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Status Report

Within the framework program

Plastics in the Environment

Sources • Sinks • Solutions

Analysis of Microplastics

Sampling, preparation and detection methods

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Table of contents

1. Introduction

The subject area "Plastics in the Environment" is multifaceted and encompasses various aspects. Alongside many unresolved "research questions", microplastics (MP) analysis in particular has proven to be a very complex task. There are still many unanswered questions in this relatively new field of research. At the same time, it is undeniable that MP are ubiquitously present and detectable. This has already been demonstrated by numerous studies. However, it has been difficult to validate and reproduce the results and consequently to draw comparisons between them. Even after several years of experience, the analysis of MP is still a major challenge for many laboratories; standardized and validated methods are still under development.

It is therefore all the more important to engage in an interdisciplinary discussion on sampling, sample preparation and detection methods. This status report summarizes the current state of knowledge on these topics from the last three years of work within the research focus "Plastics in the Environment - Sources • Sinks • Solutions" and its precursor projects. It presents proven methods for specific research questions and thus forms an important basis for the further development and harmonization of MP analysis.

1.1 The research focus "Plastics in the Environment“

The German Federal Ministry of Education and Research (BMBF) is funding a total of 20 collaborative projects and a scientific accompanying project with around 40 million euros over the period 2017-2021 as part of the research focus "Plastics in the Environment - Sources • Sinks • Solutions" (www.bmbf-plastik.de). The aim of the research is to scientifically assess problems associated with plastic waste in their entirety for the first time and to close existing knowledge gaps.

More than 100 institutions from the fields of science, industry and practice are involved in what is currently the world's largest research focus on the impact of plastics on the environment. The research projects can be assigned to five different topic areas, which are organized along the entire life cycle of plastics:

- Green economy: material flows, value chains, technologies
- Consumption, consumer behavior, trade and production, governance
- Recycling technologies
- Input pathways, transport, decomposition and retention in the limnic systems
- Seas and oceans as sinks and accumulation areas

The interdisciplinary nature of the research focus is intended to provide a better understanding of the environmental impacts of undesirable plastic inputs, starting from soils, through river basins, and into the oceans. In addition, approaches to reducing and preventing inputs will be identified and implemented. The spectrum of topics covers the improvement of certain plastic materials with regard to their environmentally compatible degradability. It also encompasses the investigation of input pathways into soils and water bodies, the analysis of possible toxic effects on aquatic organisms as well as the strengthening of environmental awareness among consumers. As key users and implementers of innovations, companies are involved in the development and realization of the research projects since the beginning.

1.2 About this status report

This status paper was prepared within the framework of the cross-cutting issue 1 "Analytics and reference materials" of the research focus. It summarizes the contents of the project discussions and coordination within the cross-cutting issue. The organization of this ongoing process of development or elaboration has been supported by several events.

The status report presents a summary of the physicochemical methods for the analysis of MP used in the research focus "Plastics in the Environment". The present recommendations are based on the current state of knowledge of the analysis of MP and are directed at actors in science as well as potential users in practice.

Scientists from the following collaborative projects of the research focus participated in the preparation of the status report via workshops, contributions and feedback loops: EmiStop, ENSURE, MicBin, MicroCatch_Balt, MikroPlaTaS, PLASTRAT, PLAWES, RAU, REPLAWA, RUSEKU, SubμTrack and TextileMission as well as the two predecessor projects MiWa and BASEMAN.

2. General principles

2.1 Objective

Different methods are necessary to answer the respective questions and goals. This includes not only the detection methods, but also the related sampling and sample preparation procedures extending to the statistical evaluation of the results. At the end, we include an overview of strengths and limitations of different methods depending on the problem to be addressed. A major objective is the safe and traceable examination of the transport and input paths of MP into different environmental media, such as water and soil, using the respective appropriate measurement and examination methods.

A schematic representation of the interrelations of MP analysis is shown in Figure 1. The MP analysis consists of three sub-steps, which are complex in handling and analytical results: sampling, sample preparation and detection.

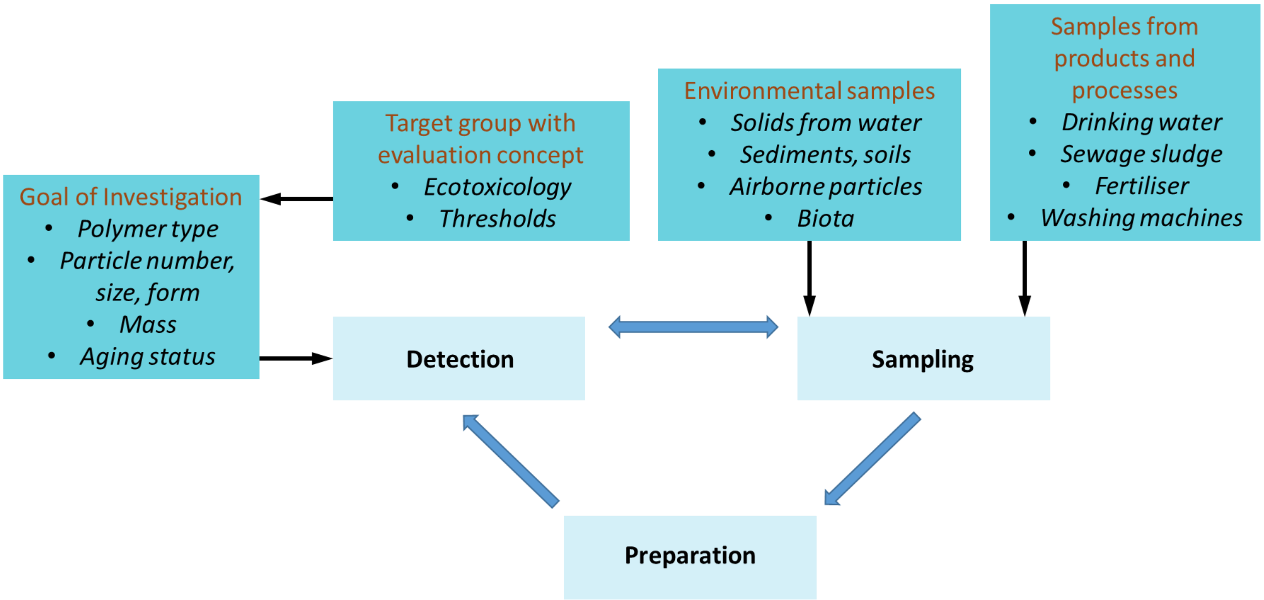

Figure 1: Schematic representation of the connections in MP detection

Usually the objective of a measurement or a measurement program is based on a clear question or a technical or evaluation concept, which is based on necessary evaluation parameters, e.g. integration into an overall ecological context, limit values for monitoring.

Based on the specific question, a suitable detection method is selected, which generates different MP information: polymer type, contents, number, shape, size, degradation state.

The environmental area to be sampled e.g. water, soil / sediment but also the product area to be sampled, which includes compost, sewage sludge as well as drinking water, determines the sampling procedure. The sampling procedure must ensure that a representative portion of the medium is sampled with sufficient analyte content for the selected detection method.

The sample preparation depends on the environmental matrix to be analyzed (e.g. quality of natural accompanying organic materials, proportions of inorganic substances), the sample quantity to be analyzed and the chosen detection method. Consequently, different procedures are necessary depending on the environmental sample and the detection method.

The following questions should therefore be answered at the beginning of every MP analysis:

- Which objective is to be achieved with the measurements?
- What kind of results shall be achieved with the measurements?
- Which environmental media / which products are to be sampled?

2.2 Definitions[1]

In German the terms plastic or macroplastic and microplastic adapt the English term "plastics" and are colloquial terms for plastic or microplastic. By definition, the term plastics covers only thermoplastics, including polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), polyamide (PA), polymethyl methacrylate (PMMA) and polyvinyl chloride (PVC), and thermosetting plastics, including epoxy resins (EP), polyurethane resins (PUR) and polyester resins (UP). Elastomers built up from synthetic polymers, e.g. styrene-butadiene rubber (SBR), ethylene-propylene-diene rubber (EPDM). Products based on predominantly synthetic polymers (e.g. textile fibers, coatings, tires) are also taken into account in current research activities. These materials and products can also release microparticles that are identified as synthetic polymers. For simplification, all the materials mentioned above

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[1] Terms and definitions of plastics in the environment, Cross Cutting Issue 3 “Terms and Definitions”.

are colloquially summarized in this document by the term "plastics / microplastics (MP) / macroplastics".

The classification of processed, natural polymers (e.g. viscose fibers, cellulose hydrate) or chemically modified, natural polymers (e.g. cellulose acetate, vulcanized natural rubber) is still open, since they can hardly be distinguished as MP particles from naturally occurring polymers.

2.3 Classification

It is recommended to classify MP analyses into size classes in accordance with Table 1. This improves the methodological applicability of procedures (feasibility of filtration, detection limits in the detection method, among others) and enables the classification of particle quantities and contents in impact analyses (e.g. for environmental assessment).

*Table 1: Particle size classification (*assuming spherical particles with a density of 1 g/cm3)*

Classification	Large microplastic	Microplastic					
Particle size classes /µm	5,000 – 1,000	1,000 – 500	500 – 100	100 – 50	50 – 10	10 – 5	5 – 1
Average particle size in size class / µm	3,000	750	300	75	30	7.5	3
Mass of an individual particle* / mg	14.13	0.221	0.014	2.2E-04	1.4E-05	2.2E-07	1.4E-08
Number of particles in 14.13 mg (Ø = 3 mm particle)	1	64	1 000	6.4E+04	1.0E+06	6.4E+07	1.0E+09

The classification is based on a numerical model and the "historical definition" with the numbers 1 and 5 mm. The following size classes are proposed: 5,000 - 1,000 µm, <1,000 - 500 µm, <500 - 100 µm, <100 - 50 µm, <50 - 10 µm, <10 - 5 µm, <5 - 1 µm. The maximum dimension of a particle or film fragment or the length of a fibrous structure defines the classification into size classes. The analysis of plastic particles < 1 µm ("nanoplastic") is not considered in this document.

The table also shows in which relation to the size class the mass of a spherical, individual particle (with density 1 g/cm^3) decreases or the number of particles at a defined mass (e.g. 14.13 mg) increases.

This classification was developed specifically for MP and therefore differs from other classification systems. The particle sizes and the particle size distribution have an influence on the material properties of the solid(s). In the different technical and scientific areas (e.g. water, soil, air, civil engineering, geology), different classification systems with different limits for the characterization of solids have been developed[2]. A universal particle classification system therefore does not exist.

2.4 Representation of results

The aim is to depict a uniform presentation of the results (exception biota, see 6.5). Therefore, the following information should always be provided in the future:

i. MP number (possibly polymer type) per volume for sampled waters / liquids / air volume (**number / L**) or per total dry mass for sampled solids (**number / kg**) including indication of the investigated particle size range.
ii. MP mass (possibly polymer type) per volume for sampled waters / liquids / air volume (**µg / L**) or per total dry mass for sampled solids (**mg / kg**) including indication of the investigated particle size range.

An accurate description and traceable documentation of the amount of the environmental aliquot sampled, the prepared laboratory sample and the test sample shall always be provided. Details can be found in the individual chapters on detection, sampling and preparation.

It should be noted that the conversion between particle masses and particle numbers is highly erroneous due to necessary assumptions (e.g. spherical particle shape) and was

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[2] In the field of soil science in Germany, the classification of soil types is based on the grain size composition of the mineral soil substance according to the predominant grain fraction into the main soil types clays, silt, sands and loams. The limiting sizes are based on the numbers 63 µm and 2 mm. The value 63 µm is also used in the Surface Water Ordinance (Oberflächengewässerverordnung) for the specific characterization of solids with regard to heavy metals.

only performed for illustrative purposes in Table 1. A conversion between particle numbers and masses is generally not recommended.

