



Microplastics In Europe's Freshwater Ecosystems:
from sources to solutions

STANDARD OPERATION PROCEDURES (SOPs) FOR MICROPLASTIC (MP) SAMPLING AND ANALYSIS

MP ASSESSMENT IN FRESHWATERS (DELIVERABLE 2.1)

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Abbreviations and terms

Abbreviation/ term	Explanation
Bft	Beaufort (scale for wind speed measurement from 1-13)
Bulk sample	A sample that has not been pre-sieved or pre-filtered and contains the whole sample matrix.
ESR	Early Stage Researcher
FPA	Focal plane array
FTIR	Fourier transform infra-red
GF	Glass fibre
GPS	Global positioning system
IR	Infra-red
LOD	Limit of detection
LOQ	Limit of quantification
MP	Microplastic
MPSS	Munich Plastic Sediment Separator
μFTIR	Micro-FTIR
μRaman	Micro-Raman
N	Number
Pyr-GS-MS	Pyrolysis gas chromatography mass spectrometry
Sampling location	A specific ecosystem region or location where the MPs are to be assessed. E.g., A specific river catchment.
Sampling site	A specific part/area of the sampling location, that could be coupled to an address or GPS coordinates. E.g., the 0-2 km upstream part along a river before reaching a harbour.
Sampling point	The specific point or spot within the sampling site, where the sample is taken. E.g., Five points of the surface water (0-1 m depth) along the middle of a river at 0 km, 0.5 km, 1 km, 1.5 km and 2 km).
SiMPle	A freeware for the fast detection of microplastic materials in environmental samples. (Aalborg University, Denmark and Alfred Wegener Institute, Germany)
Size category	A group to which MPs within a certain size class are attributed to. E.g., MP size category A.
Size class¹	A group of a defined range of MP sizes. E.g., MP size class 0-1 μm.
SOP	Standard operation procedure
TED-GC-MS	Thermal extraction/desorption-gas chromatography-mass spectrometry
WWTP	Waste water treatment plant

¹ Size class and category are often used as synonyms. The main difference is that 'category' often suggests a predefined classification and that 'class' refers to a group including items with countable, common characteristics.

Abbreviation/ term	Explanation
QA	Quality assurance
QC	Quality control

Beneficiaries

#	Short Name	Full legal name
1	UBT	Universität Bayreuth
2	AAU	Aalborg Universitet
3	ENPC	Ecole Nationale des Ponts et Chaussées
4	EVONIK	Evonik Technology & Infrastructure GmbH
5	Fraunhofer	Fraunhofer UMSICHT
6	HHL	HHL Gemeinnützige GmbH
7	KI (NIC)	Kemijski institut (National Institute of Chemistry)
8	NTNU	Norges Teknisk-Naturvitenskapelige Universitet
10	UGOT	Goteborgs Universitet
11	UiB	Universitetet i Bergen
12	UoP	University of Plymouth
13	VUA	Stichting VU (Vrije Universiteit Amsterdam)
14	Univie	University of Vienna

PUBLISHABLE SUMMARY

Since the beginning of plastic production, approximately 8.3 billion tonnes of plastic have been produced worldwide, of which 60 % ended up in the environment. Microplastics (MPs) are defined as plastic particles ≤ 5 mm and pollute aquatic ecosystems worldwide, often entering freshwaters in urban areas. MPs affect the environment by releasing toxic compounds, accumulating pollutants, harming organisms, and accumulating in the (human) food chain. The understanding of MPs in freshwater systems is crucial, and rivers are acknowledged to transport MPs into marine ecosystems.

The comparability of different study results for MP assessment in the environment is difficult because studies are carried out using widely different methodologies. Difficulties for standardising or harmonising methodologies for MP sampling, sample processing and analysis are manifold. Various aspects influence the quality and the results of studies, such as: diverse sampling locations, heterogeneous samples, available equipment and scientific question. Those aspects are often interdependent, hampering easy and straight forward standardisations. Hence, an underlying concept of standardisation, namely to agree upon certain ways to proceed and accomplish tasks has not fully been developed for MP assessment in freshwaters. However, there is ample consensus in the scientific (and non-scientific) community for the need to develop standards for MP assessment.

Nets/trawls for large volume surface water sampling are popular, and the use of a filter-cascade pump set-up for water column sampling has many advantages. Collecting sediment with plastic-free grabbers or corers are suitable. Appropriate sample processing steps (digestion, density separation), and combination/repetition of procedures depending on the sample content (inorganic/organic matter) are necessary, followed by MP particle size fractionation. To comprehensively analyse MPs (determine shape, size, colour, polymer type), a coherent combination of available, complementary techniques (FTIR, Raman, optical microscopy, Pyr-GC-MS) is essential. Quantification (mass, concentration, MP sizes) is important to better understand MP pollution, hence, the acquired data should be reported meticulously in a transparent way using consistent units. Methods for MP assessment depend on the study design, the characteristics of the targeted MPs, the available equipment and more. Therefore, we advise ensuring to provide all data necessary to comprehend the research results and applying good practices along all procedures. Although it is not possible to always apply the same methods for MP assessment in freshwaters among different projects, the recommendations given in this report allow for better comparability of the research results.

The project LimnoPlast (2020-2023) aims to assess the sources, fate and sinks of MP in urban freshwater environments and link social, technical and environmental science. Fifteen Early Stage Researcher (ESR) projects have been designed to investigate the MP contamination in Europe's freshwater systems and tackles the various knowledge gaps. Three of the projects (ESR 3, 4 and 15) will directly assess MP in the water systems in Aalborg/Aarhus (Denmark), the Greater Paris catchment of the Seine river (France) and the City of Amsterdam catchment (The Netherlands), respectively.

In the frame of the LimnoPlast project, we conclude that it is inevitable to agree on certain SOPs, while the individuality of each executing project team is taken into account. This report (Deliverable 2.1) contains a method review for sampling, sample processing and analysing MPs in urban freshwater systems, focusing on sediment and water samples. We discuss different procedures regarding flexibility, suitability and representativeness and draw conclusions for recommendations for harmonized strategies (or SOPs) regarding the objectives of the three LimnoPlast projects.

The main questions and aspects that are touched upon in this report are:

- What are the different steps needed for reliable quantification and characterization of MP?
- Which criteria are essential to choose a suitable method?
- Where to sample MP (sampling locations)?
- How does the targeted size of MP influence the sampling methods?
- What influences the matrix has on the sample processing, and how could this be harmonized? What quality assurance (QA) and quality control (QC) processes should be implemented?
- What MP characteristics should be analysed and reported (particle size, shape, type of polymer, mass)?

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1 INTRODUCTION

Plastics are polymers, produced (since the 1950s²) by the synthesis of fossil fuel (synthetic polymer) or bio-based polymers and comprise different chemical compositions (i.e., type and additives), and hence characteristics. Globally, 359 million tonnes (2018) of plastics are produced every year; 51 % are produced in Asia and around 17 % in Europe (Plastics Europe 2019). Different synthesis processes result in two major plastic groups: thermoplastics and thermosets (Table 7, 7.1.1). Their main distinction lies in the molecular structure; thermosets are cross-linked and therefore resistant to re-moulding or re-heating (permanent), while thermoplastics can be re-used or re-cycled. Plastic applications vary widely from packaging to fishing gear, clothing, high-tech coating, additives in dyes, construction material, or ingredients in personal care products (PCPs) (GESAMP 2015). A major drawback to this extensive use is the resulting environmental pollution. Mismanagement in all steps of the life of plastics and plastic products leads to global contamination of the environment, i.e., air, soil, water (ecosphere). An estimated 8 million tonnes of plastic waste enter the oceans annually, often through rivers, in the form of visible plastic litter but also of small particles, so-called microplastics (MPs).

Since the start of research on MP pollution, studies have adopted various definitions for MP size categories and size classes (Figure 1). The most used definitions consider MPs as plastic particles ≤ 5 mm (Hartmann et al. 2019). This latter definition will also be used within the LimnoPlast project. Plastic particles of the size between 1-5 mm are considered large MPs, from 1 μm -1 mm small MPs, and the particle size category $< 1 \mu\text{m}$ is referred to as nanoplastics. Plastic particles between 5-25 mm are so-called mesoplastics, and particles > 25 mm are considered macroplastics. Although, MP particle sizes seem to be roughly well-defined by now, there is still discrepancy on how to determine the diameter of a heterogeneously shaped MP particle, as fibres, for example, can be very thin (μm) but long (cm) at the same time (Dris et al. 2016; Treilles et al. 2020).

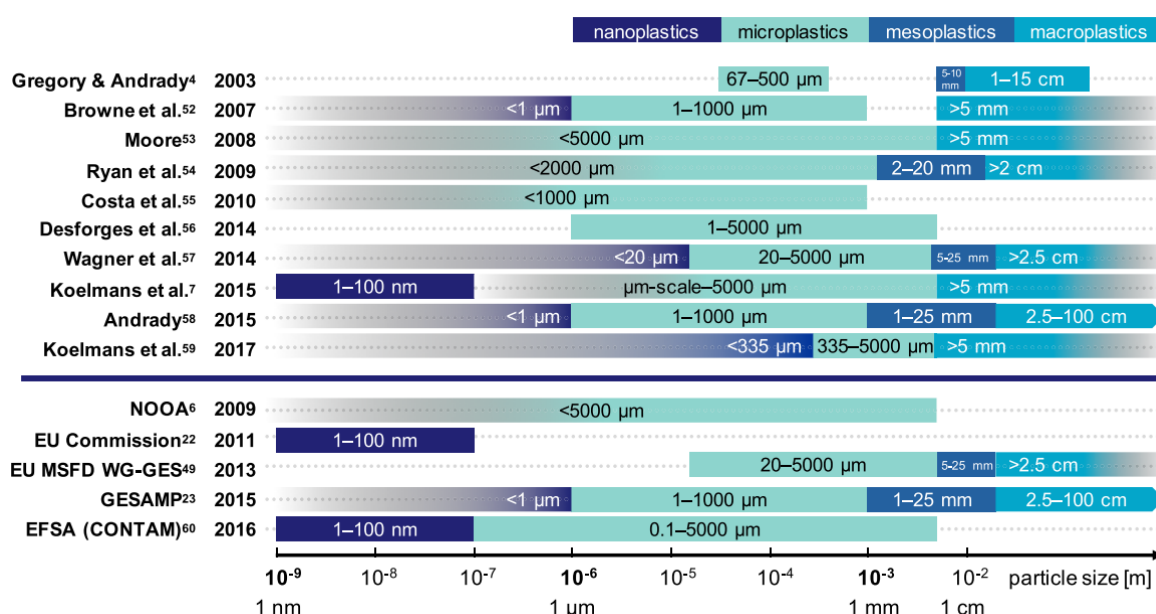


Figure 1. Examples of differences in the categorization of plastic debris according to size as applied (and/or defined) in scientific literature and in institutional reports. It should be noted that this does not represent an exhaustive overview of all used size classes. From Hartmann et al. (2019)

Freshwater systems (lakes, rivers, ponds, etc.) hold various sources, sinks and pathways on pollution with MPs as they vary widely in ecological and hydrological structures. Major MP inputs can be from terrestrial surfaces (runoff, wind, flooding) and the atmosphere (dry or wet deposition) (Dris et al. 2016). The degradation of macro- and mesoplastic contributes additionally to the MP occurrence in the environment. Urban pathways and

² <https://www.unenvironment.org/interactive/beat-plastic-pollution/>

anthropogenic activity, such as stormwater and road-surface runoff, combined sewer overflows, and influent from wastewater treatment plants (WWTPs) (Fahrenfeld et al. 2019) are likely primary sources releasing MPs into freshwater systems, as well (Mao et al. 2020).

In accordance with the distribution of plastic types in global plastic production, the most frequently contaminating MP types in the aquatic environment are PE~PP>PS>PVC>PET (Koelmans et al. 2019). The average amount of MPs in freshwaters from different studies, presented as a mean abundance of particles m^{-3} in a review by Li et al. (2018), can be calculated to 600 521 particles m^{-3} , which extrapolates to a global contamination of freshwaters³ (lakes and rivers) of $1.07 \cdot 10^{20}$ particles; that equals 107 000 000 000 000 000 particles. Such an extrapolation is biased and in general, the reported data on MP pollution worldwide suffer from a lack of comparability. Therefore, the establishment of standardized (i.e. suitable, reproducible, reliable, comparable) sampling and analysis methods (and monitoring approaches) is mandatory (Löder and Gerdts 2015).

The full analytical chain of MP assessment in freshwaters consists of four main steps: Sampling (see section 3.1), sample processing (or MP extraction) (see section 3.2), polymer identification and MP characterisation (see section 3.3) and quantification and data reporting (see section 3.4). There exist various state-of-the-art sampling techniques depending on the sampling location and the scientific question. MP sampling techniques differ in their application options for, e.g. different depths, surface areas, volumes, MP sizes, replication, equipment, and therefore their choice matters. In freshwater environments, MPs are embedded in different matrices, the water phase or the sediments or inside of organisms (biota). Commonly applied techniques for sampling MPs in the water phase are trawls or nets for concentrated samples, bottles or automated water samplers for bulk samples (Barrows et al. 2017; Eriksen et al. 2018; Stock et al. 2019). Sediment matrix is sampled with grabbers or sediment cores (Frias et al. 2018; Scherer et al. 2020). MPs ingested in organisms can also be investigated via biomonitoring, where MPs are commonly assessed in the soft tissue, the gut-contents or the whole organisms (Kazour 2017; Klein et al. 2016; Leslie et al. 2017; Stock et al. 2019). The aspect of biomonitoring is beyond the scope of this report and will not be further addressed.

