toronto: Academic Press, 1996.

314. N. Peez, M.C. Janiska, W. Imhof. "The First Application of Quantitative (1)H NMR Spectroscopy as a Simple and Fast Method of Identification and Quantification of Microplastic Particles (PE, PET, PS)". Anal. Bioanal. Chem. 2019. 411(4): 823–833.

315. H. Wijesekara, N.S. Bolan, L. Bradney, et al. "Trace Element Dynamics of Biosolids-Derived Microbeads". Chemosphere. 2018. 199: 331–339.

316. I. Acosta-Coley, D. Mendez-Cuadro, E. Rodriguez-Cavallo, et al. "Trace Elements in Microplastics in Cartagena: A Hotspot for Plastic Pollution at the Caribbean". Mar. Pollut. Bull. 2019. 139: 402–411.

317. A. Bellingeri, E. Bergami, G. Grassi, et al. "Combined Effects of Nanoplastics and Copper on the Freshwater Alga *Raphidocelis subcapitata*". Aquat. Toxicol. 2019. 210: 179–187.

318. A.B. Silva, A.S. Bastos, C.I.L. Justino, et al. "Microplastics in the Environment: Challenges in Analytical Chemistry–A Review". Anal. Chim. Acta. 2018. 1017: 1–19.

319. P.J. Anderson, S. Warrack, V. Langen, et al. "Microplastic Contamination in Lake Winnipeg, Canada". Environ. Pollut. 2017. 225: 223–231.

320. M. Eriksen, S. Mason, S. Wilson, et al. "Microplastic Pollution in the Surface Waters of the Laurentian Great Lakes". Mar. Pollut. Bull. 2013. 77(1-2): 177–182.

321. S.A. Strungaru, R. Jijie, M. Nicoara, et al. "Micro- (Nano) Plastics in Freshwater Ecosystems: Abundance, Toxicological Impact and Quantification Methodology". TRaC, Trends Anal. Chem. 2019. 110: 116–128.

322. C. Cardell, I. Guerra. "An Overview of Emerging Hyphenated SEM-EDX and Raman Spectroscopy Systems: Applications in Life, Environmental and Materials Sciences". TRaC, Trends Anal. Chem. 2016. 77: 156–166.

323. S. Ghosal, J. Wagner. "Correlated Raman Micro-Spectroscopy and Scanning Electron Microscopy Analyses of Flame Retardants in Environmental Samples: A Micro-Analytical Tool for Probing Chemical Composition, Origin and Spatial Distribution". Analyst. 2013. 138(13): 383–3844.

324. A.L. Dawson, S. Kawaguchi, C.K. King, et al. "Turning Microplastics into Nanoplastics Through Digestive Fragmentation by Antarctic Krill". Nat. Commun. 2018. 9(1): 1001.

325. I. Ferreira, C. Venancio, I. Lopes, et al. "Nanoplastics and Marine Organisms: What Has Been Studied?". Environ. Toxicol. Pharmacol. 2019. 67: 1–7.

326. R. Lehner, C. Weder, A. Petri-Fink, et al. "Emergence of Nanoplastic in the Environment and Possible Impact on Human Health". Environ. Sci. Technol. 2019. 53(4): 1748–1765.

327. M. Hassellv, R. Kaegi. "Analysis and Characterization of Manufactured Nanoparticles in Aquatic Environments". In: J.R. Lead, E. Smith, editors. Environmental and Human Health Impacts of Nanotechnology. Chichester, UK: John Wiley and Sons, 2009. Pp. 211–266.

328. S. Lambert, M. Wagner. "Characterisation of Nanoplastics During the Degradation of Polystyrene". Chemosphere. 2016. 145: 265–268.

329. S. Lambert, M. Wagner. "Formation of Microscopic Particles During the Degradation of Different Polymers". Chemosphere. 2016. 161: 510–517.

330. J.A. Gallego-Urrea, J. Tuoriniemi, T. Pallander, et al. "Measurements of Nanoparticle Number Concentrations and Size Distributions in Contrasting Aquatic Environments Using Nanoparticle Tracking Analysis". Environ. Chem. 2010. 7(1): 67–81.