2.5 Quality control

At all times during analysis (sampling, preparation, detection), maximum "plastic-free" working conditions must be ensured. This includes avoiding the use of plastic sampling and laboratory equipment and using alternatives made of metal, glass or silicone. An exception is the use of plastics that are not to be detected or evaluated.

Samples in the laboratory should always be handled in clean room workbenches (*laminar flow benches*), especially during sample preparation to avoid contamination via e.g. air. To avoid contamination during detection, the use of air cleaners/filters is recommended if the detection procedures cannot be operated within laminar flow benches. If this is not possible, the documentation of blank values of the room air e.g. over the measurement period is recommended.

The determination and documentation of control tests shall be performed and documented taking into account all analytical steps and under comparable conditions (same steps, same duration, same volume) for all samples (even if the working atmosphere and environment is free of plastics or low in plastics).

The documentation and determination of blank values or process blank values for the applied preparation and detection methods is absolutely necessary, since contamination during sampling, sample preparation and detection (contamination by air transfer) are critical points. Specific recommendations are given in the chapters on detection.

Determination and documentation of recovery rates using defined reference materials (particle number and/or mass for MP of different polymer types / densities, particle sizes and shapes) shall be presented for all methods. For this purpose, separate recommendations are given in the chapter on reference materials.

Likewise, examination of particle stability of different polymers under the conditions of the described sampling (e.g. pressure filtration), sample preparation (e.g. chemical preparation, ultrasound) and detection conditions shall be documented with suitable reference materials. Depending on polymer type, particle size and especially the aging condition, degradation of smaller particles (surface oxidation) and/or fragmentation of brittle, larger particles may occur.

3. Identification of research objective and research question

Specification of a detection method depends specifically on the objectives and questions of a project or an existing requirement. Currently two main paths are followed in detecting MP: 1) MP content determination of the different polymers, 2) MP particle numbers and sizes are determined specifically for each polymer. The individual characterization of specific properties of isolated MP particles is rare due to the high effort.

The detection of very small MP particles (below 5 µm) is comparatively time-consuming, but is of particular interest from a human and ecotoxicological point of view. Smaller particles are likely to have more relevant effects (e.g. transitions in the cellular domain) than larger particles. In addition, the properties of individual particles (surface morphology and chemical structure) can also be decisive for the analysis of effects. However, there are no reliable findings in this regard yet.

An overview of the research objectives and detection methods is shown in Figure 2 and will be defined in more detail in the following sections.

3.1 Content determination

From a regulatory point of view, MP contents are an important parameter to estimate the basic occurrence of polymers. They are useful for regular, repeated determination of MP within the scope of monitoring and for controlling the effect of measures against plastic contamination.

In addition to clear assignment of the polymer type, it must be defined in advance for which nominal particle size range these determinations should be made. This subdivision into size classes (Table 1) makes it possible to assign the MP contents to a specific particle size range.

Fundamentally, it must be considered that a few large particles mask many small particles in terms of mass balance. Low quantities of small particles contribute only slightly to the mass.

3.2 Particle determination

The determination of particle number, size and shape gives a detailed picture of the occurrence of MP in environmental samples. Particle shape, number and size can differentiate the particles of the different polymers. Basically, it can be said that in terms of numbers, significantly more small than large particles are found.

The detection of very small particles (< 5 µm or < 10 µm, respectively) is very complex and only marginally possible for real samples so far. As a rule, samples have to be prepared in advance for particle determination.

3.3 Characterization of specific properties

The individual characterization of specific properties of isolated particles, e.g. aging condition, surface structure or condition and the analysis of additives can be relevant for the evaluation of interactions with the environment, but also of sources, input paths and retention. Such analyses can be important for the evaluation of toxicological properties, but they require prior, sometimes very complex, isolation of individual particles.

Monitoring, high MP contamination	Detailed investigation, low MP contamination	Sources and fate, toxicological investigation
Determination of MP contens	Determination of MP particle numbers	Determination of MP particle properties
Polymer type Polymer mass	Polymer type Particle size Particle form	Polymer type Additives Particle size Particle form Surface morphology

Figure 2: Identification of examination objective and result

4. Selection of the detection method

A basic distinction is made between three different detection methods. Spectroscopic methods are used to detect characteristics of the specific chemical structure of polymers and to assign reference spectra. In thermoanalytical methods, the sample is pyrolyzed under inert conditions and specific decomposition products of the individual polymers are detected. Finally, certain plastics can also be broken down through chemical processes and specific decomposition products of polymers or elements from additives or from plastic itself can be detected and used for classification. Chemical processes do not include those that dissolve MP as a largely intact polymer and thus extract it from the matrix (see Chapter 6.6).

Spectroscopic detection methods facilitate the determination of polymer types as well as the number, size/size distribution and shape of MP particles. Thermoanalytical/chemical detection methods determine polymer types and MP contents. Both detection methods are complementary to each other. Relatively speaking, the obtained data can be compared between the approaches; with the current technical possibilities, however, the data cannot be superimposed from one onto the other.

When measuring real samples using purely imaging techniques (e.g. light and electron microscope) and particle counting techniques (e.g. light scattering, laser scattering, Coulter Counter) there is a high risk of misinterpretation, because MP particles cannot be identified clearly compared to e.g. naturally occurring particles. Therefore, it is essential to perform these tests with reference and blank samples.

4.1 Spectroscopic methods

Infrared spectroscopy

Sebastian Primpke, Gunnar Gerdts[3]

One of the most common methods for the non-destructive determination of the chemical structure based on covalent bonds of a known substance/chemical is infrared spectroscopy. This method uses mid-infrared radiation (MIR) to excite the covalent bonds of a chemical compound to oscillate by absorbing the irradiated energy. The different proportions of bonds in a molecule and thus different excitable vibrations result in specific spectra that act like a "fingerprint" and can be matched via databases or with other data analysis methods. The sample must be free of water; otherwise its IR spectrum will cover the spectrum of the analyte.

In general, detection by IR (infrared) or FTIR (Fourier-transform infrared spectroscopy) offers different measurement principles based on reflection and/or transmission. In transmission measurement, the sample is completely penetrated by the IR radiation, resulting in a spectrum of the complete particle. However, this type of measurement is susceptible to total absorption above a certain particle or layer thickness and materials with a high soot content, whereby either spectral details or the complete signal is lost. When using reflection, the IR beam is directed onto the sample and the reflected radiation is detected; however, this requires a highly reflective sample. This method is therefore performed on highly reflective surfaces (e.g. gold-coated filters). By means of attenuated total reflection (ATR), in which a crystal is pressed onto the particles and the spectrum at the interface between crystal and sample is measured, the surface structure of the particle in particular can be detected.

Due to this variety of measurement possibilities, infrared spectroscopy is often used for the detection of MP. ATR-FTIR spectrometry is very common for larger particles

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[3] S. Primpke, R.K. Cross, S.M. Mintenig, M. Simon, A. Vianello, G. Gerdts, J Vollertsen, 2020, Journal of Applied Spectroscopy, 3702820917760.

S. Primpke, S.H. Christiansen, W. Cowger, H. De Frond, A. Deshpande, M Fischer, E. Holland, M. Meyns, B.A. O'Donnell, B.E. Oßmann, M. Pittroff, G. Sarau, B. Scholz-Böttcher, K. Wiggin, 2020, Applied Spectroscopy 2020, Vol. 74(9) 1012–1047.

(approx. > 500 µm). Smaller particles (approx. <500 µm) can be examined on a filter / window fixed with a µ-ATR objective, which is manually or automatically brought into contact with the particles to be examined. However, this bears the risk that single particles will adhere to the crystal of the ATR objective and thus be carried away or hard particles will damage the crystal.

Measurements in reflection and transmission are mostly used with a (confocal) FTIR microscope (µ-FTIR) for the detection of particles <500 µm. By using so-called focal plane array (FPA) detectors, the measurement throughput can be significantly increased. Depending on the detector size and manufacturer, the FPA detectors can analyze areas of approximately 0.7 mm × 0.7 mm in one measurement (about 1 min, up to 16,384 spectra).

When using IR microscopes, the particles can be selected prior to detection. Marking the particles is done either by the user or an image analysis algorithm. The particles should be isolated as far as possible in order to allow a clear identification. That said, a so-called chemical mapping can be carried out, in which the spectral information of the examined area is displayed in spatial resolution, for example using a grid. The resolution varies depending on the optics and detector size used. This imaging measurement has the advantage that large filter areas can be analyzed in a short time (e.g. 14.8 mm × 14.8 mm, ~ 4 hours measuring time). By using IR transparent filter materials, the particles can be quickly prepared for the measurement, e.g. by filtration onto a suitable substrate. Aluminum oxide is most commonly used as filter substrate, despite the limited measuring range (3600-1250 cm^{-1}), because it is comparatively inexpensive. Alternatives are filter membranes made of silicon or metal coated polycarbonate filters.

A new possibility is to use laser-based IR systems that utilize a special laser (µ-IR). These have the advantage of significantly higher radiation power compared to conventional IR sources. Each wavenumber is tuned individually and thus a spectrum is obtained, which is much less computationally intensive than the Fourier transformation, but more time consuming in the measurement. Due to the high power, there is no need to cool the detector with liquid nitrogen. In combination with FPA detectors, short measurement times for large areas (e.g. 12 mm × 12 mm, ~ 36 minutes measurement time) at a pixel resolution of 4.2 µm can be obtained.

All of the imaging measurement procedures generate large amounts of data, for which various software tools are currently available for evaluation. These use well-known software tools such as R, Python or Matlab or commercial solutions and different algorithms/approaches for spectrum assignment. A freely available variant that can analyze both large data sets and individual spectra is the siMPle software (www.simple-plastics.eu).

The detection limit for µ-IR spectroscopy depends on the instrument used, the measurement parameters and the optics; usually particles down to a size of 10 µm can be identified and quantified.

To use this method effectively for particles smaller than 500 µm, chemical or enzymatic sample preparation is usually necessary to remove the (in)organic matrix particles and reduce the number of potential particles. However, these measures are not always sufficient, so that a partial analysis is performed. In such a case, at least 50 particles (>500 µm) or 50% of the filter area (<500 µm) should be examined to obtain a representative result.

For reliable IR results, blank values need to be determined.

Ideally, process blank samples cover contamination from sampling, preparation and detection. These determine both internal and MP contamination caused by sampling and preparation and help to find and eliminate sources of contamination. The number of process blank samples is coupled with the number of samples and therefore at least one process blank value should be determined for every five samples.

Laboratory blank values become necessary if, for example, preparation and detection take place in separate laboratories. These determine internally caused MP contaminations and help to find and eliminate sources of contamination. Based on the laboratory blank values the limit of detection (LOD) for the laboratory can be determined. For the LOD determination, at least 3 (optimally 10) laboratory blank values are recommended.

Near infrared spectroscopy

Mathias Bochow[4]

The near infrared (NIR) is the spectral range of the electromagnetic spectrum adjacent to the MIR, in which we find the so-called overtones and combination bands of the (fundamental) absorption bands located in the MIR. As in vibrational spectroscopy in the MIR, molecules are stimulated to vibrate by electromagnetic radiation. The energy thus absorbed by the molecules is missing in the reflectance spectra as well as in the transmittance spectra. Since the spectral ranges in which this stimulation occurs are molecule-specific (spectral fingerprint), the position, shape and intensity of the absorption bands present in the spectrum can be used to identify and quantify substances. For this purpose, automated statistical methods from the field of chemometrics are usually used and a comparison with reference spectra from spectral databases is performed.