After sampling, MPs are separated from the environmental matrix (water, sediment) to further identify and quantify the polymers and characterise the particles (Li et al. 2018). Extraction steps depend on the matrix in which the MPs are embedded in. Certain sub-steps can be omitted or have their order changed depending on the sample specificities. These steps include pre-filtration or pre-sieving, enzymatic or chemical digestion, density separation, and a final concentrating step on a filter or in a small volume (μL - mL) (Frias et al. 2018; Koelmans et al. 2019; Li et al. 2018; Scherer et al. 2020).

Visual identification by optical microscopy of MPs is still used sometimes, although outdated and problematic (Löder and Gerdts 2015). Established analytical techniques that allow accurate polymer identification are Fourier transform infrared (FTIR) spectrometry or microscopy (Löder et al. 2015), Raman-spectrometry or microscopy (Käppler et al. 2016), pyrolysis-gas chromatography coupled to mass spectrometry (Pyr-GC-MS) (Fischer and Scholz-Böttcher 2017) or thermal extraction/desorption-gas chromatography-mass spectrometry (TED-GC-MS) (Dümichen et al. 2017; Li et al. 2020; Mai et al. 2018; Ruggero et al. 2020). FTIR and Raman spectrometry are particle-related, non-destructive, analytical approaches while Pyr-GC-MS or TED GC-MS are mass-related, destructive, analytical methods. Mass quantification with μFTIR (density- and dimension-based calculated) compared to Pyr-GC-MS (mass-based calibration) were found in the same order of magnitude (Kirstein et al. 2021), indicating that mass quantifications could be viably when purely based on dimensions and polymer densities.

Up to date, there are no standard guidelines established for the full analytical chain of MP assessment in freshwaters. One way to approach harmonisation is the implementation of Standard Operation Procedures (SOPs). SOPs do not equal standardisation; however, they are part of harmonised methodologies. The purpose of a SOP is to provide straight-forward, detailed, clear and safe instructions on how to perform certain processes and SOPs are commonly applied in laboratories to ensure safety and health through correct handling of hazardous substances⁴. SOP development is based on a risk assessment and sets limitations that are transferable among experiments. SOPs shall ideally contain different points: Person responsible; location, time and date of

³ Numbers of global freshwater volume of lakes and rivers (sum = 178520 km^3) are taken from <https://www.usgs.gov/>

⁴ <http://www.ehs.ufl.edu/programs/lab-research/gator-tracs/standard-operating-procedures-sops/>

experiment or procedure; scope of the work; risk identification; engineering controls; step-by-step procedural descriptions; transport and storage requirements; waste disposal guidelines; and emergency procedures (University of Florida 2020). In contrast to standards, SOPs are developed by individual laboratories and may undergo modifications with time or new procedures.

Standardization or harmonisation of MP methodologies and procedures presents several levels of complexity (Koelmans et al. 2019; Stock et al. 2019). Difficulties for standardising or harmonising methodologies for MP sampling, sample processing and analysis are manifold. Firstly, the (freshwater) ecosystems to be investigated comprise various and complex conditions regarding the sampling location, surroundings, matrices and more. Secondly, there are knowledge gaps about the sources, sinks and fate of MPs in waters, including unknowns of other environmental influences (e.g., turbulence or interactions with suspended solids). These aspects make it challenging to properly phrase hypotheses, design studies, and choose suitable methodologies. Moreover, a great variety of existing methodologies, of which some have been used more commonly, while others are novel (and therefore less vetted), and the absence of standard guidelines, make it difficult for researchers to judge and conclude adequately. The interdependencies of scientific question, study design, sample, ecosystem conditions, sampling equipment, targeted MP particle sizes as well as cost efficiency and feasibility represent a unique combination for each study, hampering easy and straightforward standardisations (Figure 2). Another challenge to face regarding analytical methods and thus the sub-subsequent databases required for polymer identification is the variety of polymer compositions and the characteristics of those different types (Leslie et al. 2017; Scherer et al. 2020).

For example, different polymer densities are a major determining factor to develop and carry out efficient density separation processes.

A summarized study composition of MP concentrations in rivers by Scherer et al. (2020) shows that concentrations differing by several orders of magnitude ($LOD-10^8$ particles m^{-3} water or kg^{-1} sediment) are found in the water and the sediment phase. Even within single studies, the use of different sampling techniques resulted in (significantly) different MP concentrations found. A review by Koelmans et al. (2019) assesses various MP sampling and identification approaches of 50 studies regarding the robustness of the methods (i.e. reproducibility, precision,

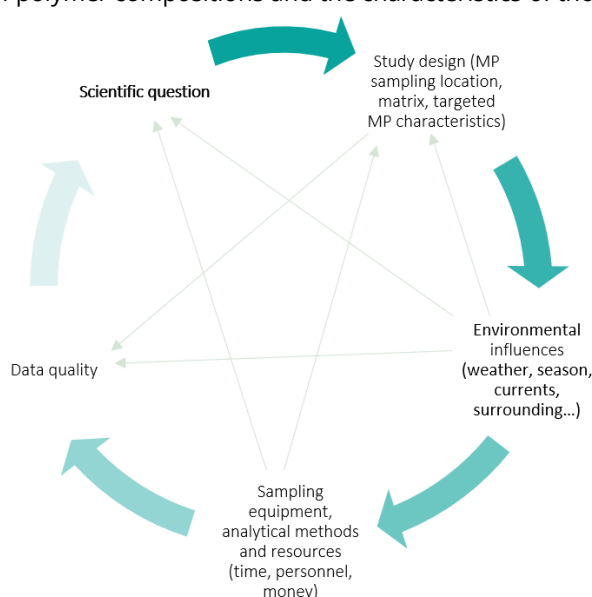


Figure 2. Inter-dependencies of different aspects for MP assessment (circular arrows from darker to lighter turquoise = showing high influence pathway starting with a scientific question, linear light green arrows = lower or more variable influences; arrows point from the influencing aspect in the direction toward the aspect being influenced); own draft.

accuracy and sensitivity) by means of a quantified quality evaluation. They conclude that, considering the up-to-date approaches (major developments in techniques were made in the last two decades), there are only few studies of high quality and they further recommend improvement on: sample processing, polymer identification, air contamination risk and positive controls. In addition to a fundamental problem for the comparability of studies, there is a difference in the targeted MP size classes, which transfers to MP shapes, as well. These points, simply lead to the conclusion that a) there should be a consensus about MP size categories to be investigated in future studies and b) more studies should target a more comprehensive assessment of MP size classes. Both examples, and the aspects discussed above, illustrate the urge for standardisation of sampling and analysis techniques in order to compare research results reliably.

2 MP ASSESSMENT IN THE LIMNOPLAST PROJECT – SCOPE AND OBJECTIVES

The project LimnoPlast (2020-2023) aims to assess the sources, fate and sinks of MPs in urban freshwater environments and link social, technical and environmental science. Fifteen Early Stage Researcher (ESR) projects have been designed to investigate MP contamination in Europe's freshwater. Three of the projects (3, 4 and 15) will directly assess MPs in the water systems in Aarhus (Denmark), in the Greater Paris area in the Seine river (France) and in the City of Amsterdam catchment (The Netherlands), respectively. In section 7.1.2, the three ESR projects are presented with their overall scientific questions and topics, their potential sampling locations and matrices, the targeted MP characteristics to be determined, as well as the methods available in the executive laboratories.

ESR project 3 aims to identify and quantify dominant MP emitting sources and the intrinsic characteristics of the MPs released from each of these sources and the location of discharging points in the Aarhus metropolitan. In this context, MP hotspots are determined, and MP distribution over space and time is investigated. It is also part of the aim to use these data in a further step to develop a model to accurately estimate annual fluxes downstream of MP hotspots.

ESR project 4 aims to determine the MP budget in the Greater Paris river catchment. The objective is to assess a range of MP size classes (10-100 µm; 100-300 µm, >300 µm) and shapes. Six potential sites of the Seine river and tributaries in the catchment will be sampled for the water phase, upstream and downstream of Paris City. The influence of agricultural sites, where WWTP sludge is commonly applied as a fertilizer will be assessed in an upstream experimental catchment. Sediment samples and benthic boundary layer samples might be taken at the same locations as for water samples. A method comparison focusing on MP sedimentation and resuspension processes might be performed at a suitable sampling sites.

ESR project 15 aims to study MP abundances in the urban region of Amsterdam which includes a large network of canals and waterways, receiving treated wastewater from three WWTPs situated in the vicinity. This project will also investigate MPs in the drinking water of Amsterdam, which is generated using freshwater from the area, linking MP occurrence in freshwaters to human exposure. Moreover, biota from aquaculture samples and human gut matrices shall be investigated on their MP concentrations.

One goal of the LimnoPlast project is to acquire comparable data among the above-mentioned projects. Therefore, recommendations for SOPs based on state-of-the-art methodologies for MP assessment in freshwaters are developed. The recommendations comprise the main procedures of MP assessment, i.e., sampling, sample processing, polymer identification and MP characterisation, as well as quantification and data reporting. The main focus is on methods for urban freshwater systems, as the recommendations are based on the scope of the three projects. In the frame of the LimnoPlast project and the targeted ecosystems, we conclude that it is inevitable to agree on certain SOPs, while the individuality of each executing project team is taken into account. Therefore, in this report, we propose

- a) that individual SOPs for the main four MP assessment steps should be developed by the individual ESR projects, with respect to their scientific question, equipment and resources, and
- b) that in these SOPs, recommendations for important aspects of each MP assessment step given in this report are included.

3 MP ASSESSMENT

To assess MPs in freshwaters, four main steps are necessary (Figure 3): sampling, sample processing, polymer identification and MP characterisation, quantification and data reporting.

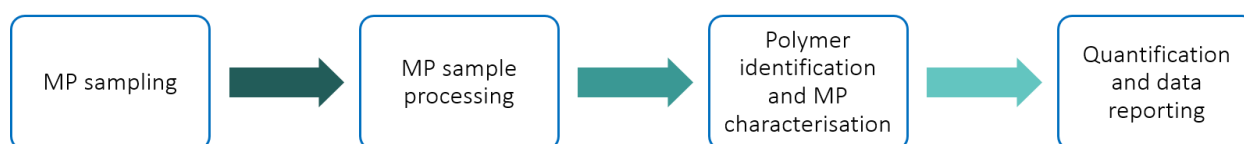


Figure 3. MP assessment chain showing the main four steps; own draft.

In the following, for each main step of the MP assessment chain, the state-of-the-art methodologies are described, followed by outlining Quality Assessment and Quality Control (QA/QC) aspects and discussing the advantages and disadvantages of the methodologies. Finally, recommendations for SOPs are given for each of the four main steps.

3.1 Sampling methods (MP assessment)

3.1.1 State of the art (sampling methods)

There has been rapid progress in the development of methods for sampling MPs in water and sediment over the past decade. Along the vertical profile in the pelagic zone of freshwater systems the surface microlayer (upper 1 mm), the surface layer (usually upper 0.5 m or 1 m), the whole water column, the sediment-water interphase, and the sediment can be distinguished (Figure 4). Different strategies for sampling are available: volume-reduced sampling (net/pre-sieved), bulk sampling (unfiltered water sample) and selective samplings (visual particle collection) of water surface, water column and sediment, although selective sampling is barely applicable for the water phase (Hidalgo-Ruz et al. 2012). Samples (water, sediment) are usually stored at +4 °C, or frozen at -20 °C. Apart from the different sampling points within the aquatic environment, different waterbodies can be sampled, such as rivers (may comprise estuaries), lakes, ponds or streams. Sampling of MPs of different sizes influences the choice of sampling methodology (Figure 5) and equipment (for sampling equipment see also section 7.1.3, Figure 7).

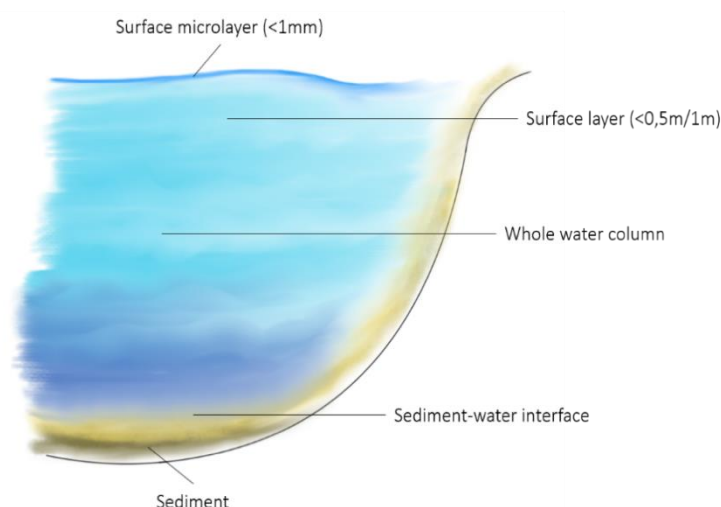


Figure 4. Illustration of a freshwater cross section and the vertical layers commonly distinguished in MP assessment and sampling. Whole water column includes water from surface to the bottom; own draft.