331. V. Kestens, V. Bozatzidis, P.J. De Temmerman, et al. "Validation of a Particle Tracking Analysis Method for the Size Determination of Nano- and Microparticles". J. Nanopart. Res. 2017. 19(8): 271.

332. P. Hole, K. Sillence, C. Hannell, et al. "Interlaboratory Comparison of Size Measurements on Nanoparticles Using Nanoparticle Tracking Analysis (NTA)". J. Nanopart. Res. 2013. 15: 2101.

333. V. Filipe, A. Hawe, W. Jiskoot. "Critical Evaluation of Nanoparticle Tracking Analysis (NTA) by Nanosight for the Measurement of Nanoparticles and Protein Aggregates". Pharm. Res. 2010. 27(5): 796–810.

334. R. Gillibert, G. Balakrishnan, Q. Deshoules, et al. "Raman Tweezers for Small Microplastics and Nanoplastics Identification in Seawater". Environ. Sci. Technol. 2019. 53(15): 9003–9013.

335. G. Wille, X. Bourrat, N. Maubec, et al. "Raman-In-SEM, a Multimodal and Multiscale Analytical Tool: Performance for Materials and Expertise". Micron. 2014. 67: 50–64.

336. R.J. Hermann, M.J. Gordon. "Nanoscale Optical Microscopy and Spectroscopy Using Near-Field Probes". Annu. Rev. Chem. Biomol. Eng. 2018. 9(1): 365–387.

337. L. Xiao, Z.D. Schultz. "Spectroscopic Imaging at the Nanoscale: Technologies and Recent Applications". Anal. Chem. 2018. 90(1): 440–458.

338. A. Dazzi, C.B. Prater, Q. Hu, et al. "AFM-IR: Combining Atomic Force Microscopy and Infrared Spectroscopy for Nanoscale Chemical Characterization". Appl. Spectrosc. 2012. 66(12): 1365–1384.

339. J.R. Felts, K. Kjoller, M. Lo, et al. "Nanometer-Scale Infrared Spectroscopy of Heterogeneous Polymer Nanostructures Fabricated by Tip-Based Nanofabrication". ACS Nano. 2012. 6(9): 8015–8021.

340. E. Pancani, J. Mathurin, S. Bilent, et al. "High-Resolution Label-Free Detection of Biocompatible Polymeric Nanoparticles in Cells". Part. Part. Syst. Charact. 2018. 35(3): 1700457.

341. M. Brehm, T. Taubner, R. Hillenbrand, et al. "Infrared Spectroscopic Mapping of Single Nanoparticles and Viruses at Nanoscale Resolution". Nano Lett. 2006. 6(7): 1307–1310.

342. F. Huth, A. Govyadinov, S. Amarie, et al. "Nano-FT-IR Absorption Spectroscopy of Molecular Fingerprints at 20 Nm Spatial Resolution". Nano Lett. 2012. 12(8): 3973–3978.

343. M. Meyns, S. Primpke, G. Gerdts. "Library Based Identification and Characterisation of Polymers with Nano-FT-IR and IR-Ssnom Imaging". Anal. Methods. 2019. 11(40): 5195–5202.

344. T. Deckert-Gaudig, A. Taguchi, S. Kawata, et al. "Tip-Enhanced Raman Spectroscopy–From Early Developments to Recent Advances". Chem. Soc. Rev. 2017. 46(13): 4077–4110.

345. B.S. Yeo, E. Amstad, T. Schmid, et al. "Nanoscale Probing of a Polymer-Blend Thin Film with Tip-Enhanced Raman Spectroscopy". Small. 2009. 5(8): 952–960.

346. K. Tiede, A.B. Boxall, S.P. Tear, et al. "Detection and Characterization of Engineered Nanoparticles in Food and the Environment". Food Addit. Contam. Part A. 2008. 25(7): 795–821.

347. G. Lespes, J. Gigault. "Hyphenated Analytical Techniques for Multidimensional Characterisation of Submicron Particles: A Review". Anal. Chim. Acta. 2011. 692(1-2): 26–41.

348. Z. Wang, S.E. Taylor, P. Sharma, et al. "Poor Extraction Efficiencies of Polystyrene Nano- and Microplastics from Biosolids and Soil". PLoS One. 2018. 13(11): E0208009.