Reflection measurements are the common measuring principle. NIR spectrometers are available as point spectrometers and imaging spectrometers. A point spectrometer can be used for contact measurements, e.g. on a particle, similar to the ATR measurement in the MIR. However, since the measuring head consists of an optical lens, whereby the position of the measuring spot cannot be determined exactly, this measuring principle is only suitable for macroplastics (particles > 1 mm). In addition, a larger area can be measured in an integrated way by increasing the measuring distance. In doing so, a single spectrum is obtained for the measured area. Based on the measured spectrum, statistical methods can be used to determine the degree of coverage of the measured spot with the target material (polymer) or the content of the target material in the matrix in mass percent. For this purpose, a calibration by reference samples with known degrees of coverage or mixing ratios of the polymer(s) and the matrix to be examined is necessary in advance. Since there are transportable, field-suitable NIR spectrometers ("IR guns") in addition to laboratory equipment, such a quantification can also be

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[4] L.K. Schmidt, M. Bochow, H.K. Imhof, S.E. Oswald, Environ. Pollut. 2018, 239, 579–589.

A. Paul, L. Wander, R. Becker, C. Goedecke, U. Braun, Environ Sci Pollut Res 2019, 26, 7364–7374.

T. Kuester, S. Zhou, K. Segl, M. Bochow, Frontiers in Environmental Science, 2020, submitted.

performed *in situ*. Furthermore, NIR spectrometers can also be introduced into a sample (e.g. into soil) by means of a measuring probe.

Experience shows that the detection lower limit for polymers in practice is about 1 m% of polymers in soil samples or a coverage of the measuring spot from 5 f%; however, this is strongly dependent on the surrounding matrix and substrate as well as the transparency and brightness of the polymer.

Imaging spectrometers resolve the area to be measured into numerous individual measurements analogous to FPA-based FTIR spectroscopy. An image of the sample is generated with a separate spectral measurement for each pixel. Depending on the optical lens used, the pixel sizes are in the range of 50 µm or larger and correspond approximately to the diameter of the smallest detectable MP particle. The analysis with methods from the field of image processing enables automatic counting and size calculation of the identified MP particles.

While NIR spectroscopy has been used for decades as a standard method for online quality assurance in food production or for online sorting of plastic packaging in recycling, the first experimental methods for MP analysis in water or soil samples have only recently become available. Due to the comparatively very high measuring speed (10 min for a 47 mm diameter filter) and relative insensitivity to contaminants such as biofilms, NIR spectroscopy allows a very high sample throughput. In any case, the sample must be filtered on glass fiber filters without organic binder before measurement and dried in a drying oven at max. 50 °C.

For NIR measurements there is currently no information available about the determination of process and laboratory blank values.

Raman spectroscopy

Natalia P. Ivleva, Dieter Fischer, Nicole Zumbülte[5]

Raman spectroscopy is a non-destructive analytical method based on the effect of inelastic light scattering on molecules. Raman spectroscopy provides "fingerprint" spectra. Both polymers (type of polymer) and additives (e.g. pigments, oxides) as well as inorganic and organic ingredients can be identified with this method. The coupling of Raman spectroscopy with confocal optical microscopy (Raman microspectroscopy, µ-Raman spectroscopy) and the use of a laser in the visible range allows a spatial resolution of down to 1 µm and even below (up to approx. 300 nm). µ-Raman spectroscopy is not sensitive to water. This enables the additional examination of MP in aqueous and (micro)biological samples.

A major drawback of Raman spectroscopy for the detection of MP in environmental samples is interference by fluorescence, which can be caused by (micro)biological, organic (such as humic acids) and inorganic compounds (such as clay minerals) in the matrix. Therefore, it is recommended to subject the MP samples to a sample preparation step prior to Raman detection. Furthermore, the choice of suitable measurement parameters (laser wavelength and power, photobleaching and acquisition time, as well as objective magnification and confocal mode) is important to minimize or avoid interference from strong fluorescence. In addition, the removal of the (in)organic accompanying matrix from complex (environmental) samples leads to a significant enrichment of the MP particles.

Despite the processing, a (high) number of natural particles are still to be expected in environmental samples. A fractional filtration of the particles into different size classes

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[5] P. M. Anger, E. von der Esch, T. Baumann, M. Elsner, R. Niessner & N. P. Ivleva, 2018, Trends in Analytical Chemistry 109 214-226.

J. Brandt, L. Bittrich, F. Fischer, E. Kanaki, A. Tagg, R. Lenz, M. Labrenz, E. Brandes, D. Fischer, K.-J. Eichhorn. 2020, Applied Spectroscopy 74(9) 1185-1197.

Y. K. Müller, T. Wernicke, M. Pittroff, C. S. Witzig, F. R. Storck, J. Klinger, N. Zumbülte, 2020, Analytical & Bioanalytical Chemistry 412 555-560.

C. Schwaferts, V. Sogne, R. Welz, F. Meier, T. Klein, R. Niessner, M. Elsner & N. P. Ivleva, Analytical Chemistry 2020 92(8) 5813-5820.

before measurement is helpful. Since a very high number of particles have to be analyzed, an automation of µ-Raman spectroscopy is necessary. Currently some commercial and open source programs (https://gitlab.ipfdd.de/GEPARD/gepard and *TUM-ParticleTyper* (doi.org/10.1371/journal.pone.02347) can be used for this purpose.

Statistical methods can be used to determine a minimum number of particles that are to be randomly selected and chemically identified on a filter. To ensure statistical certainty of the measurements, however, at least 20 % of the filter surface should be measured, since an inhomogeneous distribution of the MP particles may be present due to the process. The detection of particles > 5 µm or >10 µm can be realized for the entire filter (e.g. 10 mm or 25 mm diameter). With µ-Raman spectroscopy MP particles down to 1 µm can be examined. However, when analyzing particles this small, only a (very) small part of the filter (a few % of the surface) can be measured. If the sample is inhomogeneous, larger errors can occur when extrapolating the results to the entire filter area. Therefore, a homogeneous particle distribution on the filter must be ensured

For automated µ-Raman spectroscopy detection, gold-coated polycarbonate filters as well as silicon and Teflon filters have proven to be particularly well suited.

To realize a reliable µ-Raman detection, blank values must be determined. Laboratory blank values become necessary, if e.g. sample preparation and MP detection take place in separate laboratories. At least 3 (optimally 10) laboratory blank values are recommended. The laboratory blank values (incl. examination of ultrapure water systems, filtration system, particle & fiber input during filtration and identification & quantification) determine internal MP contamination and help to find or eliminate the sources of contamination. In addition, at least 1 process blank value should be determined for each time-limited series of measurements in order to consider the contamination during sampling, sample preparation and detection.

A combination of field-flow fractionation and µ-Raman spectroscopy has shown clear potential for the analysis of plastic particles in the submicro range. Using model samples, it has already been possible to realize chemical identification of different submicroparticle fractions (including plastic particles and inorganic particles down to 200 nm).

4.2 Thermoananalytic methods

Pyrolytic methods

Barbara Scholz-Böttcher, Georg Dierkes, Ulrike Braun[6]

At high temperatures, plastics decompose into characteristic fragments or decomposition products if the environment is free of oxygen. The generated volatile pyrolysis products refer directly to the nature of the polymer and allow conclusions about its chemical composition. To increase the analytical significance, pyrolysis systems are usually connected to a gas chromatograph, ideally with mass-spectrometric detection (GC/MS system). With respect to the instrumentation, there are two different types of pyrolysis units and their respective gas chromatographic coupling.

The online pyrolysis (Py), performed with filament, micro oven or Curie-Point pyrolizers, is usually directly connected to the injection system of the gas chromatograph. The sample is pyrolyzed in a suitable sample cup (target), under inert gas (e.g. helium) and over a ramped temperature gradient or at constant temperature (for plastics e.g. 500 - 700 °C). The resulting volatile pyrolysis products are transferred directly into the GC/MS system in a helium stream, followed by a chromatographical separation and subsequent mass-specific detection.

In Thermal Extraction Desorption (TED), the sample is decomposed under inert gas during a controlled heating process (up to approx. 600 °C) in a thermobalance. The thermobalance is connected to the GC/MS system via a thermoextraction desorption module. The thermally released volatile pyrolysis products, if requested, of a certain temperature range, are sorbed on a solid phase, then thermodesorbed in a helium stream, and identified by means of GC/MS.

The gas chromatographic separation and detection of the resulting pyrolysis products in the mass-selective detector allows the identification of individual, single MP particles,

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[6] M. Fischer, B. M. Scholz-Bottcher, Analytical Methods 2019, 11, 2489-2497.

G. Dierkes, T. Lauschke, S. Becher, H. Schumacher, C. Földi, T. Ternes, Analytical and Bioanalytical Chemistry 2019, 411, 6959-6968.

E. Duemichen, P. Eisentraut, M. Celina, U. Braun, Journal of Chromatography A 2019, 1592, 133-142.

but also the simultaneous detection of individual polymers in plastic mixtures or in natural environmental samples. For isolated particles, the generated gas chromatogram (pyrogram) is equivalent to a "fingerprint" for the respective plastic. Plastics can be reliably identified by comparison of these pyrograms with reference pyrograms from own databases or literature collections. Depending on their thermal fragmentation behavior, the pyrograms of plastics contain multiple (e.g. PE, PP, PET), few (e.g. PS) or only individual (e.g. PMMA), prominent pyrolysis products. For these pyrolysis products, the corresponding ion chromatograms can be extracted from the pyrogram recorded during GC/MS analysis. Positive signals at the corresponding retention times allow the identification of the corresponding polymers even from the more complex mixtures.

Thermoanalytical methods can also detect relevant components of tire abrasion (e.g. SBR/BR) both qualitatively and quantitatively. The analysis of SBR/BR can be carried out according to ISO/TS 21396:2017-12 (Rubber - Determination of mass concentration of tire and road wear particles (TRWP) in soil and sediments – Pyrolysis-GC/MS method) (see also chapter 4.3.)

If pyrolysis is carried out under exactly reproducible conditions, there is a correlation between the area under the signals of the indicator ions in the ion chromatogram and the mass of the respective polymer present in the sample cup. This response is linear in a system-dependent concentration range. By integrating the respective signals, the mass of the polymers can thus be determined reproducibly. This requires an external calibration of the respective polymers. The overall data quality can be improved considerably by adding an internal standard (e.g. deuterated PS).

Depending on the MP content and the amount of (in)organic accompanying matrix, quantification from complex environmental samples is possible directly or after appropriate sample preparation. The organic accompanying matrix in particular can lead to pyrolysis products that might interfere with the indicator ions and lead to rapid contamination of the system. The proportion of inorganic accompanying matrix directly affects the effective sample volume used for the measurement, since volumes used in thermoanalytical methods are limited to a few µg to mg, depending on the given method. In contrast to the organic accompanying matrix, the effect of the natural inorganic accompanying matrix on the composition and yield of the pyrolysis products is of negligible importance to a certain degree.

Thermal methods also require parallel processing of 1-2 process blank values per reprocessing series. This is obligatory in order to realistically assess any secondary contamination or carry-over caused by sample processing, and, if necessary, to be able to make appropriate corrections to the measured values. It is also recommended to integrate the samples used for calibration into the respective measuring sequence as well.

Calorimetric methods

Hajo Bitter[7]

Another category of thermoanalytical determination methods is calorimetry. In this method, phase transitions of polymers and their heat flows are used to draw conclusions about MP contents.

Differential Scanning Calorimetry (DSC) is traditionally used to determine thermal properties of plastics, such as specific heat capacities and glass-transition or melting temperatures. An empty reference crucible and a sample crucible are heated or cooled down simultaneously at constant rates in a furnace. If heat is absorbed (endothermic) or released (exothermic), temperature differences are recorded by sensors underneath the crucibles and converted into heat flows. The results are thermograms with peaks or steps.

Melting processes only exist for plastics with crystalline fractions (e.g. PE, PP, PA or PET). They are endothermic and temperature specific for these plastics. Another important parameter is the specific melting enthalpy. It is proportional to the crystalline fraction of a plastic and can be used to quantify single substances or mixtures. Plastics without crystalline fractions, such as PVC or PS, cannot be determined by this method.