- **Water net/trawl sampling:**

The most common approaches to water phase sampling are vertical and horizontal (trawling, often in transects) net samplings which permit the concentration of MPs in a small volume and the averaging of local spatial heterogeneities. Especially surface water is often sampled using nets and done along a defined surface area or length (transect) by active trawling (Wendt-Potthoff et al. 2017). In case of sufficient current velocity in a stream, nets can also be deployed stationary for passive sampling. Due to the nature of this approach, there is a high risk of clogging due to particulate matter when the mesh size is small. Common mesh sizes are about 300 µm (Stock et al. 2019). The effect of “3D positioning” of the particles (overlay of particles) in the net or sieve during sampling affects what ultimately passes through or not and is especially crucial when clogging occurs. Additionally, smaller

mesh size leads to higher dynamic pressure, which decreases the maximum possible trawling velocity and the sampled volume. Moreover, because of the dynamic pressure in the net and surface water movements, it is difficult to determine precisely the sampled water volume, which is usually measured with a flow meter installed at the net opening (Karlsson et al. 2020). If the abundance of MP particles above the mesh size is high in the sampled water, the dynamic pressure increases, and currents flowing in the opposite direction of net trawling enhance this effect. These effects apply similarly to vertical net sampling (comparable to zooplankton net sampling), which is carried out to have a depth-integrated sample. In contrast, horizontal net sampling is used to assess MP abundance at a specific horizontal layer of the water. Horizontal net sampling is influenced by wave and tidal movements, whereas vertical net sampling is more prone to turbulence in the water column and currents (Fahrenfeld et al. 2019; Klein et al. 2016; Stock et al. 2019).

- **Water pump sampling**

Pumping systems can contain filter cascade systems, meaning the water sample is concentrated on filters or sieves with different mesh sizes from larger to smaller, or single large area filters. Pump systems consist of a water (submersible) vacuum pump and a plastic or stainless-steel tube or water inlet (in the case of submersible pumps), which is inserted into the water (Tamminga et al. 2019). Sampling depths depend on the equipment (i.e. length of tube, power of pump) and the sampling location. Water is then sucked through the tube, possibly passing through a filter/sieve (cascade) or being collected as a bulk sample in sampling vessels (Karlsson et al. 2020; Stock et al. 2019). A flowmeter installed in the pump system measures the water volume (Karlsson et al. 2020). Water pump sampling systems can be automated (see next paragraph). Up to several cubic meters per hour can be pumped. Depending on the pumping duration, this technique enables the averaging of local and temporal heterogeneities.

- **Automated water sampling**

Automated water sampling is commonly done using programmable or automatically running instrumental set-ups, and various systems exist. The instruments are installed in the water or nearby and sample and store the water automatically. Some systems are bottle-based instrumental set-ups like the Multi-LIMNOS⁵ system (Hydrobios®) with automated fluid injection to prevent sample contamination. Other set-ups are more simple and collect one or several samples using a conventional, automated Niskin-bottle system. However, similar sampling systems exist for manual sample collection (see next paragraph). Also pumping systems can be automated including an installed sample storage system. The sampling volume for bottle-based automated water sampling is in the order of 1-10 litres, while water pumping systems can collect volumes up to several cubic meters.

- **Manual bulk water sampling**

Technical set-ups for water sampling are conventional water samplers, such as a Niskin bottle (for vertically depth integrated samples) or a Van-Doorn sampler (for sampling water in a horizontal manner). Hereby, the device is lowered into the water body to the desired depth and the sampler closes via a manually induced mechanism. Sampling volumes for these sampling strategies range usually between 1-10 litres. Bulk water grab/scoop samples can be collected using jars/bottles/buckets (or other) and scooping water, usually from the surface (Barrows et al. 2017; Hidalgo-Ruz et al. 2012; Mai et al. 2018). With this approach, only small water volumes (few litres) can be collected.

- **Sediment grab sampling**

Sediment samples from freshwaters are often taken using grab samplers (e.g. Van-Veen or Ekman grab sampler) (Frias et al. 2018; Stock et al. 2019). The open grab sampler is lowered carefully onto the sediment surface and with a manual mechanism the scoop/shovel of the sampler closes while incorporating sediment into the scoop. With the method of grab sampling the upper sediment layer (~10-15 cm) is collected as a disturbed bulk sample.

- **Sediment core sampling**

Using cores for sampling results in stratified, undisturbed sediment bulk samples and can, depending on the core type, collect sediments deeper than 10 cm (Mai et al. 2018; Van Cauwenberghe et al. 2015). The most common types of sediment cores are piston-cores and box-corers. If a manual core is used, the open core sampler is

⁵ <https://www.hydrobios.de/shop/multiple-water-samplers/automatic-water-sampler-multi-limnos/>

lowered relatively fast to sink several cm into the sediment. Automatic set-ups press the core actively into the sediment. Via a manual mechanism, the top of the core (for simple core sampler) or the bottom scoop (for box corer) is sealed and the corer is then uplifted (Fisher et al. 1992; Tsuchiya et al. 2019). Using this sampling methodology, depth profiles of MP contamination can be investigated via slicing the core into layers and analysing the single layer samples separately.

3.1.2 QA/QC and discussion (Sampling methods)

Concerning the aforementioned descriptions, net samplings are restricted in terms of minimum mesh size, and reported mesh sizes are usually $>50\text{ }\mu\text{m}$ and most commonly $\sim 300\text{ }\mu\text{m}$. The choice of mesh sizes (such as the aforementioned) often origin from standardly applied biological oceanography habits and are not necessarily optimized for MP sampling. Mesh size cut-off during sampling should not be seen as the real cut-off of the MP assessment and the separation of MP particles in different size classes should be included in sample processing

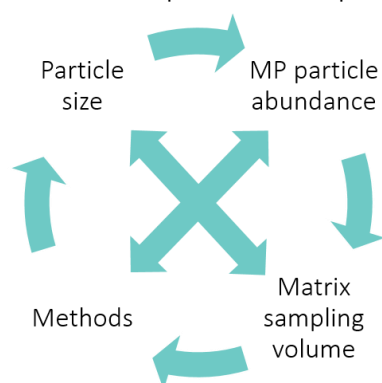


Figure 5. Interdependencies between methods, MP particle size, MP particle abundance and sample volume; arrows point from the influencing aspect in the direction toward the aspect being influenced; own draft.

in the laboratory using appropriate filters and/or sieves aiming to analyse MPs in several size categories.

A larger mesh size allows sampling larger volumes without clogging and is thus suitable for larger MP particles, which are less abundant than smaller particles (Barrows et al. 2017). Hence, to generate representative samples for larger MP sizes, larger sample volumes need to be processed to achieve appropriate MP concentration for analysis. Reversely, collecting bulk water samples amount smaller volumes (sometimes just 1 L). However, Tamminga et al. (2019) observed decreasing percentage of MP concentrations ($>100\text{ }\mu\text{m}$) with increasing sample volume, which indicates potential overestimation for small sampling volumes. The probability to sample representative amounts of larger MP particles, hence, is smaller for low-volume bulk sampling compared to large-

volume net/rawl sampling and proportions of larger MP particles were found higher in net samples than in manually collected bulk samples (Barrows et al. 2017) (section 7.1.3 Figure 8). Barrows et al. (2017) report that bulk samples contained higher proportions of non-fibrous and small-sized MPs and three orders of magnitude higher total MP amount per sample volume than trawl samples ($335\text{ }\mu\text{m}$ mesh), while the total sample volume was lower. Similarly, Tamminga et al. (2019) report higher proportions of fibrous particles in pump, compared to trawl sampling. Karlsson et al. (2020) reported MP concentrations of $0.23\text{ particles m}^{-3}$ for pump samples ($300\text{ }\mu\text{m}$ mesh) and $0.51\text{ particles m}^{-3}$ for trawl samples ($300\text{ }\mu\text{m}$ mesh). They reason, that this is partially caused by the difference in methodologies as trawl sampling included sampling the surface (micro) layer, which is known to have high MP abundance, and the pump sampled $\sim 10\text{ cm}$ below the water surface (Karlsson et al. 2020). This confirms other findings showing higher abundance of MPs at the water surface (Moore et al. 2005). At the same time Eriksen et al. (2018) suggest that larger particles may be present beneath the surface in larger proportions than at the surface. However, the overall repeatability (important for representativeness) was found to be better with pump sampling than trawl sampling (Karlsson et al. 2020). MP concentration tht can be found per sample volume also depends on the amount of MP contamination and structure of the sampling location. Mao et al. (2020) showed different MP concentrations found upstream and downstream in the Yulin River (China), and identified that river channel width was related to MP abundance, as well.

Pump sampling coupled with different sieves offers the advantages of direct *in situ* cascade size fractionating, while still being able to sample large water volumes. This may allow sampling a larger range of sizes of MP particles even with a low abundance. Another suitable combination to take into account the particle size frequency distribution is using net/rawl sampling for large sample volumes and bigger MP particles (e.g., $> 300\text{ }\mu\text{m}$) in parallel to large area filtration pumping systems with for small MP particles (e.g., $> 10\text{ }\mu\text{m}$) (University of Bayreuth, unpublished data). Only few studies (Karlsson et al. 2020; Tamminga et al. 2019) report details for the pumping system, such as tube material, tube diameter, pumping velocity, precision or information on contamination prevention measures (e.g. rinsing). Those details are crucial to assess the representativeness of

the method and to allow comparability among studies, and certain technical criteria are recommended to be taken into consideration by Bertrand-Krajewski J.-L. et al. (2000) in analogy with sampling particles in urban waters using automatic samplers or pumps: Sampling pump velocity, sampling flow (volume per time), settling velocity of targeted MP (needs to be considered with the appropriate sampling flow), tube diameter and length (tube volume), sampling point, sampling depth and most suitable depth.

Sediment sampling to assess MPs is based on conventional sediment sampling methodologies, but there might be potential to improve methods for MP inspection. The application of drill cores or sediment cores (although not box-corers, as their closing mechanism disturbs the sediment) allows the investigation of different sediment layers (mostly in lakes, where sediment layers are less disturbed than in rivers, where higher flow velocities and currents can lead to increased agitation), and therefore can be coupled to radionuclide-dating (Turner et al. 2019) as this sampling technique yield undisturbed, stratified sediment samples (Fisher et al. 1992). Selective sampling can be used for sediment samples, using tweezers or spoons to pick out visually identifiable MP particles. However, this method is limited to larger particles, visible by the naked eye (Hidalgo-Ruz et al. 2012) and not used very often.

Koelmans et al. (2019) reviewed different studies and summarized the concentration ranges of MPs sampled in the water phase in various water types, such as rivers, lakes, drinking water or WWTPs, reporting higher variability of MP concentrations in WWTP effluent > rivers > lakes. Different techniques have been used by the studies overall, whereas the sampling can be differentiated between surface water (pumping, net/trawl sampling, manual bulk sampling), wastewater (pumping, manual bulk sampling, automatic water sampling) and tap/bottled water (direct sieving/filtration). This highlights the problematics of representativeness among a variety of methods used to assess MP in similar water bodies. In general, a systematic sampling strategy for sediment sampling in a defined transect with randomised sampling points is recommended by Eerkes-Medrano and Thompson (2018), to obtain sample representativeness of the sampling site. The sampling protocol (or SOP) must be optimized for the respective sampling/study side and scientific question, while maintaining basic standard principles that allow comparison with other studies.

Along all process steps for MP assessment, preventing or minimising the risk of plastic contamination is crucial. Nonetheless, different sampling devices are made from or contain certain amounts of plastics (e.g. net-trawls, PP tubes, plastic hoses in pumping equipment, or plexiglas tubes in core samplers). For example, aluminium, glass or stainless-steel tubes for cores should be preferred (Tsuchiya et al. 2019). Most studies and reviews report sample storage (*in situ* sample transfer from sampling device to transport device) in glass jars, that have been pre-cleaned by rinsing with either (filtered) sampling water or MilliQ water, or that have been burned (500 °C) to minimize the risk of contamination (Barrows et al. 2017; Frias et al. 2018; Hidalgo-Ruz et al. 2012; Leslie et al. 2017; Stock et al. 2019). Nevertheless, several studies reported the use of PVC zip-bags or other plastic-containing materials for sampling and/or sample storage (Eriksen et al. 2018; Frias et al. 2018; Karlsson et al. 2020), however, this should be avoided in any case possible. To avoid plastic contamination in samples, devices (both, plastic-containing and plastic-free) were reported to be rinsed or cleaned prior to use with filtered fluids. Also, water or alcohol during rinsing to collect sample rests from the sampling devices should be filtered prior to use. Aerial contamination is minimized by good sample handling practice, for example direct sealing of open device ends when not in use, fast sample transfer into storage containers and air contamination blanks should be run in parallel (Dris et al. 2016). Koelmans et al. (2019) recommend samples to be stored directly after sampling and further treatment to be done back in the laboratory under more controlled conditions. Inter-sample contamination is tackled by rinsing sampling devices with either filtered sampling water or MilliQ water between samples taken. Besides, sample contamination is usually quantified through the processing of blanks. Many articles recommend to run procedure blanks and air contamination blanks in triplicate (N=3) (Dris et al. 2018, Frias et al. 2018, Koelmans et al. 2019), Stock et al. 2019).