349. M. Correia, K. Loeschner. "Detection of Nanoplastics in Food by Asymmetric Flow Field-Flow Fractionation Coupled to Multi-Angle Light Scattering: Possibilities, Challenges and Analytical Limitations". Anal. Bioanal. Chem. 2018. 410(22): 5603–5615.

350. J. Gigault, B. Pedrono, B. Maxit, et al. "Marine Plastic Litter: The Unanalyzed Nano-Fraction". Environ. Sci.: Nano. 2016. 3(2): 346–350.

351. D. Magri, P. Sanchez-Moreno, G. Caputo, et al. "Laser Ablation as a Versatile Tool to Mimic Polyethylene Terephthalate Nanoplastic Pollutants: Characterization and Toxicology Assessment". ACS Nano. 2018. 12(8): 7690–7700.

352. M. Baudrimont, A. Arini, C. Guegan, et al. "Ecotoxicity of Polyethylene Nanoplastics from the North Atlantic Oceanic Gyre on Freshwater and Marine Organisms (Microalgae and Filter-Feeding Bivalves)". Environ. Sci. Pollut. Res. Int. 2020. 27(4): 3746–3755.

353. G. Balakrishnan, M. Deniel, T. Nicolai, et al. "Towards More Realistic Reference Microplastics and Nanoplastics: Preparation of Polyethylene Micro/Nanoparticles with a Biosurfactant". Environ. Sci.: Nano. 2019. 6(1): 315–324.

354. D.M. Mitrano, A. Beltzung, S. Frehland, et al. "Synthesis of Metal-Doped Nanoplastics and Their Utility to Investigate Fate and Behaviour in Complex Environmental Systems". Nat. Nanotechnol. 2019. 14(4): 362–368.

355. L. Pessoni, C. Veclin, H. El Hadri, et al. "Soap- and Metal-Free Polystyrene Latex Particles as a Nanoplastic Model". Environ. Sci.: Nano. 2019. 6(7): 2253–2258.

356. T.R. Zhang, C.X. Wang, F.Q. Dong, et al. "Uptake and Translocation of Styrene Maleic Anhydride Nanoparticles in Murraya Exotica Plants as Revealed by Noninvasive, Real-Time Optical Bioimaging". Environ. Sci. Technol. 2019. 53(3): 1471–1481.

357. F. Bessa, J. Frias, T. Kögel, et al. Harmonized Protocol for Monitoring Microplastics in Biota. https://www.researchgate.net/publication/332157735_harmonized_protocol_for_monitoring_microplastics_in_biota: 2019 [accessed 2 April 2020]. doi: 10.13140/RG.2.2.28588.72321/1.

358. A. Filguerias, J. Gago, M.L. Pedrotti, et al. "Standardised Protocol for Monitoring Microplastics in Seawater" https://www.researchgate.net/publication/331936034_standardised_protocol_for_monitoring_microplastics_in_seawater: 2019 [accessed 2 April 2020].

359. J. Frias, E. Pagter, R. Nash, et al "Standardised Protocol for Monitoring Microplastics in Sediments" https://www.researchgate.net/publication/326552185_standardised_protocol_for_monitoring_microplastics_in_sediments: 2018 [accessed 2 April 2020].

360. C. Zarfl. "Promising Techniques and Open Challenges for Microplastic Identification and Quantification in Environmental Matrices". Anal. Bioanal. Chem. 2019. 411(17): 3743–3756.

361. A.A. Koelmans, N.H. Mohamed Nor, E. Hermsen, et al. "Microplastics in Freshwaters and Drinking Water: Critical Review and Assessment of Data Quality". Water Res. 2019. 155: 410–422.

362. J. Li, H. Liu, J. Paul Chen. "Microplastics in Freshwater Systems: A Review on Occurrence Environmental Effects, Methods for Microplastics Detection". Water Res. 2018. 137: 362–374.

363. L. Mai, L.J. Bao, L. Shi, et al. "A Review of Methods for Measuring Microplastics in Aquatic Environments". Environ. Sci. Pollut. Res. Int. 2018. 25(12): 11319–11332.

364. W.F. Wang, J. Wang. "Investigation of Microplastics in Aquatic Environments: An Overview of the Methods Used, from Field Sampling to Laboratory Analysis". TRaC, Trends Anal. Chem. 2018. 108: 195–202.