Two pieces of information are processed for accurate detection: Individual plastics are identified on the basis of the melting temperatures, the area of a melting peak then gives the enthalpy of melting. For calibration, different masses of known plastics are weighed, melted and the areas and temperatures of the resulting melting peaks are determined. In the case of samples of unknown composition, the first step is to search for melting peaks. The peak temperatures are determined from in-house databases or scientific

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[7] Bitter, H., Lackner, S., Chemosphere 2020, 258, 127388.

literature. Then the areas of the identified melting peaks are determined and converted into masses using calibration lines or literature values.

Maximum sample quantities per measurement are in the range of 30 to 40 mg, the limit of detection (LOD) is about 50 µg (polymer dependent). The latter is comparatively high, which makes the method less susceptible to process or laboratory blank values, but absolutely necessitates sample preparation to increase the MP content of a sample.

4.3 Chemical methods

Stephan Wagner[8]

An alternative analytical approach for the determination of plastics in environmental samples is based on the detection of individual chemical components of plastics. If parts of the polymer, additives or individual elements contained in the plastics are detected, these are referred to as chemical methods.

The use of additives depends on the type of polymer and the use of the plastic. For example, only a few additives are permitted for materials in contact with food, whereas both organic and inorganic additives are used in construction materials (insulation material, drainage pipes, tarpaulins) and tires. Elements such as iron or titanium, for example, are used as pigments or bromine and zinc in flame retardants. Zinc is also a component of tires. The detection of such elements enables the determination of a MP content if the proportion of material/additive added to the polymers is known.

This chapter presents a chemical process approach using the example of tire abrasion. Tire material consists of 30 or more individual components. For a quantification of tire abrasion, it is therefore necessary to define marker compounds, with which the amount of tire abrasion in an environmental sample can be determined. In this context, the element zinc is used as a marker compound for quantification.

As zinc is contained in tires in stable and relatively high concentrations (approx. 1 m%), the environmental concentration of tire abrasion can be determined by zinc analysis. In addition to the determination of the element zinc as a marker compound, thermoanalytical methods such as Py-GC/MS or TED-GC/MS can also be used to qualitatively and quantitatively determine the marker compounds for SBR/BR in tires (see 4.2).

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[8] S. Wagner, T. Hüffer, P. Klöckner, M. Wehrhahn, T. Hofmann, T. Reemtsma, 2018 Water Reseach 139, 83 – 100.

P. Klöckner, T. Reemtsma, P. Eisentraut, U. Braun, A.S. Ruhl, S. Wagner, 2019, Chemosphere 222, 714 – 721.

As a result of the rolling friction between vehicle tires and road surface, small tire particles are worn off from the tire tread, which then form aggregates with mineral particles. These aggregates are called tire and road wear particles (TRWP). During sample preparation, the TRWPs have to be separated from other zinc-containing particulate materials (zinc from e.g. steel refinements). Density separation is a common procedure in MP analysis for the separation of matrix components (see chapter 6). For density separation, sodium polytungstate solution diluted with water to a density of 1.9 g/ml is used with subsequent centrifugation. The TRWP rise and accumulate in the floating fraction. This fraction is separated from the rest of the sample, dried and weighed. The dried sample is chemically digested using microwave-assisted acid digestion to dissolve the zinc components as completely as possible into the aqueous solution.

The quantification of the zinc concentration in this solution is carried out by inductively coupled plasma optical emission spectrometry (ICP-OES) or by ICP mass spectrometry (ICP-MS). The measured zinc concentration is used to determine the content of zinc in the light fraction of the solids (which floated during density separation), which in turn is used to calculate the content of tire abrasion in the total weighed sample. This calculation is based on an average zinc content in tire treads of approx. 1 m%. Based on the assumption that TRWP consists of 50 m% of tire abrasion particles and 50 m% of mineral particles, the content of TRWP in the total sample can be determined.

Depending on the sample matrix, particles containing zinc that do not originate from tire abrasion may also be present in the light fraction. If the TRWP concentration is very low and there are zinc sources other than tires, an incorrect determination is possible. Analysis of environmental samples has provided indications that density and zinc concentration may vary as TRWPs age in the environment. Zinc can leach out of the particle and TRWP aggregates may break. Both processes would result in misquantification.

4.4 Comparison of methods

A comparison of the methods is shown in tables 2 and 3. The determined values / data are based on the measurements of environmental samples. The existing detection methods differ - independent of the parameters for sampling and preparation - in their methodological performance and feasibility per measurement. These include the analyzable sample mass or particle number within the scope of a measurement, the detection limits with regard to particle size and mass, the necessary preparation of the sample in the measuring device, and the measuring and evaluation time per measurement.

The detection methods differ from one another in the type of result and performance of the measurement result. This includes information on the type of polymer and possible additives, the aging condition, the determination of particle number, size, shape and surface condition as well as contents.

Table 2: Overview of methodical parameters for detection methods, without consideration of the sample composition in particular (organic/inorganic) and sample preparation; abbreviation of methods, see appendix.

Properties	Spectroscopic						Thermoanalytical			Chemical
	µ- Raman	IR / FTIR (µ-/FPA-)	µ ATR-FTIR	ATR-FTIR	NIR	NIR Imaging	Py-GC/MS*	TED-GC/MS	DSC	ICP-MS
Sample template for measurement	Prepared filter residue	Prepared filter residue	Isolated particles	Isolated particles	Sample	Prepared filter residue	Isolated particles / prepared sample	Sample	Isolated particles / prepared sample	Sample
Maximum analyzable mass in sample	undefined	undefined	undefined	undefined	undefined	undefined	µg-mg	mg	mg	mg
Maximum particle-number per filter	10^3 – 10^5	10^3 – 10^5	undefined	undefined	undefined	10^3 – 10^5	undefined	undefined	undefined	undefined
Dimension measuring time (real environ. sample)	d - h	d - h	min	min	min	h - min	h	h	h	min
Lower detection limit (in practice)	1 – 5 µm	10 µm	25 - 50 µm	500 µm	~ 1 m% ***	50 µm, 5 f% ***	0.01 - 1 µg (abs)**	0.5 – 2.4 µg (abs.)**	~ 50 µg	

** Various superstructures of the pyrolysis unit (e.g. Curie point, filament, micro furnace). ** depending on polymer type and pyrolysis unit and inorganic dilution. *** m% = mass percent, f% = area percent*

Table 3: Overview of result generation of the detection methods (abbreviation of the methods, see appendix, OF = surface).

Properties	Spectroscopic						Thermoanalytical			Chemical
	µ Raman	IR / FTIR (µ-/FPA-)	µ ATR-FTIR	ATR-FTIR	NIR	NIR Imaging	Py-GC/MS*	TED-GC/MS	DSC	ICP-MS
Type of polymer	yes	yes	yes	yes	yes	yes	yes	yes	partially crystalline	just tires
Detectable additives	pigments	no	no	no	no	no	yes	no	no	no
Aging condition	surface oxidation	partly	surface oxidation	surface oxidation	no	no	oxidation	no	no	no
Particle number size, shape	yes	yes	yes	yes	no	yes	no	no	no	no
Contents	no	no	no	no	no	no	yes	yes	yes	yes

5. Selecting the Sampling Method

Sampling generally involves representative extraction of specific MP particles from an environment in which a large number of natural particles often occur. MP particles can also be embedded in natural matrix components. There are two basic points to consider when taking samples:

- Representative occurrence of the feature carriers relevant to the material to be examined
- Availability of a sufficient amount of the analyte to be tested depending on the requirements of the detection method (LOD = limit of detection, or LOQ = limit of quantitation)

Sampling programs and methods are therefore determined by the environmental medium or product to be sampled, the research question and the related detection methods.

In the following sections, key features for sampling under different framework conditions will be mentioned. Each soil type, body of water, product and installation has special features which must be regarded and considered throughout the sampling process.

When setting up examination programs, the spatial and temporal conditions should also be considered as far as plastic inputs and outputs are concerned (point sources, diffuse sources, continuous, discontinuous). These may be rather similar (e.g. sampling air) or vary, depending on the different media, e.g. combined sewer overflows or organic fertilizers.

5.1 General

Claus Gerhard Bannick, Mathias Ricking, Nathan Obermaier

To achieve the different goals for a specific examination requires a suitable sampling method as well as the appropriate technical equipment. Examination goals could be:

- the uniform characterization of environmental media (e.g. water, soil, air), biota (e.g. fish, mussels), materials (e.g. compost, sewage sludge, construction waste) or products (e.g. organic fertilizers, foodstuffs)
- the determination of an average concentration, e.g. for freight calculations
- the determination of magnitude of a possible dispersion of contents over time

- the determination of a spatial distribution / temporal dynamic within a medium, biota, material or product
- the determination of compliance with quality standards - e.g. limit values (quality control measurements)

The required sample volume for liquids or quantity for solids depends on the sampling method as well as on the number, size, shape and, if necessary, the spatial and temporal distribution of the MP particles (analyte) to be examined. This is matrix- or installation-specific. Not all particles occur everywhere at the same frequency.

Regarding the methods mentioned in Chapter 4, the integral methods (thermoanalytical / chemical) differ from the imaging methods (spectroscopic) regarding the general framework conditions for the sampling.

For thermoanalytical methods, the main focus of interest besides representative collection is the aspect of a sufficient analyte mass. This is determined by LOD / LOQ, which are polymer-specific. Obtaining a sufficient analyte mass is especially important in the range of small and very small MP particles. The spectroscopic methods focus on a representative detection of the feature carriers, as they are able to detect single particles up to a certain size.

Table 4 shows the correlation between the required analyte mass by the TED-GC/MS and the detection limit for spherical, round particles of different sizes.

The LOD of the four most commonly found polymers for the TED-GC/MS are in the range of 0.06 - 2.4 µg (absolute). The required analyte mass is based on the polymer with the highest LOD. Different polymers occur in the matrices to be investigated in different contents and size compositions as well as in different temporal and spatial distributions. Thus, a representation of all polymers to the same extent by a shared sampling method is not achievable.

Table 4: Calculated particle numbers depending on LOD for spherical particles of different polymers.

	Density in g / mL	**LOD / µg**	**Number of particles for different particle diameters of:**					
			3 µm	7,5 µm	30 µm	75 µm	300 µm	750 µm
PE	0.95	2.2	283,061	10,484	164	10	0	0
PP	0.9	0.14	19,014	704	11	1	0	0
PS	1.05	0.08	9,313	345	5	0	0	0
PET	1.38	0.24	21,258	787	12	1	0	0

Table 4 shows that it takes 283 061 PE particles of 3 µm diameter to achieve the LOD of the measuring method. With decreasing LOD, the number of particles required is also reduced: with PS, only 9 313 particles are needed to achieve the LOD. If the larger particle ranges are considered, e.g. 300 µm particles, the LOD of PP and PET is achieved with just one particle. Statistically speaking, the LOD would be achieved by PS with less than one large particle. For the detection of PE, 10 particles of 75 µm are required. The same correlations apply to fibers and film fragments. The required sample quantity is based on the polymer with the highest LOD. When searching for specific polymers, analogous considerations must be made.

Before starting the actual sampling program of a medium, preliminary investigations are necessary to determine the order of magnitude of the MP content to be investigated.

For the design of an optimal, representative sampling for the above-mentioned questions, the type of sampling, the sampling frequency or the sample quantity can be derived from the preliminary investigations in relation to the subsequent detection method. Fundamentally, statistical validation, i.e. multiple repetition of sampling, (sample preparation) and detection is required, especially with regard to the spatial and temporal variance of the media to be investigated.

5.2 Water samples

Claus Gerhard Bannick, Mathias Ricking, Nathan Obermaier[9]

In contrast to dissolved and homogeneously distributed compounds, the representativeness of a water sample with particles of different sizes and qualities increases with the sample water volume. MP are part of the suspended solids in the water. The derivation of fixed sample volumes is not possible in relation to the previous versions because of the different requirements and goals. Preliminary tests must always be carried out for the corresponding medium.