3.1.3 Recommendations for SOPs (Sampling methods)

Table 3. SOP recommendations for aspects of MP sampling methodology

Aspect	Recommendation
Particle size range	<ul style="list-style-type: none"> The recommendations are dependent on the scientific question, the targeted MP types and size ranges, the sampling method used, as well as on laboratory equipment. We recommend a comprehensive coverage of MP sizes (LOD-5000 µm) and to categorise as following: LOD-10 µm, 10-100 µm, 100-300 µm, 300-1000 µm, 1000-5000 µm. This allows comparability of results from many studies as well as to draw a holistic image of the worldwide MP load in the environment. It is however possible to work only on a sub-range of MP size classes, using ideally the categorisation recommended above in order to allow the comparability with other studies The drawback of such an overall standardization suggestion is the feasibility – determining a larger range of size fractions implies appropriate sampling, processing, and analysis equipment, increased time effort and therefore costs.
Sampling location	<ul style="list-style-type: none"> We recommend assessing the representativeness of the sampling location (depending on the scientific question) and the specific sampling sites and points at the sampling location. We recommend to pre-test the heterogeneity of the sampling site for uncertainty control under different hydrodynamic conditions. We recommend point sampling in freshwaters to reduce heterogeneity, but to sample possibly several points on the sampling site (N≥3). We recommend a systematic transect sampling for horizontal trawl samplings. When no spatial or temporal integrative sampling techniques (horizontal trawl sampling, pumping over longer period/several hours) are used, at least triplicates have to be considered to enlighten variability.
Sampling volumes	<ul style="list-style-type: none"> The sampled volume has to be selected in relation to the targeted MP type and range of size classes in combination with the sampling method used and the matrix sampled. We recommend larger sampling volumes for matrices that potentially have a lower MP abundance. Specific tools like stochastic modelling can be used to balance targeted MP sizes classes, sampling volume and number of samples (N) under consideration of influencing effects like clogging. Sample volume must be recorded as reference unit.
Sampling depth	<ul style="list-style-type: none"> The sampling depth is depending on the scientific question, accessibility and feasibility (equipment). We recommend considering different depths in freshwaters, and to consider whether to include the surface microlayer (top 1mm) or not, when sampling surface water (<1m). See also sampling location. For sediments either surface sediment (e.g. 5 cm top layer) or core sampling (e.g. 10 cm top layer) with the additional option to dating. Sediment coring in flowing water (rivers) is not necessarily suitable for dating approaches, due to the sediment-bed movements and disturbances, especially in the upper sediment layer.
Equipment	<p>Water:</p> <ul style="list-style-type: none"> The equipment depends on the targeted MP particles size and the sampling site. We recommend to take bulk samples of appropriate volume for higher abundant MP particles. A stainless-steel pump with or without cascade sieving is highly recommended to sample larger volumes, especially for potentially rare particles.

Aspect	Recommendation
	<ul style="list-style-type: none"> We recommend net trawl sampling for surface transect sampling of high volume through-put, but not for smaller volumes. <p>Sediment:</p> <ul style="list-style-type: none"> We recommend the use of sediment cores or grab samplers. In case of sediment cores, we highly recommend to avoid instruments with plastic tubes. A glass-made or stainless-steel sampler should be preferred. Otherwise the polymer type of the tube needs to be excluded from the analysis.
<i>In situ</i> processing	<ul style="list-style-type: none"> We do not recommend <i>in situ</i> processing in the field to minimize the risk of contamination (air contamination, fibres from clothing, etc.). Further processing should ideally take place in a controlled laboratory environment.
Description of sampling location	<ul style="list-style-type: none"> We recommend to describe in detail the hydrological and geological conditions of the water body and other potential influencing factors for MP abundance (e.g. WWTP outlet upstream).
Number of samples	<ul style="list-style-type: none"> We recommend a minimal replication of N=3 for samples taken, related to the uncertainty of the sampling strategy (Brüge et al. (2020)). For each sampling location, stochastic modelling tools can be used beforehand, to assess the relation of number of samples, replications, and uncertainties.
Flow velocity	<ul style="list-style-type: none"> We recommend to measure flow velocity entering the trawl/net during sampling to calculate the water volume sampled and to assess the flow velocity of the targeted sampling site before actual sampling and determine variabilities, to be able to interpret the results accordingly.
Measurement of additional (environmental) parameters	<p>General:</p> <ul style="list-style-type: none"> GPS coordinates of sampling site and sampling points Time and date of sampling Duration of sampling (minutes) Depths of sampling (see also sampling location) Weather conditions of the past 48 hours prior to sampling: precipitation (mm) and intensity (mm/h in hourly time steps) (optional), wind speed (km h⁻¹, m s⁻¹, Bft) (optional), radiation (W m⁻²) (optional), temperature (°C) (optional), extreme events (particularly intense, frequent and/or long storms, heat/cold periods or precipitation events) (optional) <p>Water sampling:</p> <ul style="list-style-type: none"> For moving waters: Water flow (m³ s⁻¹) and velocity (m s⁻¹) For standing waters: vertical temperature profile Conductivity (µS cm⁻¹) Algal biomass density (mg L⁻¹) (optional) Suspended solids concentration (mg L⁻¹) (or turbidity) (optional) Granulometry of suspended solids (optional) <p>Sediment sampling:</p> <ul style="list-style-type: none"> Sediment dry weight and density (matrix-related parameters) Organic carbon fraction (optional) Granulometry (optional)

Aspect	Recommendation
Sample storage and transport	<ul style="list-style-type: none"> We strongly recommend to use plastic-free materials like glass jars or metal boxes and store samples in the dark. Water samples should be stored at 4 °C or below, sediment samples should be stored at +4 °C or frozen at -20 °C.
Contamination prevention in the field and in the laboratory	<ul style="list-style-type: none"> We recommend to avoid equipment, material or textiles (including clothing of persons present during sampling) containing plastic or plastic components. We recommend to work clean, fast and accurate to minimise aerial contamination. Samples and open equipment (e.g. outlets, tube openings) that will be in contact with the sample should always be covered with aluminium foil, cork, wood or other non-plastic alternatives. All equipment used should be cleaned appropriately in the laboratory before sampling and partially <i>in situ</i> (glass: burning oven 500 °C, etc., net: rinsed with filtered sample water, tap water or reverse osmosis water). In between samples equipment should be cleaned appropriately to prevent inter-sample contamination. However, we recommend to report the sequence of samples to trace back in case accumulation of MPs may occur with sample sequence.
Blanks	<ul style="list-style-type: none"> We recommend the processing of procedural blanks along all steps during the sampling and air contamination blanks, ideally with a replication of N≥3.
Quantitative/qualitative (LOD/LOQ)	<ul style="list-style-type: none"> We recommend to run procedural blanks and to only consider samples with a concentration superior to the concentration in the blank.
Potential of influence by external factors (wind, rain, ...) / dependencies	<ul style="list-style-type: none"> We recommend sampling in dry weather conditions, with low winds, unless another research question is the focus. In urban areas, we recommend to not sample directly after a strong rain event, unless wanted. We recommend to assess the conditions of the sampling location and external influences carefully and plan accordingly.
Other aspects (clogging potential, ...)	<ul style="list-style-type: none"> When the filters or sieves clog, we recommend to exchange the clogged filter with a new one and continue. Analyse all of the sub-sampled filters. For any vertical net sampling, we recommend making a trial to assess the depth from which on clogging does not occur. The presence of biomass and suspended solids in the water phase should be taken into account for any kind of volume-reduces samples.

3.2 Sample processing (MP assessment)

3.2.1 State of the art (Sample processing)

Sample processing (i.e., MP extraction) comprises the separation of MPs from their environmental matrix (water, sediment) and, in particular, the removal of organic and inorganic matter (other than MPs) either physically, chemically, or enzymatically, or as a combination of those. The separation of MPs from the sample is commonly approached by two main steps: density separation and digestion (Figure 6). In a last step, the sample is concentrated for polymer identification and MP characterisation. Different samples (volume-reduced, bulk) from

different matrices require partially different treatment procedures, which themselves can affect the MPs (e.g., melting, size reduction) and thus their identification.

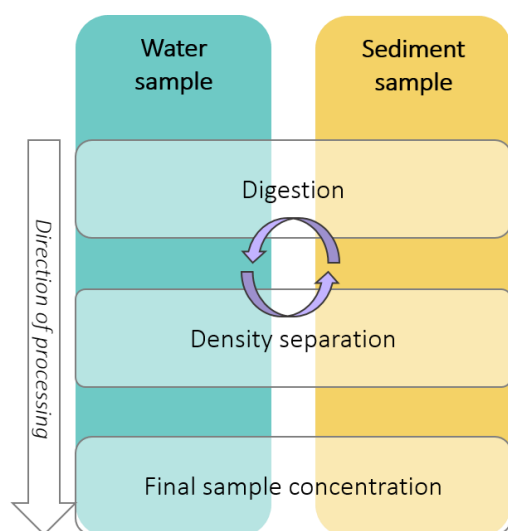


Figure 6. MP sample processing procedures for water and sediment samples. The circled arrow indicates that the order of processing could be switched, according to the sample conditions (i.e. organic and inorganic matter content); own draft.

Sample preparation:

In volume-reduced samples (e.g., net/trawl samples or pre-sieved pump samples), a first size fractionation is given by the nature of the sampling method and sometimes also for bulk samples *in situ* sieving steps are included in the sampling strategy. Bulk or volume-reduced water samples are mainly in a liquid phase, containing either sampling water and/or rinsing fluid from collecting MPs from the net/trawl cod-end. To remove the liquid phase and/or fractionate the samples in MP size categories, samples can be primarily sieved or filtered on pre-cleaned stainless steel meshes or glass fibre (GF) filters of different mesh/pore sizes using ideally a glass or stainless-steel filtration unit (Löder et al. 2017). The concentrated and/or fractionated samples can now undergo further digestion and/or density separation. Very clean water samples, like tap water, may be directly filtered for polymer identification) without further processing (Stock et al. 2019).

Sediment samples from sediment grab sampling usually contain water (often rich in organic matter content) that can be expressed as water content (%) and can be wet-sieved (Scherer et al. 2020). Even if the samples have been undergoing *in situ* sieving (volume reduced sample), the sample may still contain some sample and/or rinsing fluid. Sediment core samples especially may have a standing water column on top of the sediment and water can be removed either *in-situ* or in the laboratory through suction using a vacuum pump and a tube inserted into the column and subsequently/or through overflow using a sediment core slice apparatus (often plastic-containing material) to press the matrix column up until the sediment surface. The sediment core matrix can be collected as a bulk sample in a vessel or different sediment layers can be collected by slicing. If samples contain a larger content of inorganic and/or organic matter (especially sediment samples), the sample may be drained only, carefully restraining most of the matter in the vessel. The remaining matter in the vessel may undergo a density separation and/or digestion step before further filtration.

Sample processing - digestion

The main principle of digestion is the destruction of natural organic matter by energy transfer through chemical reactions using chemicals and especially enzymes. Frequently used enzymes are cellulase (for cellulose), lipase (for fats), chitinase (for chitin) or proteinase or protease (for proteins) (Löder et al. 2017). Enzymatic digestion requires time (several days), elevated temperature, and suitable pH conditions to reach optimal efficiency and enzymatic activity. The appropriate combination of enzymes to be applied, depends on the organic sample content. Enzymatic digestion finds application often in samples containing high organic materials, e.g. WWTP effluents or river samples with high organic load, and bottom sediments. However, water samples after a rain event (and increased surface runoff or combined wastewater sewer system overflow) may contain also slowly biodegradable matter (e.g., particulate solids) (How et al. 2020) and inorganic matter (e.g., mineral particles) (Galfi et al. 2017). Enzymatic and chemical digestions are complementary and different digestions can be performed

subsequently, to destroy all kinds of organic matter in the sample. Simon (2018) used, for example, cellulase enzymatic digestion followed by hydrogen peroxide (H_2O_2) oxidation on WWTP samples. A comprehensive modular approach based on an enzymatic-oxidative treatment has also been suggested by Löder et al. (2017) for the purification of water, wastewater, and sediment samples.

Chemical digestions are done by oxidation (H_2O_2 , Fenton reaction: H_2O_2 + ferrous sulfate [FeSO_4]), or using acidic solutions (nitric acid [HNO_3], hydrochloric acid [HCl]) or alkaline solutions (sodium hydroxide [NaOH], potassium hydroxide [KOH]). The chemical solution is prepared to the desired concentration (mol L^{-1}) and added to the sample. Most chemical digestions last several hours to days. Most commonly, samples undergo chemical digestion in 1-2 days, to minimise chemical reaction risk on the MP particles as several polymers might be affected by strong and long chemical treatment (Löder et al. 2017; Treilles et al. 2020). Hereby, the chemical, concentration, exposure time and temperature play major roles on the performance and the effect on MP characteristics (e.g. reduction in dimension, tenacity or infra-red spectra) for certain polymer types (Treilles et al. 2020).

Sample processing – density separation:

Density separation by flotation with chemical solutions or flow-induced (elutriation) is used for both, water and sediment, samples. The principle of density separation is to allow low-density materials such as MPs (ranging between $0.9\text{--}2.1\text{ g cm}^{-3}$) to float (buoyancy) and high-density mineral matter to sink down. Therefore, the solution should have a higher density than the MP polymers to be extracted. Another alternative is oil extraction, where the remaining oil traces are removed using ethanol afterwards (Ball, H et al. 2019; Stock et al. 2019)

For sediment samples, the separation of the inorganic matrix is often gravity-based using elutriation (Claessens et al. 2013) or devices like the Munich Plastic Sediment Separator [MPSS] or equivalent with addition of zinc chloride (ZnCl_2) solution (Imhof et al. 2012; Ivleva et al. 2017). In general, the sample is mixed with a separation solution, such as sodium chloride (NaCl), sodium bromide (NaBr) or sodium polytungstate (Na_2WO_4), in a separating funnel, MPSS or glass apparatus and let to settle for typically 24 hours, then the supernatant containing the floating fraction is collected for further analysis (Hidalgo-Ruz et al. 2012).

Different separation solutions are more effective for certain polymer types and some can even impair MPs due to the chemical properties. The recovery rate is the efficiency of the separation to recover MPs in the lightweight fraction and good separation processes achieve around 90-100 %. The techniques achieve different recovery rates for different polymer types due to the polymer and solution density properties (see section 7.1.4 Table 9).

New developments for separating MPs from matrix are coming, such as the hydrophobicity-based microplastics separator (μSEP) (Renner et al. 2020) or electrostatic separation (so far used only for larger MPs), which is based on electrical charge of particles and the time needed for discharge. As the polymer discharge time is low, MPs can be separated from the matrix, and although high recovery rates are promising, efficient application on heterogenic natural samples and small particles is difficult (Nguyen et al. 2019).

3.2.2 QA/QC and discussion (Sample processing)

Regarding digestion, the use of strong acidic, alkaline or oxidative chemicals in the above-mentioned procedures can cause degradation, melting and discoloration of MP particles and should be considered carefully (Treilles et al. 2020). Problems for MPs occur for acidic digestion: melting, destruction (above $60\text{ }^\circ\text{C}$), yellowing; and alkali digestion: discoloration and degradation. For a good quality and efficiency of the removal of organic from the MP samples multi-step treatments (modular approaches), such as the application of proteinase K and H_2O_2 to degrade biofilms and organic material or Fenton reaction to remove organic compounds, are often recommended (Löder et al. 2017; Mai et al. 2018). The combination of several methods have been tested and were partially really efficient. A comprehensive modular approach based on an enzymatic-oxidative treatment has been suggested by Löder et al. (2017) for the purification of samples from various environmental matrices, and is often used (Karlsson et al. 2020; Ruggero et al. 2020). For density separation procedures, care has to be taken to choose a suitable separation solution and the different density properties of the potential MP types. Agglomerates of MPs with organic (e.g. biofouling) and/or inorganic matter can cause false density separation as the particle density of the agglomerate might differ significantly from the pure MP particle density (Chubarenko et al. 2016). An appropriate combination or repetition of digestion and density separation could be useful to avoid that. Moreover, when assessing different polymers of different density properties (additives in polymers

might also change the density properties (Chubarenko et al. 2016)), additional subsequent density separations have to be carried out on the settled particle fraction from the previous separation.

Size fractionation of MPs can take place at various moments during the sample processing using sieves or filters of different mesh sizes. Whereas relatively clean water samples may be fractionated before sample processing, it should be considered (in all samples) that biofilm or algae growth on MP particles and agglomerates with other organic and inorganic matter could distort the actual MP particle size and cause false fractionation. This could even lead to the loss of MP particles smaller than in the targeted fraction during further processing when organic and inorganic matter is finally removed and the MP particles are purified.

During sample processing, the samples are exposed to different environmental conditions (high temperatures, air, chemicals), handled by different people, and are in contact with various materials in different steps during certain amounts of time (from seconds to days). Therefore, the sample processing is one of the most crucial parts to minimise the risk of contamination. Sample handling, filtration and other processes take ideally place under laminar flow cabinet. Otherwise samples should be kept covered always. Laboratory surfaces are recommended to be cleaned prior to analysis as are materials to be used (e.g. tweezers). For pre-cleaning equipment, the stainless-steel filters can be muffled at 500 °C for 2-3 hours or rinsed with filtered reverse osmosed water, filtered ethanol and again with filtered reverse osmosed water and undergo ultrasonification in the rinsed solution, before being rinsed again with filtered reverse osmosed water. GF filters are pre-cleaned by filtration with filtered reverse osmosed water and subsequent burning in the muffle oven at 500 °C for 2-3 hours. All equipment (vessels, filtration units, etc.) should be clean (rinsing with HNO₃ solution, or intensive rinsing with filtered or ultrapure water and dried) and some studies recommend to filter all solutions prior to use on 1 µm (Frias et al. 2018) or 0.2 µm (Wendt-Potthoff et al. 2017). When transferring the sample onto a filter or to another vessel, the vessel that contained the sample should be rinsed with filtered reverse osmosed water, filtered ethanol and again with filtered reverse osmosed water to collect all MPs from the sample.

The processing of replicate procedural blanks in parallel to the preparation of the samples are imperative to determine the grade of contamination. Additional air filtration can give an idea of potential airborne contamination. Koelmans et al. (2019) recommend the avoidance of synthetic textiles for lab clothes. Blanks should be analysed similarly to the samples. If the MP concentration of a polymer in the sample is less than the concentration in the corresponding procedural blank (LOQ), then the respective MP polymer is not included in the data. More generally the mean blank concentration has to be subtracted from the sample concentration. In sense of a conservative approach, mean blank values can be rounded up to the next integer prior to subtraction. Positive controls, i.e. spiking samples with known number and type of polymers, are suggested to estimate losses or analytical uncertainties/errors and determine recovery rates during all steps of processing MPs (Müller et al. 2020).

3.2.3 Recommendations for SOPs (Sample processing)

Table 4. Aspects and recommendations for SOP of sample processing.

Aspect	Recommendation
Particle size range (adaptation)	<ul style="list-style-type: none"> Depending on scientific question, targeted MP type and range of size classes, and sampling method used, as well as on laboratory equipment. We recommend a comprehensive coverage of MP sizes (LOD-5000 µm) and to categorise as following: LOD-10 µm, 10-100 µm, 100-300 µm, 300-1000 µm, 1000-5000 µm.
Procedures	<ul style="list-style-type: none"> We recommend size fractionation at the end of the sample processing, when all organic and inorganic matter has been removed and MP particles are pure. Succession of organic matter degradation and density separation steps, with blanks run in parallel. We recommend to develop a suitable strategy for sample processing based on the sample type and content and combine density separation and digestion steps in a manner to achieve optimal results and minimal effects on the MPs. We recommend to keep chemical processes as long as necessary and as short as possible, and therefore we recommend to follow established processes or

Aspect	Recommendation
	<p>prior to processing test the efficiency and error ranges of the methods. In this context, we recommend to publish detailed processing protocols (SOPs for sample processing).</p> <ul style="list-style-type: none"> We recommend to keep the exposure of MPs to higher temperatures as low as possible and do not exceed temperature 60 °C. Reactions involving oxidations should be temperature controlled (cooling) due to the exothermic reactions. We strongly recommend, to work to the actual health and safety rules of the executive laboratory.
Equipment	<ul style="list-style-type: none"> We strongly recommend to avoid equipment, material or textiles (including clothing of persons present during sampling) containing plastic or plastic components. Ideally stainless-steel or glass materials are used, or exotic polymers like polytetrafluoroethylene (PTFE) which are rarely found in the environment and can be excluded from the analysis.
Use of toxic chemicals	<ul style="list-style-type: none"> Toxic or hazardous chemicals should be avoided, and if used their consumption shall be kept to a minimum.
Sustainability	<ul style="list-style-type: none"> We recommend to re-use or recycle chemicals and material if possible and in general their consumption shall be kept to a minimum.
Contamination potential	<ul style="list-style-type: none"> We recommend to avoid equipment, material or textiles (including clothing of persons present during sampling) containing plastic or plastic components. Ideally stainless-steel or glass materials are used, or exotic polymers like polytetrafluoroethylene (PTFE) which are rarely found in the environment and can be excluded from the analysis. Samples need to be covered whenever possible and we recommend to work clean, fast and accurate, to minimise aerial contamination. In this context, the use of laminar flow boxes during sample processing is highly recommended. Samples and open equipment (e.g. outlets, openings) that will be in contact with the sample should always be covered with aluminium foil, cork, wood or other non-plastic alternatives. All equipment used should be cleaned appropriately before sampling in the laboratory and partially <i>in situ</i> (glass: burning oven 500 °C or HNO₃-rinsed, others: three times rinsing with filtered tap water, filtered ethanol and filtered reverse osmosis water). In between samples equipment should be cleaned appropriately to prevent inter-sample contamination. However, we recommend to report the sequence of samples to trace back in case accumulation of MPs may occur with sample sequence. We recommend to store chemicals in clean glass containers with non-plastic lids. However, if chemical or solutions are stored in a plastic canister or in containers with plastic lids, we recommend filtration (0.2 µm) before use to reduce possible MP contamination.
Sample storage and handling	<ul style="list-style-type: none"> We recommend to use plastic-free materials like glass jars and store samples in the dark. Water samples should be stored at 4 °C or below, drained sediment samples at -20 °C. Samples should always be covered with a plastic free lid, aluminium foil or other plastic-free materials.
Quantitative/qualitative (LOD/LOQ)	<ul style="list-style-type: none"> If the concentration of a MP polymer in a sample is less than its concentration in the corresponding blank, then the polymer should not be considered (LOQ). More generally the mean blank concentration has to be subtracted from the sample concentration. However, we recommend to report all samples, also those below the LOQ. See section 3.4 for how to report the data.

Aspect	Recommendation
Other aspects (...)	<ul style="list-style-type: none"> We recommend to document any changes from the SOP and other possible uncertainties. We recommend, in analogy with Table 3 (heterogeneity of sampling site, matrix and measurement of additional parameters), to determine the organic and inorganic matter content of the sample prior to sample processing to allow for the appropriate choice of sample processing strategy, chemicals and enzymes.
Uncertainties	<ul style="list-style-type: none"> When the filters or sieves clog, we recommend to exchange the clogged filter with a new one and continue. Analyse all of the sub-sampled filters. Report results from several filters that were supposed to be one sample as sum of the filters.

3.3 Polymer identification and MP characterization (MP assessment)

3.3.1 State of the art (Polymer identification and MP characterization)

Polymers can be chemically identified (polymer type, e.g. PET) by different spectrometric methods and additionally be characterized by their origin, shape, degradation state, dimensions, size and colour using visual methods (e.g. optical or scanning electron microscopy [SEM]). Shapes of MPs range from fibres (majority), fragments (very common), films and beads to foams, but many more exist (MSFD Technical Subgroup on and Marine Litter 2013).

Visual identification of MPs alone is outdated and problematic (Löder and Gerdt 2015), because precision is limited and polymers cannot be chemically identified. Established analytical techniques for accurate polymer identification are Fourier transform infrared (FTIR) spectrometry (Löder et al. 2015; Thompson 2004), Raman spectrometry, which both can be coupled with microscopy (Frias et al. 2018; Käßler et al. 2016; Krishna et al. 2016), and pyrolysis-gas chromatography coupled to mass spectrometry (Pyr-GC-MS) (Fischer and Scholz-Böttcher 2017; Fries et al. 2013) or Thermal Extraction/Desorption-gas chromatography-mass spectrometry (TED-GC-MS) (Dümichen et al. 2015, 2017; Li et al. 2020; Mai et al. 2018; Ruggero et al. 2020). All these approaches can be seen complementary, whereby FTIR and Raman spectrometry are particle-related analytical approaches and Pyr- or TED-GC-MS are mass-related analytical methods.

Binocular microscope and SEM:

For visual MP characterization, filters or windows containing the (pre-treated) sample or selected particles are inspected under a binocular microscope using different magnification (e.g. x40) (Fries et al. 2013). Using a stereomicroscope enables to determine particles until μm size (e.g. $10\ \mu\text{m}$ (Barrows et al. 2017)), however misinterpretation can occur (Löder and Gerdt 2015). They may be sorted with tweezers, counted (more suitable for larger MPs) and colour, shape and dimensions can be determined. Generally, colours of MPs are determined as blue, black, transparent, white, red, green and others (Li et al. 2020). The polymer type, however cannot be specified using this method (Li et al. 2018; Müller et al. 2020). Dimensions can be measured using imaging software and/or using filter grid lines as reference (Barrows et al. 2017). Transparent and white particles should be inspected using fluorescence microscopy (Hidalgo-Ruz et al. 2012). SEM enables to inspect the morphology of MP particles to a very high resolution, and hence degradation patterns may be determined (Fries et al. 2013).