Based on previous experience and the "normal distribution" shown in Table 1, there are usually significantly more particles present in the smaller size ranges than in the larger size ranges. Regarding the representativeness of the detected characteristic carriers, the sample volume for the detection of MP in the lower µm range could be smaller (in the milliliter or liter range), because the statistical probability of obtaining a representative proportion of the small particles to be examined is very high for numerically high particle occurrences. This is especially true for the spectroscopic methods. The masses obtained here, however, are usually not sufficient to be detected by a chemical or thermoanalytical method. If the sampling method aims to cover the entire size range down to the upper µm-range, considerably larger quantities of water must be filtered (up to several cubic meters). The higher the MP content and thus the number of relevant feature carriers, the smaller the sample volume can be.

Depending on the sampling method, different sampling devices are required and/or suitable. Not only must they be usable in the respective specific situations, they must also be able to generate the necessary sample quantities.

In the following standards of the ISO 5667 series, additional information and instructions on sampling and sampling devices are available:

- ISO 5667-4 Sampling from lakes, natural and man-made
- ISO 5667-6 Sampling of rivers and streams

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[9] C. G. Bannick, R. Szewzyk, M. Ricking, S. Schniegler, N. Obermaier, A. K. Barthel, K. Altmann, P. Eisentraut, U. Braun, 2019, Water Research 149, 650-658.

- ISO 5667-8 Sampling of wet deposition
- ISO 5667-9 Sampling from marine waters
- ISO 5667-11 Sampling of groundwaters
- ISO 5667-17 Sampling of bulk suspended solids

In the following the most important water and water body types are outlined with regard to special features of sampling for MP studies.

Potable water (tap water / bottled water)

Potable water is largely free of solids, because it has been purified. This also includes MP. However, pipes or fittings within residential supply lines can introduce MP into drinking water. To investigate MP in drinking water in a representative way, very large volumes of water (several cubic meters) should be sampled.

MP particles in drinking water from plastic bottles originate during production of bottles and bottle caps. These are mainly MP particles from materials used for this purpose (PE, PP, PET). Even unscrewing and twisting bottle caps can generate particles that can enter drinks. For subsequent thermoanalytical investigations, a sample of at least 8 - 10 liters should be prepared. For spectroscopic investigations, approx. 0.25 - 1 L may already be sufficient.

Groundwater

Due to the filtering and buffering function of soils, groundwater is largely protected from particulate pollution. Depending on the soil substrate and the resulting soil pores, only very small particles are usually displaced into the groundwater. Generally, these are particles in the lower µm range. Therefore, large sample volumes (several cubic meters) shall be taken to obtain sufficient attribute carriers for the detection methods. Exceptions to this are weak soils on fissured rocks (Rendsina soils) where it is possible for larger particles to enter the aquifers via preferential flow.

Rainwater / Wet Deposition

Wet deposition contains airborne solids that are suspended during precipitation. The MP sizes to be expected here are in the lower µm range (see Chapter 5.4). It is not yet possible to give recommendations for sample volumes for direct sampling of MPMP in wet deposition due to the lack of research.

Surface water / sea water

When sampling surface waters and marine waters, a distinction must be made between sampling of the water surface and sampling of the water column. The concentration of solids in flowing or running water is primarily determined by the velocity of the flow/current and thus by the (out-)flow of the water in question. The higher the flow or flow velocity, the greater the erosion force and the longer the period during which the solid particles remain in suspension.

The currents in marine waters are subject to influences such as tides, ocean currents and atmospheric conditions. Especially in coastal areas, local peculiarities can occur.

The flow in rivers depends on the prevailing climatic conditions (high tide / low tide) and is usually turbulent, although the turbulence varies greatly. Ideally, samples should be taken from turbulent, thoroughly intermixed water. If a laminar flow is present, turbulence should be generated for sampling, if possible.

When sampling from canals (waterways) it should be considered that the direction of flow is variable, the flow rate varies considerably and may depend to a greater extent on ship movements (e.g. number of locks) than on prevailing weather conditions. It should be remembered that stratifications and flows are more pronounced in canals than in rivers under quieter conditions. The passage of ships can have a noticeable short-term influence on the water quality of a canal, especially on the concentration of suspended solids.

Lakes must be distinguished according to size, depth, inflows and outflows. Different, dynamic flow systems are established.

For all surface waters and marine waters, all sizes of MP are to be expected not only due to discharges, but also the degradation of macroplastics. The recommended volumes can vary considerably due to the strong dependence on the specific particle composition and water quality.

Wastewater

Wastewater is produced in households and industries as well as in the collection and drainage of rainwater. As a 3-phase mixture (aqueous phase, solids and air inclusions), it differs significantly from the previously mentioned water sample types, in some cases with high solid contents, depending on the origin. In addition to MP, raw wastewater also contains large amounts of macroplastics and is transported via sewer systems to sewage treatment plants. In case of heavy precipitation, however, it may also be discharged directly into water bodies (combined sewer overflows).

Due to the efficiency of the treatment in the wastewater treatment plant, only particle sizes < 500 µm are relevant in the treated wastewater. Sampling of road runoff can be done via manhole shafts. Here, the use of automatic samplers which can be triggered automatically at a certain water level is recommended. Due to the high concentrations of tire abrasion and other MP, 50-100 liters of sample volume are sufficient for this purpose.

Sampling concepts

In principle, sampling of the different areas can be performed by single sampling (this is especially suitable for preliminary investigations) or by integrative sampling over a longer period of time (several days).

For single sampling of large sample volumes (e.g. surface water, clear run, groundwater), direct fractional filtration on site as well as sampling with a flow-through centrifuge and subsequent fractionation in the laboratory have proven successful so far. In both cases, water volumes of 2 - 4 cubic meters per working day can be considered realistic. Fractionation in the laboratory is much more precise than under field conditions.

For integrative sampling, sedimentation boxes as well as automatic samplers which can take up several cubic meters of water over longer periods of time (days) are suitable. Especially pump-driven sedimentation boxes provide predictably consistent results. In both cases, the fractionation of the solids is carried out in the laboratory.

Table 5 compares the requirements for representative sampling volumes for the different water types.

Table 5: Summary of orders of magnitude of the water volumes to be sampled depending on water type and detection method (based on literature references and own experience)

	Raw sewage, road runoff	**Treated wastewater** [1)]	**Surface waters** [1)]	**Wet deposition**	**Potable and ground-water**	**Bottled water**
Volume (imaging methods)	0.1 m^3	1 - 2 m^3	1 - 2 m^3	1 m^3	500 l	1 l
Volume (integral methods)	0.1 m^3	1 - 2 m^3	>1 m^3	1 m^3	> 20 m^3	10 l

[1)] *Data may vary depending on MP contamination*

5.3 Solid and sludge samples

Claus Gerhard Bannick

In principle, the same requirements apply to the sampling of solids and sludge as to the sampling of water. In this case, the aim is to extract a representative amount of MP while ensuring that a sufficient quantity of the analyte is sampled. Similar to the water sector, the usual procedures cannot be applied to MP sampling in every case scenario. In legal regulations concerning soil protection, fertilizers or waste, there are already specifications for sampling, whereby particularly micropollutants are targeted. The following regulations are relevant:

- Federal Soil Protection and Contaminated Waste Ordinance
- Ordinance on the utilization of sewage sludge, sewage sludge mixture and sewage sludge compost (Sewage Sludge Ordinance)
- Ordinance on the utilization of biowaste on land used for agriculture, forestry and horticulture (Biowaste Ordinance)
- Ordinance on the marketing of fertilizers, soil additives, growing media and plant additives (Fertilizer Ordinance)
- Ordinance on Sampling and Analysis Methods for Official Fertilizer Control (Fertilizer Sampling and Analysis Ordinance)

Many references can also be found in the methods manual of the Association of German Agricultural Analytic and Research Institutes. (VDLUFA) or in the "Principles of soil sampling" of the Advisory Board for Soil Sampling at the Federal Ministry for Environment, Nature Conservation and Nuclear Safety (BMU).

The biggest challenge in sampling is determining the appropriate sampling quantity. In most cases, MP have to be enriched via density separation before detection. Depending on the MP contents in the solids or slurries, it may be necessary to adjust the size of the field sample.

In general, we can distinguish between two different approaches to the sampling of solids: in-situ sampling for soils – that is, in the original soil compound – and sampling (soil) material in stockpiles, as bagged goods or other containers as well as in/from dynamic systems (e.g. conveyor belts).

Soils

Guidance on basic sampling considerations can be found in the following ISO standards:

- ISO 18400-205 - Guidance on the procedure for investigation of natural, near natural and cultivated sites
- ISO 18400-203 - Investigation of potentially contaminated sites

Stockpiles

- Guidance on sampling bulk materials and piles is given in the above-mentioned regulations. In addition, the following documents should also be consulted: DIN EN 932-1 Test for general properties of aggregates - Part 1: Methods for sampling
- LAGA PN 98 Guideline for the procedure in physical, chemical and biological investigations in connection with the recovery/disposal of waste (Länderarbeitsgemeinschaft Abfall, LAGA)

Given that examinations in and on soils have only been carried out sporadically to date, no general statements can be made at present.

5.4 Atmospheric samples/ airborne particles

Jens Reiber

MP are not yet included in the current regulations (national and EU) and thus not taken into account in air quality measurements. However, an orientation for sampling MP in air can be based on immission measurements of fine dust (PM10, PM2.5). Existing measurement methods could be adapted and applied to airborne MP.

Particulate matter (PM) is the term used to describe particles in the air that do not immediately sink to the ground but rather remain in the atmosphere for a certain time. Depending on the size of the dust particles, the fine dust is divided into fractions: PM10 refers to all dust particles whose aerodynamic diameter is less than 10 µm. A subset of the PM10 fraction are the finer particles whose aerodynamic diameter is less than 2.5 µm (PM2.5). The smallest of them, with an aerodynamic diameter of less than 0.1 µm, are called ultrafine particles. This section provides an overview of the current legal norms on the subject of air, basic procedures for a measurement campaign as well as standards and guidelines for measuring particles and fibers in the air. Studies of air pollutants to determine air quality are listed in European and national legal standards (Table 6).

Table 6: Selection of legal norms concerning air

EU-level	Council Directive 96/61/EC of 24 September 1996 concerning integrated pollution prevention and control - IPPC Directive
	Directive 2008/50/EC of the European Parliament and of the Council of 21 May 2008 on ambient air quality and clean air for Europe
Federal level	Law for the protection against harmful environmental effects caused by air pollution, noise, tremors and similar processes (Federal Immission Control Act (BImSchG)) plus various ordinances e.g. 39th BImSchV - Ordinance on Air Quality Standards and Emission Ceilings
	First General Administrative Regulation Pertaining to the Federal Immission Control Act Technical Instructions on Air Quality Control - TA Luft

Air samples are taken according to a defined measurement planning and documentation, which includes criteria for measuring point density / minimum number of sampling points, sampling duration / averaging period, duration of a measurement program, sampling times, measurement frequency and applied measurement principles (continuous versus discontinuous measurements).

A distinction must be made between sampling of the outside air and the indoor air. A determination of the measuring strategy and the measuring procedures is further dependent on the following target parameters.

Gravimetric and chemical analyses are carried out after collecting the samples on a filter. In general, suspended particle measurements can be carried out using appropriate sampling heads both with and without fractioning according to particle size (PM10, PM 2.5, > PM10). A sampling pump can also be used to suck a defined volume of air through a gold-coated core pore filter in order to collect the air particles and fibers for subsequent gravimetric and/or chemical analyses (see Institute for Occupational Safety and Health of the German Social Accident Insurance, IFA, Table 7).

A selection of standards and guidelines on particle and fiber sampling are listed in Table 7 and can be used as a guide for planning MP studies in the "Air" compartment. The list is not exhaustive and the documents cannot yet be evaluated with regard to MP sampling.

Table 7: Standards and guidelines for measuring particles and fibers in the air (ambient and indoor air)

Standard / Guideline	Designation
DIN EN 12341	Ambient air - Standard gravimetric measurement method for the determination of the PM10 or PM2.5 mass concentration of suspended particulate matter; German version EN 12341:2014
DIN EN 16450	Ambient air - Automated measuring systems for the measurement of the concentration of particulate matter (PM10; PM2.5); German version EN 16450:2017
DIN ISO 16000-34	Indoor air - Part 34: Strategies for the measurement of airborne particles (ISO 16000-34:2018)
VDI 2463 Blatt 1	Particulate matter measurement - Gravimetric determination of mass concentration of suspended particulate matter in ambient air - General principles
VDI 2463 Blatt 7	Particulate matter measurement - Determination of suspended particulate matter and gaseous chemical compounds in ambient air and indoor air - Active sampling via low-volume sampler (LVS)
VDI 2463 Blatt 8	Particulate matter measurement - Determination of suspended particulate matter in ambient air and indoor air - Non-fractioning sampling system for low-volume samplers (LVS)
VDI 2463 Blatt 11	Particulate matter measurement - Measurement of mass concentration in ambient air - Filter method; Digitel DHA-80 filter changer
VDI 4320 Blatt 2	Measurement of atmospheric depositions - Determination of the dust deposition according to the Bergerhoff method
VDI 3786	Environmental meteorology - Meteorological measurements - Fundamentals
VDI 3786 Blatt 2	Environmental meteorology - Meteorological measurements - Wind
VDI 3786 Blatt 3	Environmental meteorology - Meteorological measurements – Air temperature
IFA-Arbeitsmappe 7485	Fibers, general, inhalable
VDI 4300 Blatt 1:1995-12	Indoor-air pollution measurement - General aspects of measurement strategy
VDI 4300 Blatt 11:2013-12	Measurement of indoor air pollution - Measurement strategies for determination of airborne particles in indoor environment - PM2.5-fraction

Moreover, it should be noted that the measuring procedures include gravimetric (quantitative) evaluations. A qualitative differentiation of the particles / fibers with respect to MP is not described. Therefore, procedures for the identification and quantification of plastics are recommended.

5.5 Biota samples

Katrin Wendt-Potthoff, Christian Laforsch

The systematic sampling of MP in biota as a purely analytical concept for the presence of MP in the environment (as is the case for water, soil or air), can only be implemented to a limited extent. It should not be understood as an equivalent, harmonized technique for the analysis of MP in the environment. The degree to which such work is representative must be carefully classified and discussed.

This chapter, however, should also allow a classification in a harmonized analysis, which can include the detection of MP in biota from e.g. feeding and exposure experiments.

Sampling of MP in organisms in the environment must take into account the different living environments, different behavioral patterns and nutritional strategies of the animals as well as the different dimensions and the resulting potential uptake of MP particles by the organisms. For comprehensive statements on the exposure of biocoenoses to MP, representatives of different size classes, nutrition types and positions in the food web should be investigated. If the aim is to compare living organisms from several sites for their exposure to MP, the organisms should be selected according to bioindicator criteria (e.g. wide distribution and easy availability, no protected or endangered species, known routes of exposure).

In the case of contamination with MP via ingestion, it should be explained according to the reference to the biology of the relevant organism (scale of ingested food, type of ingestion such as filter feeders, suspension feeders, grazers or predators.)

Sampling procedures for different types of organisms are not bound to existing, systematic sampling protocols; existing work usually documents individual samples. The purchase of organisms such as mussels at local markets or in grocery stores, as well as samples from commercial fisheries, are pragmatic and resource-efficient. They also provide insights into potential consumers as well as information on the potential exposure of individual groups of organisms from the respective environmental compartments. However, the degree to which these samples are representative with regard to the contamination of organisms and the corresponding environmental compartment must be considered with caution. In most cases, neither the exact sampling location nor the time are known, nor is there any precise information on possible intestinal cleansing phases, as is the case with bivalve molluscs for excreting sand. During such phases, part of the MP is excreted, which depends on both the duration of passage in the animal and the nature of the MP. Accordingly, organisms collected in the field should either be fixed immediately or subjected to a defined intestinal cleansing phase, if possible. During the fixation process, a careful and quickly effective method should be applied, as the organisms can empty their digestive tract under stress.

Depending on their size, living organisms can be used for further analysis in their entirety (e.g. mussels, small worms, etc.). For this purpose, the organisms should be cleaned from the outside beforehand in order to remove adhering particles, unless the object of the specific study is the adhesion of MP. The procedure applied must be documented. Subsequently, the organisms can be decomposed by acids, alkalis or enzymes. The concentrations, temperatures and exposure times must be specified (see Chapter 7.3). Alternatively, only the stomach or intestine is considered (fish, birds, larger worms, etc.) or excretions can be analyzed. The latter allows for non-invasive testing, which is ecologically beneficial and allows for repeated sampling in labelled animals or during experiments. Attention should be paid to fresh or at least undisturbed excreta. In the case of small translucent organisms or sectional preparations, combinations of microscopic methods may also be used for detection. Some organisms can be made more translucent beforehand by maceration.

The problem of the different conditions for chemical or biological sample preparation and the blank value problem as well as the possible degradation of MP during preparation is already addressed in this document. In addition, microscopic techniques must be used to prove whether particles are present in the stomach contents, in the intestinal lumen, in the tissue or outside the organisms, and that no carry-over of particles into other tissues has occurred during cutting procedures.

The recording of MP in biota should be documented in terms of quantity or mass per individual. Since the size of the individuals plays a role, it should be recorded. In addition to the dimensions of the organisms, it can also be useful to indicate the wet mass. If microscopic methods are used, the dimensions of the particles should be indicated, since they are important for the interpretation of biological processes. When analyzing excretions, the mass of the sample under investigation should be recorded.

6. Selecting of the treatment method

The selection and sequence of the treatment method depend on the environmental matrix to be analyzed as well as the detection method (see Figure 1). For this purpose, recommendations for individual work steps are given below. The selection and combination of these steps and their chronological sequence must be determined individually on the basis of the sample at hand, the selected detection method, and the expected MP particle numbers or MP content in the sample.

In principle, the application of all the treatment steps described here involves the risk of particle loss, contamination from other sources, and fragmentation of particles, especially aged particles. Therefore, the number of treatment steps must be kept to a minimum and only carried out under protective conditions.

In general, a distinction is made between processes for removing the inorganic matrix (density separation, electrostatic separation), processes for removing the organic matrix (chemical treatment, enzymatic treatment) and processes for complete extraction of the MP particles (chemical extraction).

A systematic evaluation of the integrity of MP grades, sizes, geometries and aging conditions with regard to the subsequent detection methods is still pending. Therefore, no generally valid recommendations can be given so far.

Spectroscopic methods for the determination of MP particles require the removal of the organic and inorganic matrix. This reduces the total number of particles, which leads to a shortening of the measuring time and counteracts a possible overloading of the filters. Interfering fluorescence caused by natural organic substances in Raman microspectroscopy is minimized.

For thermoanalytical methods, the removal of the organic matrix can have the advantage that the content of natural organic substances in the sample is reduced and the MP content is concentrated. Furthermore, possible co-elution can be avoided. Concentration can significantly increase the detection sensitivity. In principle, however, the separation of the organic matrix is not necessary for TED-GC/MS.

6.1 General

Transport

Transport or storage before sample preparation does not have to be refrigerated as far as MP detection is concerned. However, cooling the samples proves to be advantageous as it prevents biological fouling.

Hygienization

Initially - also depending on the subsequent sample preparation and the detection method - it must be determined whether hygienization of the samples is necessary. Sterilization is generally recommended for the analysis of samples from wastewater, waters with high wastewater contents, sewage sludge and biowaste. A variety of methods can be used, each with specific limitations, e.g. steam sterilization (if appropriate, melting of PE particles), radiation sterilization using beta radiation, UV radiation (if appropriate, polymer degradation), chemical sterilization using ozonization or reactive chemicals (if appropriate, chemical degradation of particles).

Drying

Depending on the type of field sample, it may be useful to first dry the sample or a sample aliquot to determine a dry sample weight. This will also prevent organic fouling during prolonged storage. Drying should be carried out at room temperature up to a maximum of 60 °C (preferably at a lower temperature), since above this temperature window, conventional types of plastics (PE, PP, PS, PET, PA, PVC) exhibit specific phase transitions (glass transition temperature, melting temperature), which involve altered degradation mechanisms. A gentler alternative is the use of freeze-drying, which also avoids agglomeration of the sample material.

Homogenization

The preparation of the dry field sample to a laboratory sample may initially include adequate sample homogenization (careful mortaring, rotary sample divider / Cross-Riffling method), particularly for sediment / soil samples. It should be noted, however, that mechanical stress may cause degradation of particles.

However, depending on the sample in question, homogenization may also lead to segregation effects of the particles depending on size, density and surface properties.

Fractionation / Sieving

The division of the samples into different size classes is advantageous during sampling (avoidance of filter cake) as well as during further processing steps and the subsequent detection procedure.

The sieve fractions according to Table 1 are only approximate, since non-spherical particles (especially fibers) cannot be classified by a size exclusion method. It should also be noted that mechanical stress may cause degradation of particles.

However, for the thermoanalytical and chemical detection methods, fractionation according to Table 1 is recommended. The processing of the field sample to a laboratory sample can initially include coarse sorting, which excludes particles and fragments above the MP definition (> 5 mm / > 1 mm). Additional manual sorting of visually or microscopically detectable MP particles > 500 µm can be useful if technical limitations exist for the subsequent detection procedures.

For screening lines < 100 µm, wet screening is recommended to avoid excessive dust formation or loss of particles.

6.2 Density separation

Density separation is used for samples with a high inorganic matrix content. An overview of the possible methods is presented in Table 8. For an effective density separation, the use of a saturated metal saline solution is necessary. If required, this enrichment step may have to be performed several consecutive times. For large sample volumes (e.g. sediments, soils) the metal saline solution should be inexpensive and non-toxic. If necessary, the saline solution may be reused through purification. Different metal saline solutions can separate different polymers. The data can be significantly influenced by additives, processing and aging of the MP particles, as well as by biological growth or agglomeration with natural particles.

The volume of the solutions as well as the applied sample masses depend on the applied, representative environmental sample as well as the applied detection method. As a rule, work is carried out at room temperature and with a separation time of 12 - 24 hours. The excess can be sucked off with a pump and transferred directly to a filter for detection. Subsequent rinsing with water is required.

Table: Overview of conditions used in density separation

Polymer	**Density**		**Possible saline solutions**					
PP PE SBR EPDM	< 1 g/mL	NaCl (1.2 g/cm^3) pH = 7 Low toxicity	Na-wolframate (1.4 g/cm^3) pH = 3 Low toxicity	NaBr (1.5-1.6 g/cm^3) pH = 6-9 Moderate toxicity	$ZnCl_2$ (1.6 g/cm^3) pH = 5 High toxicity	Potassium formate (1.6 g/cm^3) Low toxicity	NaI (1.8 g/cm^3) pH 6-9 Moderate toxicity	Na-poly wolframate (1,9 g/cm^3) pH = 3 Low toxicity
PS PA PMMA EP	1 - 1,2 g/mL							
PET UP	1,2 - 1,4 g/mL							
PVC PUR TRWP	> 1,4 g/mL							

Centrifugation can be performed to support the separation effect if desired. For a (supporting) separation by means of hydrophobic interactions (e.g. silicone oils, kerosene oils) no generally valid recommendations can be given so far.

6.3 Electrostatic separation

Kristina Enders, Franziska Klaeger, Matthias Labrenz, Friederike Stock[10]

Electrostatic separation relies on the functional principle of separating materials by means of different electrostatic charging or discharging potentials. Thus, a separation of MP (low conductivity) from mineral materials (higher conductivity on average) can be achieved. Electrostatic separators as the hamos Korona-Walzen-Scheider (KWS) make use of this principle and are used in the recycling industry in form of large machines. At this point in time, the method has only been investigated for MP research in a few studies. Using a small prototype model of a KWS, it could be shown that a separation of sediments takes place and MP is thus enriched. This initial enrichment allows large sample volumes to be examined for MP, although further separation methods, such as density separation, remain crucial (at least for particle-based detection methods).