Staining combined with fluorescence/UV microscopy:

Tagging (or staining) MPs with a staining agent such as Nile red and subsequent analysis under fluorescence microscope can be used for some common polymer types as PP, PE, PS, nylon, PUR and in lesser quality also for PET and PVC (Ruggero et al. 2020), and has been found accurately applicable for MPs in larger size classes (e.g. $0.63\ \text{mm}$) (Hengstmann and Fischer 2019). Different staining colours are available: green, blue, orange or red (Ruggero et al. 2020). The sample should be on a Polycarbonate (PC) membrane to avoid background fluorescence. Staining agents are then applied on the sample, covered and left to incubate in the dark under elevated temperature for 10 minutes. Particles can be counted and dimensions may be assessed. Staining agents

are not specific for certain MPs and false positives (detection of other particles (non-polymers)) can occur. The efficiency of this method is still questioned (Ruggero et al. 2020).

FTIR and μ FTIR spectrometry:

With FTIR and, when FTIR is coupled to a microscopy (μ FTIR), the IR spectra of particles are identified using attenuated total reflection (ATR), and reflectance or transmission mode. The principle of FTIR is based on the energy absorption of chemical bonds in a particle during radiation with IR light (commonly mid-IR range 5000-400 cm^{-1}) due to molecular vibrations. The spectra (usually measured by wavenumber [4000 – 600 cm^{-1}] versus absorbance) can be compared to reference spectra in a database to identify the exact polymer type or polymer composition (Mai et al. 2018). (μ)FTIR spectrometry is often used to complement visual analysis but can also - and is more and more - be used alone. This method requires the dry sample on a filter, a transmission glass or a reflection glass window. Particles $<20\text{ }\mu\text{m}$ and colourless particles are often not suitable for (μ)FTIR as they could lack absorbance. ATR is suitable for larger particles ($>500\text{ }\mu\text{m}$) (Mai et al. 2018). Manually, the filter can be mapped obtaining images and IR spectra of the mapped area. Additional automated (μ)FTIR features are Focal Plane Arrays (FPA), which enable fast IR imaging of a full glass window or filter area and can be used for small particles down to $10\mu\text{m}$ (FPA- μ FTIR) (Löder et al. 2015; Löder and Gerdts 2015; Mai et al. 2018; Simon et al. 2018). FTIR imaging and microscope-coupled FTIR also enables to acquire and analyse pictures of the samples to characterise the particles (shape, dimensions, colour). White and transparent MP particles, surface irregularities, and particles $<20\text{ }\mu\text{m}$, as well as water (interference due to its absorbance) and other organic materials are problematic for FTIR analysis, but fluorogenic and coloured (problems occur only with the colour black due to very high IR absorbance) materials are suitable.

Raman and μ Raman spectroscopy:

Raman spectroscopy uses the principle of exciting molecules with monochromatic light photons of a certain laser wavelength (500-800 nm (Löder and Gerdts 2015)) and detecting back-scattering light (stokes and anti-stokes) as a spectrum (for polymers ranging between 150-3400 cm^{-1} (Müller et al. 2020)), which is specific due to vibration of chemical bonds in the particles⁶. For this principle, the molecules need to have a chemical bond to be excited (polarised) (Krishna et al. 2016; Li et al. 2018) It can be used (as hybrid technique with a micro-spectrometer) to identify very small particles (μ Raman) down to $1\text{ }\mu\text{m}$ because the analysis area is small (μm range). This technique can be coupled to a microscope, known as Raman microscopy (hybrid technique), which allows point specific analysis and determining spatial distribution (mapping) with spectral imaging (e.g. raster scanning). μ Raman is fast, compared to FTIR, and has good spectral resolution (3–9 cm^{-1}) and spatial resolution (μm -range) (Krishna et al. 2016; Müller et al. 2020). Similar to FTIR, by using image and microscopy of the Raman technique, pictures of the samples can be taken to further characterise the MP particles (shape, dimensions, etc.). Interferences can occur with fluorogenic particles or background fluorescence. When combined with confocal laser-scanning one can investigate polymers three-dimensional or inside tissues (Löder and Gerdts 2015).

GC-MS-coupled methods:

Pyr-GC-MS

The principle of Pyr-GC-MS is based on fast thermal degradation of the sample under pressure (pyrolysis) followed by detection of pyrolysis products of polymers in the GS-MS (Dümichen et al. 2015). Hereby, the measured pyrolysis products give information about the concentration, molecular composition and structure, of polymer compounds present in the sample (Gomiero et al. 2019). The method is destructive and only used for polymer identification (including analysis of organic additives) and mass quantification (Fries et al. 2013; Gomiero et al. 2019; Kirstein et al. 2021). This method originally requires the feed of a single particle with a specific maximal mass and size (determined by thermal desorption tube diameter) into the instrument and therefore is commonly applied for larger MPs ($>500\text{ }\mu\text{m}$) after visual sorting (Fries et al. 2013; Li et al. 2018; Löder and Gerdts 2015), although studies determining MP down to nano-size and $1\text{ }\mu\text{g}$ weight using Pyr-GC-MS are evolving and promising (Hermabessiere et al. 2018; Müller et al. 2020; Ter Halle et al. 2017). Moreover, Fischer and Scholz-Böttcher (2017) developed a Pyr-GC-MS approach that facilitates the measurements of a MP sample filter after purification.

TED-GC-MS:

⁶ <https://www.thermofisher.com/>

The principle of TED-GC-MS is thermal degradation of the sample and subsequent composition analysis of the gaseous decomposition products in the GC-MS ion chromatograms (Dümichen et al. 2015). TED-GC-MS allows even for the analysis of untreated MP samples, however, this technique is also limited to the measurement of a maximum sample amount of 20 mg (Dümichen et al. 2017; Elert et al. 2017). The total mass per polymer type can be determined by the weight percent of certain decomposition products specific to polymer type (calibration required) and is reported per unit sample mass.

Polymer identification with reference databases:

The final step of polymer identification includes the use of reference databases. Different studies use different databases for the evaluation of the potential MP particle spectra or GC-MS ion chromatogram peaks. However, the MP spectra can deviate for different degradation states of the polymer, thus this need to be taken into account during identification of polymers and the development of databases. The identification of MP degradation state could successfully be determined using Raman spectroscopy, but cannot be determined using GC-MS coupled methods (Elert et al. 2017). The exhaustive and time consuming manual analysis of large FTIR imaging data sets is meanwhile extremely facilitated by the availability of automated MP identification software, such as SiMPle⁷, and sometimes also self-established ones. In this context, the application of Random Decision Forest Classifier-based automated MP analysis is much faster than currently available alternative which are based on spectral comparisons and thus highly promising (Hufnagl et al. 2019). Most frequent polymer types detected in freshwaters are: polypropylene (PP), polyethylene (PE), polystyrene (PS), polyethylene terephthalate (PET), polyamide (PA), polyvinyl chloride (PVC), poly urethane (PU), with most identifications of PP and PE (Li et al. 2020).

3.3.2 QA/QC and discussion (Polymer identification and MP characterization)

Chemical polymer characterization is absolutely necessary and pure visual sorting and counting is outdated (Löder and Gerdtz 2015). Visual sorting under the binocular microscope can be hampered when MP particles are attached to inorganic or organic matter or when the sample still contains many particles other than MPs (non-purified sample) (Barrows et al. 2017). However, Scherer et al. (2020) found that visual MP analysis (numerical) is comparable to Pyr-GC-MS analysis (mass-based concentration) for quantification of MP particles (125-5000 µm) from sediment samples and in a comparability study from Müller et al. (2020) visual sorting for determining polymer total particle number scored best, followed by µFTIR, even for small MPs down to 2 µm. Raman spectroscopy can be well used to determine the degradation state of a MP particle, while this is less suitable with FTIR spectroscopy (Elert et al. 2017). The smallest size fraction of MP particles that can be determined in a sample (LOD) depends first on the sampling and sample processing methodology and further on the polymer identification methodology (Hidalgo-Ruz et al. 2012). FTIR methods can only process MPs >10 µm, while Raman methods can analyse down to 1 µm. Even though GC-MS-coupled methods are limited in maximum particle size and/or mass, manual downsizing of particles can be done.

Crucial aspects for Raman and FTIR spectroscopic approaches are different instrumental settings and measurement limitations. FTIR is rather fast, popular for MP assessments, versatile in its application, and very accurate. But very small particles (below 10 µm) can not reliably be analysed, transmission mode is not applicable for thick, bulky particles, and the compounds to be identified need to have a bipolar moment (Krishna et al. 2016; Li et al. 2018). Raman spectroscopy has a good spatial resolution and particles in down to 1 µm can be measured. Raman can be combined with confocal analysis, and water does not hamper analysis, but fluorescence, and this method is more time consuming than FTIR (Löder and Gerdtz 2015). Both techniques, especially when used in combination with microscopy, require thoughtful and reasonable configurations: utilisation of accessories (e.g., light source, sample background), instrument settings (e.g., magnification, aperture), measurement mode (reflectance, transmission, ATR), background calibration, program settings (scan area, number of scans, resolution).

One important distinction between methods is the feasibility and sample through-put capacity. The methods differ in their processing time so that for µFTIR spectrometry (transmission mode) is depending on the scanning area and the objective used. For example, for a 10 mm diameter window scanned with a 15 × objective using FPA-FTIR, time needed is about 4.5 hours. GC-MS-coupled methods are well established for measurements of organics

⁷ <https://simple-plastics.eu/about.html>

in general and sample analysis is rather fast, but limited to one particle per measurement (Elert et al. 2017; Müller et al. 2020). A single shot and double shot analysis with could take 18 and 36 minutes, respectively (Vollertsen J., Liu F., and Molazadeh M., 2020, pers. comm.). Another advantage of gravimetric-based methods (GC-MS-coupled) is that other organic additives from a MP particle can be identified as well (Mai et al. 2018).

Using self-established databases for polymer identification can, as it was the case in a study in the river Elbe by (Scherer et al. 2020), resulted in misinterpretations or non-detectability of polymer types when potential cross-linked polymers are not included in the reference database. FTIR or Raman spectral analysis should use comprehensive, extensive and well-managed and updated databases, mentioning that open access solutions and the opportunity of steady (controlled) optimization by users are key to establish better and standardized MP identification.

Given the large variety of polymers, the characteristics of MPs found in the environment, and the methodological limitations, no single identification methods (from those discussed in this report) can generate a full dataset on MP polymer identification and characterisation solely. Therefore, a thoughtful combination of complementary methods should be implemented, depending on the scientific question and study design, to obtain a good quality and comparability of the results.

3.3.3 Recommendations for SOPs (Polymer identification and MP characterization)

Table 5. Aspects and recommendation for SOPs for polymer identification and MP characterisation

Aspect	Recommendation
Particle size range	Binocular microscope: <ul style="list-style-type: none"> • >500 µm FTIR spectrometer (ATR mode): <ul style="list-style-type: none"> • >500 µm µFTIR spectrometer (transmission/reflectance mode): <ul style="list-style-type: none"> • 10-500 µm (lower limit depends on the instrument). µRaman spectrometer: <ul style="list-style-type: none"> • ≥1 µm Pyr-GC-MS: <ul style="list-style-type: none"> • Not applicable, the total mass of the polymer needs to be >LOD for detection >LOQ for quantification.
MP characterisation	<p>We recommend to assess, if possible: polymer type, MP particle size, particle numbers, concentration per sample unit, MP particle colours, shapes, major and minor dimensions, mass (if available or extrapolated), origin and degradation status.</p> <p>See section 3.4.3 Quantification and data reporting for more details.</p>
Units for Particle assessment	Binocular microscope: <ul style="list-style-type: none"> • Number of suspected MPs, should always be confirmed with spectrometry FTIR spectrometer (ATR mode): <ul style="list-style-type: none"> • Number of confirmed MPs µFTIR spectrometer (transmission/reflectance mode): <ul style="list-style-type: none"> • Number of confirmed MPs µRaman Spectrometer: <ul style="list-style-type: none"> • Number of confirmed MPs Pyr-GC-MS: <ul style="list-style-type: none"> • Mass of each polymer particle.
Method and procedure	Binocular microscope:

Aspect	Recommendation
	<ul style="list-style-type: none"> For large particles, visual inspection can allow to easily remove obviously natural particles. All other particles are considered as suspected MPs and need to be further analysed with a spectrometric method after photo-documentation. <p>FTIR spectrometer (ATR mode):</p> <ul style="list-style-type: none"> Particles are handled manually with tweezers and analysed one by one in the ATR mode. <p>µFTIR spectrometer (transmission/reflectance mode):</p> <ul style="list-style-type: none"> The sample (or a sub-sample if too much particles are present) is filtered or concentrated in a very small volume (µL-mL) on an adequate material (with no interference with IR light within the wavelengths of interest, e.g. aluminium oxide or silicon filter, or zinc selenide (ZnSe) window). The sample is then fully analysed (IR mapping) in order to confirm the nature of all particles. <p>µRaman spectrometer:</p> <ul style="list-style-type: none"> The (sub-)sample needs to be filtered on an adequate small filter (the less time is required for mapping a small area) or window (avoiding background fluorescence, e.g. anodisc filter). As the spatial resolution of the µRaman spectrometry is high, a full spatial mapping is time-consuming. The mapping of a small surface should be considered. It is possible to use an automated visual mapping (spectral image mapping) and detection of all particles, followed by direct identification of these particles. <p>Pyr-GC-MS:</p> <ul style="list-style-type: none"> The subsamples need to be filtered on an adequate filter suitable for pyrolysis and can be fully analysed with Pyr-GC-MS afterwards. After introduction of the full mass of the sample or a sub-sample in the pyrolysis cup, the different types of polymers are easily pyrolysed. The generated pyrolysis products are characterized by specific ions (m/z) and retention time. For qualitative approach, calibration (internal or external) is required. Up to date, only external calibration (i.e. using virgin polymer) are used. Since this kind of calibration does not account for the matrix effect, high uncertainties on mass assessment are expected.
Settings	<p>Binocular microscope:</p> <ul style="list-style-type: none"> 40x magnification or preferred, using grid allows for dimension reference. <p>FTIR spectrometer (ATR mode):</p> <ul style="list-style-type: none"> The spectral range measured is usually 4000-400 cm⁻¹. A total number of 8 scans minimum is required. It is recommended to use 16 scans and a spectral resolution of 4-8 cm⁻¹. <p>µFTIR spectrometer (transmission/reflectance mode):</p> <ul style="list-style-type: none"> The spectral range measured for µFTIR imaging of filters should cover as much as possible from the range 4000-400 cm⁻¹; However filter materials like aluminium oxide limit the range to 1250 cm⁻¹ at the lower wavenumber range. The number of recommended scans depends on the instrument and goes from 1 scan to 64 scans. We advise to test whether it is possible to decrease the number of scans without losing information and spectral quality (the same number of MPs is found without a decrease in the matching factor). A spectral resolution of 4-8 cm⁻¹ is recommended. <p>µRaman Spectrometer:</p>

Aspect	Recommendation
	<ul style="list-style-type: none"> The spectral range for the laser wavelength 785 nm should cover 50-3940 cm^{-1}, for laser wavelength 532 nm cover spectral range of 50-4000 cm^{-1}. Objectives range between x10-x100 and should be aligned to the desired spatial resolution. Adjust settings to limit fluorescence and increase spectral quality (Hermabessiere et al. 2018) <p>Pyr-GC-MS:</p> <ul style="list-style-type: none"> Pyrolysis can be performed at different temperatures depending on the devices. Specific ions and retention time for each polymer should be confirmed. Calibration curves should be performed in the same conditions as the real samples. Optimized working parameters recommended: thermal desorption (first-shot) at 100-300 °C with a rate of 50 °C min^{-1}; 600 °C for the second shot (pyrolysis) with a split ratio of 1:10 and an injection temperature of 300 °C .
Data processing	<p>Binocular microscope:</p> <ul style="list-style-type: none"> Visual observations of the MP particle characteristics (such as colour, dimensions (e.g. measurements using ImageJ software), shape) should be documented (e.g. using an Excel table), photographed (microscope camera) and statistically and/or graphically analysed. <p>FTIR spectrometer (ATR mode):</p> <ul style="list-style-type: none"> Using self-made or custom IR spectral databases (siMPle®) for evaluation of the sample spectra. <p>µFTIR spectrometer (transmission/reflectance mode):</p> <ul style="list-style-type: none"> Using open access software like siMPle® or commercial products like purity microplastics finder for automated analysis of large imaging data sets. <p>µRaman spectrometer:</p> <ul style="list-style-type: none"> Using open access software or commercial products for automated analysis of large imaging data sets, e.g. OMNIC® <p>Pyr-GC-MS:</p> <ul style="list-style-type: none"> Using open access software or commercial products for automated analysis of large imaging data sets, e.g. Agilent Chemstation®
Contamination potential	<ul style="list-style-type: none"> We recommend to avoid equipment, material or textiles (including clothing of persons present during sampling) containing plastic or plastic components. We recommend to work clean, fast and accurate to minimise aerial contamination (installation of analysis machines in clean rooms has to be considered) and to cover samples whenever possible. Samples and open equipment (e.g. outlets, openings) that will be in contact with the sample should always be covered with aluminium foil, cork, wood or other non-plastic alternatives.
Uncertainties and drawbacks	<p>Binocular microscope:</p> <ul style="list-style-type: none"> Limited determination of MP characteristics and no polymer identification possible. <p>FTIR spectrometer (ATR mode):</p> <ul style="list-style-type: none"> Due to the fact that ATR- FTIR is a surface technique, impurities at the MP particle surface lead to bad spectra. In such cases, we recommend to gently cleaned the MP particle surface with alcohol and cotton sticks and repeated the measurement afterwards. Compare the results.

Aspect	Recommendation
	<p>μFTIR spectrometer (transmission/reflectance mode):</p> <ul style="list-style-type: none"> • Impurities on the sample filters/windows can lead to a spectral overlay from MPs and matrix. • Problems for the spectra identification can occur with white, transparent, or black MPs, and sample impurities. • Difficulties in identification with automated software tools and thus loss of particles from the analysis. Thus, the sample needs to be as clean as possible (purified) and the filters/windows should have one distinct layer of single particles, which often implies splitting the sample on several filters/windows. <p>μRaman spectrometer:</p> <ul style="list-style-type: none"> • Total particle count could be questionable. • Interferences with fluorescence and polymer additives (e.g. dyes). • Time-consuming when mapping larger areas <p>Pyr-GC-MS:</p> <ul style="list-style-type: none"> • No information about other MP characteristics can be obtained. • It is a destructive method. • Only single particles can be analysed.

3.4 Quantification and data reporting (MP assessment)

3.4.1 State of the art (Quantification and data reporting)

Reported by most studies are total number of particles and/or particle concentration, particle size classes, particle shapes, particle minor and major dimensions, polymer type and density, pigment particles, and sometimes particle mass (usually extrapolated). Data is reported often as a combination of the above-mentioned parameters, for example number of particles per polymer type and shape (Eriksen et al. 2018).

For identified polymers, the database used, the date of the matching and the match (%) or Hit Quality Index (HQI) should be reported. Most often reported shapes of MPs are fibres, spheres, foils and fragments (Scherer et al. 2020; Stock et al. 2019). However, different studies report also shapes such as film, foam, pellet, line, bead, flake, sheet, granule, paint or nurdle (Koelmans et al. 2019). Colours can be found in indefinite variety (black, white, transparent, grey, silver, brown, purple, blue, turquoise, green, yellow, orange, pink, red (Scherer et al. 2020), however, blue, red, transparent, white, black, green, yellow, and other are most commonly determined (Bruge et al. 2020; Karlsson et al. 2020).

Commonly, MP particles are measured in their major and minor dimensions using microscope imaging (Kirstein et al. 2021) or directly under the microscope. The spatial resolution of the microscope (LOD) of the microscope set-up is the limiting factor for measuring dimensions. Based on these measurements, the polymer densities and certain assumptions about the particle geometrical shapes, further calculations can be performed and so the polymer mass in the sample estimated (Bruge et al. 2020; Simon et al. 2018). The major and minor dimensions of MP particles can be defined as the “(...) longest continuous axis in the centre of the particle, (...)” and the “(...) longest axis perpendicular to the major axis.”, respectively (Simon et al. 2018). Further assumptions need to be made in order to generalise the MP shapes, as it is not yet feasible to precisely define each single MP particle. Therefore, the ratio of the thickness to the minor dimension is assumed to be equal to the ratio of major to minor dimension. Moreover, the shape for most MP particles is presumed ellipsoid, while for fibres the shape is presumed cylindrical (Kirstein et al. 2021).

The volume is calculated using the dimension measures according to the presumed shape. To calculate the mass of MPs sampled, the density of different polymer types according to general databases is used. The mass can then be calculated via multiplying polymer density per summed up volume of the particles of the respective polymer (Simon et al. 2018). When using GC-MS coupled methods the particle mass can be calculated by comparing the mass-based results to calibration curves of standardized polymers (Kirstein et al. 2021).

3.4.2 QA/QC and discussion (Quantification and data reporting)

Due to the differences in scientific question and subsequently differently targeted MP characteristics to be investigated studies report the data in various ways. Reviews, such as Hidalgo-Ruz et al. (2012; Koelmans et al. (2019) or Stock et al. (2019), mention that the variety of shapes and other MP characteristics lead to partial miscalculations, as different shapes can have significantly different volume to surface ratios and therefore can have quite different masses and settling behaviours.

The determination of the MP colour also depends on the analytical equipment used and precise identification of the colour can be hampered by sample processing and discoloration effects due to the use of certain chemicals (Ruggero et al. 2020). The particles of each polymer type should be sorted into size categories as mentioned before (section 3.3.3) according to their maximum dimension and numbers normalized to sampling unit (m³ or kg dry weight). The same can be done with shape parameters or colour.

The MP particle concentration per sample is estimated by the mean of all analysed MP particles and has to be mentioned with its uncertainty interval (Brüge et al. 2020). Three or more replicates are necessary to be analysed. When less than 10 concentration values are available non parametric statistics have to be used (median and box-and-whisker plots). When more than 10 concentration values are available, the mean and standard deviation can be used (Brüge et al. 2020).

MP particle number and MP concentration should be reported for different size classes and polymer or shape types and the total MP per sample. In general, we recommend to provide a very detailed supplementary with as much detailed quantified information as possible about the MP characteristics for all the different combination (e.g. colour per size class, MP particle number, mass per sample, polymer type, and so on). Those values should be reported ideally without the blanks subtracted, but with blank results reported accordingly. In the best case this list contains every single MP particle with an ID and its respective characteristics.

3.4.3 Recommendations for SOPs (Quantification and data reporting)

Table 6. Aspects and recommendation for MP quantification and data reporting

Aspect	Recommendation
MP particle size range	<ul style="list-style-type: none"> We recommend to report the MP size classes (e.g. LOD-10 µm, 10-300 µm, etc.) that were determined, as well as to report the following six MP characteristics per size category (i.e. particle number, concentration, colours, dimensions, shapes, mass)
MP particle numbers	<ul style="list-style-type: none"> Particle number and MP concentration should be reported for different size classes, polymer types, shapes, etc. and the total MP per sample.
MP concentration	<ul style="list-style-type: none"> We recommend to report the MP concentration (abundance) data per sample volume or dry mass.
MP colours	<ul style="list-style-type: none"> We recommend to report the MPs colour distribution among the recommended size classes, shapes and polymer types.
MP dimensions	<ul style="list-style-type: none"> We recommend to report the MPs major and minor dimensions.
MP shapes	<ul style="list-style-type: none"> We recommend to report the MP shapes that were determined. Shapes to be covered should ideally be all shapes differentiated as detailed as possible. Moreover, we recommend to report minimum: fibres, fragments, spheres, foils, and 'others'.
MP mass	<ul style="list-style-type: none"> We recommend to report the MPs mass either from Pyr-GC-MS methodology or from weighing (might only be applicable for large MPs) or by calculations. We recommend to always report and explain all formula and assumptions used for transparency.
MP degradation	<ul style="list-style-type: none"> We recommend to report the MPs degradation state, if possible.

Aspect	Recommendation
MP polymer type	<ul style="list-style-type: none"> We recommend to report the database used, the date of the matching, and the match (%) or Hit Quality Index (HQI).
Blanks	<ul style="list-style-type: none"> For clarity, we recommend to report the results of MPs with and without subtraction of blanks and report the results of the blanks in addition. In case blanks are directly subtracted from the samples, the raw data should be included in supplementary materials. This helps to be precise about the LOD achieved.
Uncertainties	<ul style="list-style-type: none"> We recommend to report the uncertainty interval for each sample. Three or more replicates are necessary to be analysed. When less than 10 concentration values are available non parametric statistics have to be used (median and box-and-whisker plots). When more than 10 concentration values are available, the mean and standard deviation can be used (Bruge et al. 2020).
Other aspects (...)	<ul style="list-style-type: none"> Unidentified shapes, colours, particle numbers and more should be reported as “unidentified” or “inconclusive”. More generally, we recommend to report malfunctioning procedures (“No-Gos”), to support better methods validation and development in the scientific community.

4 CONCLUSIONS

Even though standardisations for microplastic (MP) assessment methodologies are not established yet, established standard operating procedures (SOPs) are crucial for the reliability, quality and comparability of research outcomes. This consequently effects the development and application of efficient measures and policy decisions regarding the reduction of MP pollution in the environment. To this end, in this report we reviewed and discussed state-of-the-art methodologies for MP assessment in freshwater systems and derivate recommendations for SOPs taking into consideration the scope of three individual LimnoPlast research projects. The scientific questions of the three LimnoPlast projects deal with MP assessment in the water and the sediment of urban freshwater systems. Despite the different directions in the hypotheses (human exposure to MP, MP abundance, spatial MP distribution), harmonised sampling, sample processing and analytical methods will be used in these projects.