This method has limitations with respect to particle size and the nature of the sample matrix. High recovery rates (up to 100 %) were achieved for large MP particles (>2 mm) and decreased with decreasing size (~45 % at 20 µm). It was found that the presence of very fine-grained sediments may further reduce the recovery rates of individual MP sizes. Generally, a fine-grained, organic-rich sample matrix led to a poorer separation performance, which insufficiently reduced the sample volume. The mineral composition of the matrix also demonstrated an influence on the mass reduction.

Electrostatic separation using the KWS can be recommended for predominantly sandy samples where larger MP (> 500 µm) are investigated and that require a reduction of large initial volumes, as may be the case for typical Baltic Sea beach sand samples.

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[10] S. Felsing, C. Kochleus, S. Buchinger, N. Brennholt, F. Stock, G. Reifferscheid, 2018, Environmental Pollution 234, 20–28.

K. Enders, A.S. Tagg, M. Labrenz, 2020, Frontiers in Environmental Science 8: 112, K. Enders, R. Lenz, J.A. Ivar do Sul, A.S. Tagg, M. Labrenz, 2020, MethodsX 7: 100784.

Given the variability of recovery rates, especially for small MP, it is recommended to use internal standards. Furthermore, it should be considered that electrostatic separation, especially if small MP are investigated, requires subsequent processing steps. With each additional step, potential additional losses and contamination risks must be taken into account.

6.4 Chemical treatment

Jutta Kerpen, Oliver Knoop[11]

For chemical treatment, oxidative, acidic or alkaline digestion methods are suitable. The most sensitive point in chemical processing is the potential degradation, mechanical damage or discoloration of MP particles, whilst achieving optimal removal of all organic components from the sample. These organic components can vary greatly depending on the type of sample and may include microorganisms/biofilms, plant residues, humus or organisms (e.g. mussels, worms, fish, tissue samples). Based on chemical treatment. organic components can be removed and thus MP particles isolated.

Acidic digestions, for example with nitric acid (HNO_3) or hydrochloric acid (HCl) proved to be unsuitable, as many polymers are sensitive to acids and degrade easily. Besides, low-dose acids do not remove organic matter adequately. Alkaline digestions also show degrading effects on some polymers, but are more often used for biological samples. For example, 10 v% potassium hydroxide (KOH) as well as sodium hydroxide (NaOH) attack some polymers (e.g. PET, PLA) and only partially achieve good removal rates for organic matter.

Oxidative methods, usually based on 10 to 35 v% hydrogen peroxide (H_2O_2), are being used more often recently, with different temperatures and extraction times of hours to

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[11] Al-Azzawi, Mohammed S.M.; Kefer, Simone; Weißer, Jana; Reichel, Julia; Schwaller, Christoph; Glas, Karl; Knoop, Oliver; Drewes, Jörg E. 2020. *Water* 12, no. 9: 2445. DOI: 10.3390/w12092445.

Wolff, Sebastian; Kerpen, Jutta; Prediger, Jürgen; Barkmann, Luisa; Müller, Lisa (2019): *Water Research X* 2, p. 100014. DOI: 10.1016/j.wroa.2018.100014.

days being reported. At temperatures up to 40°C, low-percentage solutions leave the MP particles largely undamaged, although the organic components are occasionally insufficiently decomposed. At higher concentrations and temperatures, a good removal performance can be achieved, though slight changes in the MP particles can be observed. During the so-called Fenton reaction, the formation of hydroxyl radicals from hydrogen peroxide is catalyzed via iron (II). This can greatly accelerate the sample extraction (minutes to hours) and shows good removal rates for organic components of e.g. sewage sludge, with only minor effects on different MP particles. However, iron oxides can precipitate during the Fenton reaction, which interfere with subsequent spectroscopic detection of small particles. Besides H_2O_2, sodium hypochlorite (NaClO) can be used as an oxidizing agent.

The MP particles can become larger by swelling or smaller by degradation or fragmentation. The latter causes an increase in the particle number and thus, possibly, to excess findings. The spectra of the polymers in the subsequent detection by FTIR or µ-Raman can also be influenced by oxidation on the surface and thus make identification difficult or impossible. Therefore, stability tests must be carried out for the respective polymers and recovery rates as well as blank values must be determined for the chemical treatment method used.

The choice of digestion method must therefore be adjusted to the nature of the sample, the expected polymers and the detection method. When using digestion methods that can strongly degrade certain polymers, this must be taken into account and clearly stated in the documentation. Two validated treatment processes for wastewater and sewage sludge are listed below as examples.

- Treatment of the concentrated sample with Fenton ($FeSO_4$ at pH 3 and continuous addition of 30% H_2O_2. Dissolution of precipitated iron particles by addition of conc. H_2SO_4 for 30 seconds and immediate subsequent filtration.
- Treatment of the concentrated sample with 50% H_2O_2 at 50°C for 24 hours, repeated concentration, treatment in NaClO (12 v%) at room temperature 6 d.

6.5. Enzymatic Processing

Jens Reiber

The use of enzymatic preparation methods aims to extract MP gently from organic foreign substances and to obtain it as particles. Most enzymatic preparations are further supplemented by at least one oxidative step with H_2O_2 (see 6.4). The applied enzymes, each of which decompose other compounds, require different conditions (reaction buffer, pH, temperature, etc.) under which they can optimally catalyze the processes. If multiple enzymes are used on one sample, they may have to be used consecutively.

The selection of enzymes depends on the composition of the matrix (Table 9). Between the enzymatic steps, which are performed in solution, volume-reducing steps such as filtration or sieving are necessary. After the enzymatic treatment, MP are usually present on substrates or dispersed in the solvent. The following is a list of common enzymes along with the bonds that are cleaved and the compounds that are decomposed.

Table 9: Overview of common enzymes

Enzymes	**Splitting**	**Degradation**
Cellulase	endo-1,4-beta-glycosidic bonds	Cellulose
Protease	peptide bonds	Proteins
Lipase	ester bonds of triacylglycerides	Fats and oils
Amylase	1,4-α-D-glycosidic bonds	Starch
Chitinase	1,4-β-poly -N-acetylglucosamine bonds	Chitin
Pectinase	1,4- α-D-galactosiduronic bonds	Pectin

A disadvantage is the duration of an enzymatic treatment. An enzymatic step can take several days of incubation time. A further disadvantage can be an incomplete degradation of the natural organic compounds.

6.6 Chemical extraction

Corinna Földi, Georg Dierkes[12]

Under standard conditions (room temperature and normal pressure), common polymers (including PE, PP, PET) are not soluble in the usual extraction agents. They can, however, be dissolved by increasing the extraction temperature and the extraction pressure, so that the MP to be investigated can be concentrated from larger sample volumes by means of a chemical extraction procedure. In a two-step process, both inorganic and/or organic matrix components can be depleted and MP can be relatively enriched.

For this purpose, the dry sample is first weighed into an extraction vessel, depending on the sample characteristics (expected MP concentration, degree of homogeneity, etc.) or the size of the extraction vessel up to several grams. A solvent (e.g. methanol) is added to the sample in the reaction chamber or oven and organic components of the matrix are dissolved under increased pressure and temperature conditions (e.g. 105 bar and 100 °C). The extract is then separated from the sample and discarded so that interfering substances are minimized in the subsequent detection. In a second step, the analytes are dissolved and separated from the solid phase/matrix using a suitable extraction agent (e.g. tetrahydrofuran) and again increased pressure and temperature conditions (e.g. 115 bar and 185 °C). By evaporating the extraction agent, the analytes can be sorbed onto silica gel, then homogenized and an aliquot of the sample can be quantified by thermoanalytical methods. A relative enrichment of the MP can be achieved by using a lower mass of the silica gel than for the weighed-in sample. The advantage of this method lies in its automatability and speed. As a result, even complex soil/sediment samples are ready for measurement after only a few hours. The disadvantage is the use of large quantities of partly toxic organic solvents. Since the original particles are lost with the dissolution of the polymers, the extraction method is not suitable for methods that aim to determine the MP particle count.

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[12] G. Dierkes, T. Lauschke, S. Becher, H. Schumacher, C. Földi, T. Ternes, Analytical and Bioanalytical Chemistry 2019, 411, 6959-6968.

7. Metrology and validation

This chapter summarizes the most important metrological aspects. On the one hand, this is to ensure the appropriate function of measuring equipment for MP detection by means of reference materials and interlaboratory comparisons; on the other hand, it is to provide criteria for monitoring processes and products where MP can be generated or released into the environment.

7.1 Reference materials

Korinna Altmann[13], Kyriakos A. Eslahian

Reference materials are important for the accuracy and comparability of analytical results. The properties of reference materials must be described in detail and may only deviate slightly. These include the chemical composition, the homogeneity of reference materials and the traceability of these quantities to a standard (usually SI unit) as well as a defined measurement uncertainty. Reference materials can be pure substances, in this case pure MP particle powders, or mixed in a matrix. The latter are called matrix reference materials. Matrix reference materials must be characterized with respect to all contents of certain major, minor or trace constituents.

Since MP-analysis consists of three sub-steps (sampling, sample preparation and detection) and all the individual steps are highly complex in terms of handling and analytical results, it is essential to validate the process steps with appropriate reference materials.

When selecting MP reference particles for the validation of the analysis of environmental samples, the first step is to pay special attention to the environmental relevance. The thermoplastic polymer types most frequently detected in the environment are PE, PP, PS, PET, PA and PVC; these polymer types are therefore of particular interest.

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[13] K. Altmann, Y. Hassanein, V. Wachtendorf, P. Fengler, F. Milczewski, H. Emteborg, U. Braun, Production of microplastic reference materials for validation of analytical methods. Submitted.

E. von der Esch, M. Lanzinger, A. J. Kohles, C. Schwaferts, J. Weisser, T. Hofmann, K. Glas, M. Elsner, N. P. Ivleva, Frontiers in Chemistry 8(169).

Thermoplastic polymers are rarely found in the environment in their originally synthesized form, but are rather aged or degraded by various weathering influences (humidity, UV radiation, temperature, mechanics). MP particles of these polymer types are often partially oxidized, irregularly shaped or fibrous. For this reason, special attention should be paid to the use of artificially aged materials as reference material. Fibers from textile applications or particles from tire abrasion are also frequently detected in the environment. These are not necessarily aged as they can be generated mechanically and might also be considered as separate reference materials.

MP reference particles should cover the entire size range of the MP definition (Table 1) and, if possible, be used variably with respect to their shape (including spherical particles, fibers, film fragments). Depending on the research question, either only a few particles or larger quantities are needed, which may be relevant with regard to the cost of the reference material.

It is therefore advisable to look at concrete scenarios realistically in each individual case. Specific polymer types and forms can be aged or used as initial polymer and subsequently only these can be detected and/or examined restrictively in the analysis.

Commercially available particles with a narrow size distribution are available for individual polymer types. However, the choice is limited and often the chemical composition and particle shape do not correspond to actual findings in environmental samples.

In practice, it has been shown that a size range of approx. 50 - 1,000 µm can be covered very well with cryo-ground, aged or new polymers from plastic pellets or plastic products. The manufacturing conditions have to be adapted depending on the polymer type and particle shape in question. The resulting particle size distributions are very wide (more than 200 µm in mean particle diameter), which corresponds to the environmental occurrence observed.

For particle sizes significantly smaller than 10 µm, cryo-grinding is more problematic, since on the one hand only a small proportion of the grinding material used reaches such small particle sizes, and on the other hand losses of these small particles can occur during decanting, because they are easily airborne. For these particles, mechanical grinding in solutions (including ultrasonic treatment) is recommended. This can be used for aged PS and PET (1µm - 1 mm, < 1 µm). Particles smaller than 10 µm can also be produced by a controlled polymerization synthesis, as is already common for PS particles in the field of nanomaterials. Providing MP particles in a matrix is a matrix reference material and demands special attention with regard to homogeneity control, as the particles may not be homogeneously distributed in the suspension.

For all MP reference materials, separate or embedded in a matrix, a chemical characterization of the composition, a homogeneity test with regard to the intended minimum sample quantity for an individual determination and, if required, also a stability assessment (probably less relevant) must be performed. Ideally, the homogeneity test should be recorded with adequate precision and using a method that is independent of the methods employed for interlaboratory comparisons. The result of this test must be traceable to a recognized international reference (preferably SI based) and must state a measurement uncertainty.

If the homogeneity test cannot be performed directly on a matrix reference material, a single shot application has to be performed. Here, individual containers are continuously and alternately filled with matrix reference materials and MP reference materials and only the containers of pure MP reference materials are subjected to homogeneity control. The homogeneity of the MP particles in the matrix material is thus monitored within this filling process. A container of a single matrix reference material can only be used in its entirety (one complete analysis per container); aliquoting is only possible if the final result is given as the sum of the individual measurements.

When using MP reference particles for (eco-)toxicological evaluations, it is necessary to select particle sizes with a defined composition with regard to effect-relevant particle sizes. The MP particles to be selected must be realistically aged and have environmental relevance. A homogeneous distribution and a realistic concentration in the environmental medium in question must also be taken into account.

7.2 Development of quantitative methods

Susanne Richter, Christian Hagendorf [14]

From a metrological point of view, the analysis of MP not only requires representative and meaningful methods for sampling, sample preparation and detection, but also defined and reproducible analytical procedures for the processes and materials used.

In addition to the above-mentioned reference materials, the filters and laboratory conditions used in conjunction with the final MP measurement play a central role in this type of validation of the detection methods (including the definition of process and laboratory blank values) in terms of detection limits with regard to MP size, MP contents and statistical measurement control. Depending on the available filtration materials (e.g. metal mesh, silicon, aluminum oxide, polycarbonate) as well as the metrological boundary conditions (see Chapters 4-6), central aspects for a metrologically sound method development must be determined.

In particular, procedures for a filtration process must be developed which allow for a reliable determination of blank values and recovery rates for MP particles (differentiated according to type, number, size and distribution) by means of spectroscopic methods. This is to establish a reference point for the particle number and the evaluation of the detection method.

The methodology is based on the following components:

- Implementation of specific reference materials with defined particle numbers and size distributions
- Standardized filter materials, ideally with planar surfaces and broadband optical transmission into the IR range/minimal reflection.
- Filtration and drying processes with reduced particle background (laboratory conditions, grey/clean room), appropriate laboratory equipment and materials.

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[14] S. Richter, et al. “A reference methodology for micro plastic particle size distribution analysis: sampling, filter systems and analytical approaches”, submitted.

- Correlative particle detection for the determination of size distribution and ideally MP grade

Reference and background values of these defined filtration experiments have to be determined. Handling processes in the laboratory (filtration, rinsing, drying) as well as contamination of equipment, solvents or via air are expected to be the main sources of error. Systematically comparing these with the different methodological approaches to MP detection, e.g. within the framework of interlaboratory comparisons (Chapter 7.3) or extended test procedures (Chapter 7.4), leads to a better assessment of the results obtained and an improvement in the underlying analytical procedures.

7.3 Operation of interlaboratory comparisons

Korinna Altmann, Roland Becker [15]

An interlaboratory test can be carried out with different objectives. They are used, for example, to validate analytical methods, to check the proficiency of laboratories or to characterize samples (e.g. reference materials). Proficiency testing of laboratories is a central element of external quality assurance for testing laboratories and is, as such, expressly recommended in the standard EN ISO/IEC 17025 (General requirements for the competence of testing and calibration laboratories).

Interlaboratory comparisons are generally performed by measurements carried out simultaneously by several laboratories on identical (homogeneous) subsets of the sample or material using identical or different procedures (depending on the objective of the test). An appropriate number of repeat measurements and the determination of laboratory and process blank values (see recommendations in Chapter 3-6) is required. The statistical evaluation of interlaboratory comparisons allows statements to be made about the accuracy (trueness and precision) of analytical methods, characteristic values

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[15] R. Becker*, K. Altmann, T. Sommerfeld, U. Braun, Journal of Analytical and Applied Pyrolysis 148 (2020) 104829.

of samples/reference materials and the measuring capacity of the participating laboratories.

MP is not present in the environment as a pure, defined "powder", it is integrated into the environmental matrix (e.g. water, soil, sediment, biota). Interlaboratory comparisons for the validation of analytical methods therefore require a homogeneous matrix reference material. Depending on the research question of the interlaboratory comparison, such matrix reference materials have to be specifically selected, their MP particle numbers or contents to be investigated have to be adjusted with regard to the performance of the analytical method and their homogeneity has to be assessed.

Depending on the type of plastic (density, polarity), the surface properties (oxidized surface, coating with agglomerated natural particles or biofilms) as well as the size and shape of the particle, MP particles are distributed differently throughout the environmental matrix. As a result, MP suspensions are not homogeneous and MP particles in mixtures can easily separate from the matrix.

The addition of compatibility agents (additives for the compatibility of two different substances, e.g. surfactants) to MP in solutions or the admixture of MP to natural particle quantities with comparable properties can lead to a better distribution in the medium. However, such agents or particles can cause additional preparation steps or complications/disruptions in the actual measurement.

It is difficult to produce homogeneous suspensions or mixtures. Accordingly, matrix reference materials with very low MP contents or particle numbers can only be produced to a limited extent if this is to be done in accordance with the criteria of a reference material (homogeneity of the MP contents for a defined minimum sample quantity and stability of the MP distribution, i.e. no segregation after production). Therefore, the conception of an interlaboratory study for the analysis of MP or a comparative test is a compromise which illustrates the addressed research question, the production of homogeneous, realistic matrix reference materials and the methodological boundary conditions of the process(es) to be evaluated as accurately as possible.

Validating the detection methods

To validate the detection methods in an interlaboratory test, ideally only pure MP particles are examined in a matrix that is as "inert" as possible (without a signal of its own for the result). It should be taken into account that the transfer of the test sample into the analytical instrument (e.g. preparation on filters) is a separate preparation step that cannot be attributed to the pure methodological performance of the detection procedure. The methodological boundary conditions of the different detection procedures are listed in Tables 2 and 3 (maximum analyzable mass per sample, maximum analyzable particle number per sample, detection limits).

Validating the sample preparation

The validation of the different sample preparation steps in an interlaboratory comparison must be based on the evaluation of the reduction of the environmental matrix (organic/inorganic fraction) as well as on the integrity of individual particles (i.e. particle size, particle mass). The selection of the matrix depends on the research question. The interlaboratory test for sample preparation is followed by a detection process related to particle number or mass. Accordingly, a defined particle number and/or a defined content of MP reference particles must be added to the reference material. These must be detectable within the capability of the respective detection method. It must be taken into account that the stability of the MP particles is determined in particular by the type, size and state of ageing and is therefore represented by realistic particles.

Validating the sampling procedure

The validation of the sampling in the context of an "interlaboratory test" is much more complicated. The matrix and composition of the environmental medium are of decisive importance in this case. Here, too, a defined particle count or mass of MP reference material must be added or a suitable volume sampled in order to determine the added MP within the scope of the various detection methods. What is being tested in this case is the subjective behavior of the different samplers. For this purpose, samplers are required to perform simultaneous or consecutive sampling of an installation or an area at a single location. Since MP-free environmental media are practically non-existent, it is mandatory to determine the basic MP load in advance with high accuracy for all relevant methods. In this context, it is important to determine how homogeneous or heterogeneous the respective polymer types are. It has to be taken into account that in some cases very high MP particle counts or MP contents have to be added, which can be critical in terms of costs. If required, commercially available MP particles with narrowed chemical composition or particle shapes can be used.

7.4 Testing processes and products

Claudia Heller, Monika Fuchs

This subchapter addresses the testing of specific processes and products that lead to the emission of MP into the environment and are the subject of projects within the research focus "Plastics in the Environment".

Testing the washing process of textiles in private households

The maintenance phase of clothing and textiles in the household essentially comprises the steps of washing and drying. Both processes are complex and are influenced by numerous parameters, which is why the chosen procedure must be described in detail. The following parameters must be defined for the washing process:

- Washing machine
- Washing program
- Detergent
- Laundry items incl. soiling

In general, the parameters must be described precisely and in detail and must be kept as standardized and as consistent as possible over the duration of the test. Depending on the objective of the analysis, one parameter at a time can be variable. The following shows how the parameters can be standardized with the aim of replicating the average German household.

A washing machine which is typical for German households is a drum washing machine with an opening at the front of the drum (front loader). The maximum load should be between 6 and 10 kg. In the standardization surrounding laundry care, a so-called "Wascator FOM 71CLS" is the reference washing machine.

Washing programs vary in terms of liquor ratio (water/textile ratio), reversing rhythm (direction of rotation and standstill), washing time, temperature control and spin profiles. Washing machines may also be equipped with sensors that adapt the washing program to the conditions during the ongoing process. Therefore, the wash program must be programmed to run identically for each wash cycle. Measuring equipment can be installed on the washing machine for checking purposes. The choice of washing program and washing temperature should be based on the results of consumer surveys conducted in scientific studies.

Detergent is also available in standardized form according to DIN EN ISO 6330 and DIN EN 60456. The suitable detergent must be selected in accordance with the selected laundry unit. The detergent dosage must be calculated on the basis of the hardness of the water and the selected load quantity.

There are numerous types of laundry loads in Germany, partly because of the complexity of textiles and clothing and partly because of the individual sorting habits of consumers. For Germany, eight laundry loads were identified, which represent the actual handling of laundry in the household. Within the laundry loads, different laundry items are represented, each with textile-specific characteristics. If the objective is recreating a domestic laundry cycle, one of these laundry loads should be selected and used in a simplification if necessary. A documentation of the folding of the laundry items and the loading sequence is required in accordance with DIN EN 60456. If there is another objective the DIN EN 60456 offers the possibility of using standardized laundry loads.

One of the aims of doing laundry is the removal of soil. Since there are many different types of soil, it is recommended to use a standardized type of soil that can be applied directly to the laundry. The amount of soil must be adapted to the designations light, normal, heavily soiled depending on the selected load quantity.

The entire test must be carried out in a standard climate, which, according to ISO 139, must comply with the following conditions: an ambient temperature of 20 ± 2 °C and a relative humidity of 65 ± 4 %.

8. Annex

Index of method abbreviations / technical terms

Abbreviation	**Designation**
(µ-)ATR-FTIR	Attenuated Total Reflection Fourier-Transform Infrared Spectroscopy (Microscopy)
µ-(FT-)IR	(Fourier-Transform) Infrared Spectroscopy Microscopy
µ-Raman	Raman-Microspectroscopy
FPA-FTIR	Fourier-Transform Infrared Microspectroscopy with Focal Plane Array Detector
MIR	Medium Infrared
NIR	Near Infrared
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
DSC	Differential Scanning Calorimetry
Py-GC/MS	Pyrolysis-Gas Chromatography/Mass Spectrometry
TED-GC/MS	ThermoExtraktionDesorption-Gas-Chromatography/Mass spectrometry
UV radiation	Ultraviolet radiation
PM	Particular Matter
KWS	Corona Roller Separator
LOD	Limit of Detection
LOQ	Limit of Quantification
SI	International System of Units

Abbreviations of polymers / materials

Abbreviations	**Designation**
MP	MP
PE	Polyethylene
PP	Polypropylene
PVC	Polyvinylchloride
PS	Polystyrene
PMMA	Polymethylmethacrylat
PET	Polyethylenterephthalat
PA	Polyamide
EP	Epoxy resin
PUR	Polyurethane resin
UP	Polyester resins
TRWP	Tire road wear particle
SBR	Styrene-butadiene rubber
EPDM	Ethylene propylene diene monomer rubber