Among the different methods for sampling MP in water or sediment, a suitable combination of several complementary methods can be advised. We recommend the use of nets/trawls for large volume surface water sampling, but the use of a filter-cascade pump for point and water column sampling. Sediments should be collected with plastic-free grabbers or corers if layers are investigated.

For sample processing, we recommend to choose appropriate procedures, and combination/repetition of procedures depending on the sample content (inorganic/organic matter). In general, a digestion and a density separation step should be done followed by MP particle size fractionation in a last step. Chemicals should be used carefully and procedures that are known to be detrimental on MP particles (degradation, melting, etc.) should be avoided and/or modified to be less detrimental (e.g., by lowering concentration, exposure time or temperature).

To comprehensively characterise MPs, and in particular identify polymers, we conclude that a coherent combination of available, complementary techniques (FTIR, Raman, optical microscopy, Pyr-GC-MS) should be favoured. As many research groups are not in possession of all techniques, we encourage to build collaborations for this purpose. Regardless, we recommend μ FTIR as a widely-used, fast and accurate technique, covering a large size range of MPs to be analysed.

Quantification of MPs is limited by the accuracy of measurements. As a recommendation, we advise to define minor and major dimensions of the particles and to calculate potential mass using theoretical polymer type density or use pyr-GC-MS for mass quantification. All data acquired should be reported meticulously in a transparent way using consistent units and supplementary information should be provided, so that the study design and procedures could be understood and repeated by another scientist.

The ultimate choice of the methods and procedures highly depends on the study design, the characteristics of MPs that are needed to answer the research question, the available equipment and more. Regardless, general aspects to allow improved comparability are: 1) ensuring to provide all data necessary to comprehend the results in a transparent way (sampling volumes, MP size classes, etc.) 2) applying good practices along all procedures (reason and evaluate the choice of methods and procedures used, run parallel blanks, assure appropriate technical settings, etc.). Meeting these requirements, will ensure high-quality, transparent and comparable research results. We do not believe, that a full and strict standardisation for MP assessment in the environment is realistic, but we are convinced that SOPs, harmonized strategies, shared experiences and new knowledge will help to develop reasonable, practical, and accessible methodologies, and to finally understand the impact of MPs on our environment.

Although it is not possible to always apply the same methods for MP assessment in freshwaters among different projects, the recommendations given in this report allow for better comparability of the research results. As high-standard limitations are included (such as uncertainty) in the recommendations, if followed, a high quality of the results is given. This report contributes to the advancement and implementation of harmonised methods for MP assessment. A critical assessment of this report at a later stage could definitely give new insights on pros and cons of the methods recommended here and help to initialise improved harmonised methodological strategies.

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7 ANNEXES

7.1.1 Annex A: Plastic applications

Table 7. List (non-exhaustive) of thermoplastics and thermosets and examples for their global applications. Data from Plastics Europe (2019)

	Name and abbreviation (if applicable)	Segments for application
Thermoplastics	Polyethylene (PE)	Packaging, Agriculture, Building and construction, Households
	Polyamides (PA)	Automotive, Electronics
	Polypropylene (PP)	Packaging, Automotive, Agriculture, Households
	Polycarbonate (PC)	Electronics, Building and construction
	Expanded polystyrene (EPS)	Building and construction
	Polyarylsulfone (PSU)	Automotive, Electronics, Building and construction
	Polystyrene (PS)	Packaging
	Thermoplastic elastomers (TPE)	Automotive, Electronics, Building and construction
	Polyethylene Terephthalate (PET)	Packaging
	Poly methyl methacrylate (PMMA)	Automotive, Building and construction
	Polyvinyl-chloride (PVC)	Building and construction, Households
	Fluoropolymers	Automotive, Electronics, Building and construction
Thermosets	Polyurethane (PUR)	Automotive, Building and construction
	Epoxy resins	Automotive, Electronics, Building and construction, Others
	Unsaturated polyesters	Automotive, Electronics, Building and construction, Others
	Melamine resin	Automotive, Electronics, Building and construction, Others
	Vinyl esters	Automotive, Electronics, Building and construction, Others
	Silicone	Automotive, Electronics, Building and construction, Others
	Phenol - formaldehyde resins	Automotive, Electronics, Building and construction, Others
	Urea - formaldehyde resins	Automotive, Electronics, Building and construction, Others
	Phenolic resins	Automotive, Electronics, Building and construction, Others
	Acrylic resins	Automotive, Electronics, Building and construction, Others

7.1.2 Annex B: Scope of LimnoPlast projects 3, 4 and 15

Table 8. Representation of LimnoPlast ESR projects 3, 4 and 15.

LimnoPlast project	Sample location and matrix	Scientific questions / topics	Targeted MP characterization	Available methods to the respective laboratories
ESR 3 (Denmark)	<ul style="list-style-type: none"> · Aarhus city · Freshwater · Matrices: water phase and sediment phase 	<ol style="list-style-type: none"> 1) Assessment of Sources and sinks of MPs in Aarhus area 2) Fate of MPs in Aarhus metropolitan area determined by hydrological modelling 	<ul style="list-style-type: none"> · Polymer types · Size fractions: 10-500 µm, 500-5000 µm · Shapes: all (less fibres) · Colours: all, only for larger particles · Mass: yes · Particle number per volume or dry weight sediment 	<ul style="list-style-type: none"> · UFO water pump · Sediment core sampler · Sieving, filtration · Ultrasonification · Density separation with SPT, glass separation funnel · Digestion: H₂O₂, Cellubrix enzyme, Viscozyme, Alcalase, Fenton reaction · Water bath evaporation, N₂ gas flushing · FPA-FTIR <500 µm · ATR-FTIR (Agilent) >500 µm · Pyr-GC-MS (Frontier Lab pyrolyser, Thermo Scientific GCMS) · siMPle library and OMNIC Picta
ESR 4 (France)	<ul style="list-style-type: none"> · Greater Paris area (urban) · Freshwater, river: Seine · Upstream to downstream · Low and high flow episodes, biannually 	<ol style="list-style-type: none"> 1) Determining MP fluxes upstream and downstream of the Seine river, assessing the contribution of the Paris urban environment on the MP load. 2) Assessing the contribution to the MP load in the Seine river from bordering agricultural areas. 3) Investigating the occurrence of MPs at the sediment-water interface, 	<ul style="list-style-type: none"> · Polymer types · Size fractions: 10-100 µm, 100-300 µm, 300-1000 µm, 1000-5000 µm · Shapes: all · Colours: all · Mass: yes 	<ul style="list-style-type: none"> · Niskin bottle · Pump (same as or similar to ESR 3) · Nets/trawls: 80 µm and 300 µm mesh size nets · Density separation: NaI, separation funnel · Nicolet iN10 FTIR, Size ranges LEESU ~25µm-500µm · Pyr-GC-MS (potentially) · Digestion H₂O₂ + SDS · siMPle library

LimnoPlast: Microplastics in Europe's Freshwater Ecosystems: from sources to solutions

(Coordinator: Universität Bayreuth | Contact: EU-LimnoPlast@uni-bayreuth.de | Homepage: www.limnoplant-itn.eu)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 860720

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LimnoPlast project	Sample location and matrix	Scientific questions / topics	Targeted MP characterization	Available methods to the respective laboratories
	<ul style="list-style-type: none"> Matrices: water phase and sediment phase 	<p>including small-scale experiments and potential correlation with suspended solids.</p> <p>4) Comparison of sampling methods to assess MP sedimentation and MP occurrence near sediments in rivers.</p>	<ul style="list-style-type: none"> Particle number per volume or dry weight 	
ESR 15 (Netherlands)	<ul style="list-style-type: none"> Freshwater systems (various matrices) Drinking water Fish from freshwater aquaculture (Sweden) Human gut 	<p>1) Analysis of MPs in the urban freshwater system of Amsterdam and modelling their fate and transport</p> <p>2) Linking MP abundance in freshwater (related) products (fish) and drinking water to human exposure</p> <p>3) Determine human gut samples to fill the knowledge gaps about the potential plastic particle concentrations entering human body upon ingestion</p>	<ul style="list-style-type: none"> Polymer types Size fractions: >0.7 µm, 10-500 µm, 500-5000 µm Shapes: all (filets in biota) Colours: all Mass: ng polymer per g sample (Particle number per wet or dry weight only for FTIR samples) 	<ul style="list-style-type: none"> Sediment grab sampler NaCl separation (sediments) + H₂O₂ (30 %) (for FTIR) Digestion: Solvent extraction with ASE (for Pyr-GC-MS) Bruker LUMOS ATR-FTIR microscope (>500 µm) Pyr-GC-MS (Frontier Lab pyrolyser, Agilent GC-MS) Bruker (Opus 7.5) library Gut wall samples will be freeze-dried for 48 hours, Dry weight and MP filets will be analysed sampling large volumes (depending on the MP concentrations) from the water column, using a sampling pump (>50 µm). Sample storage <i>in situ</i> in pre-cleaned (MilliQ) glass jars sediment grab samples will be taken and homogenized

7.1.3 Annex C: MP sampling devices and methodologies.

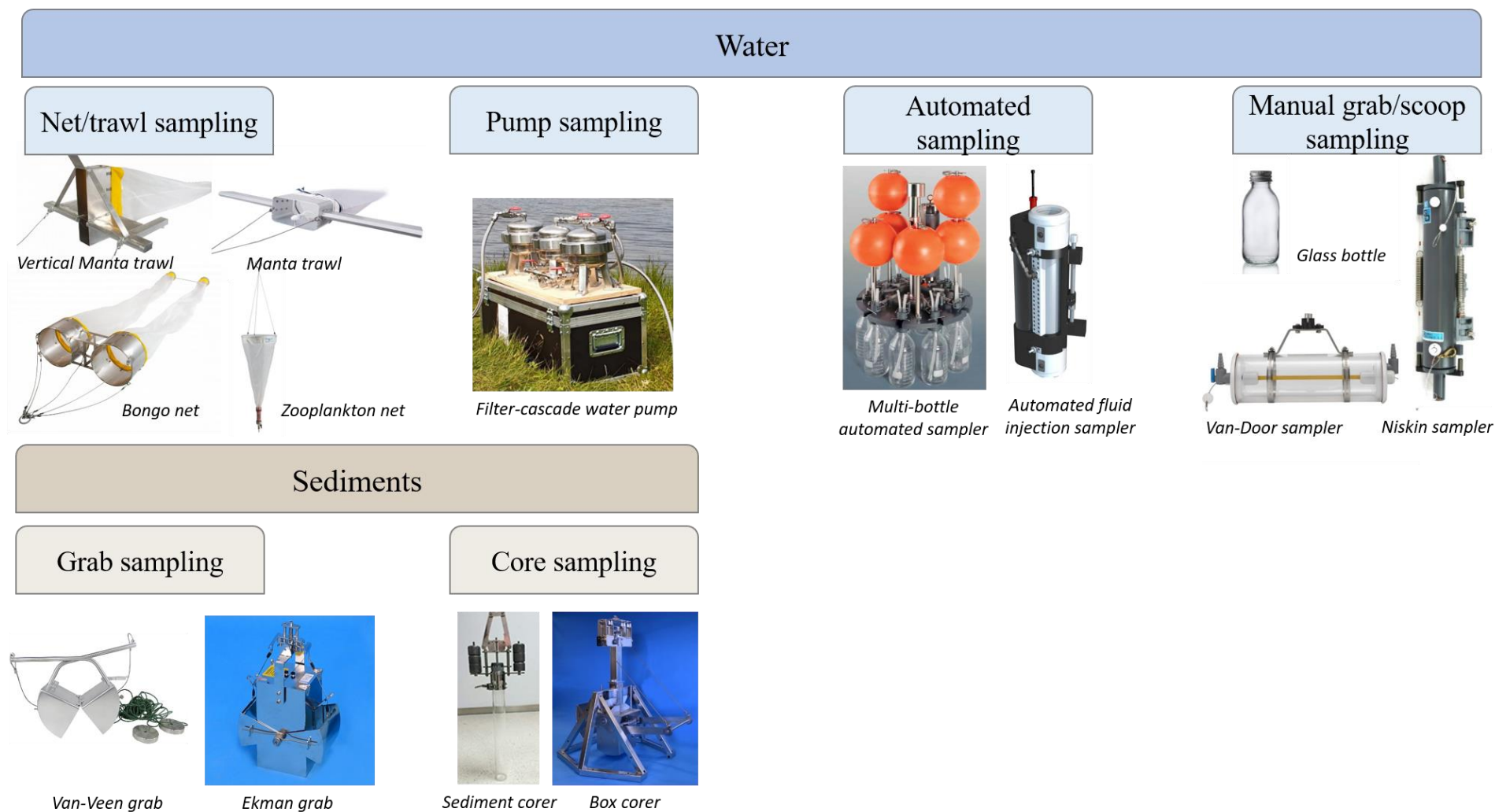


Figure 7. Examples for MP sampling devices for the different methodologies in water and sediment. Not exhaustive. Image sources: AAU, Hydrobios, KC Denmark, Feritech Global Ltd, General Oceanics

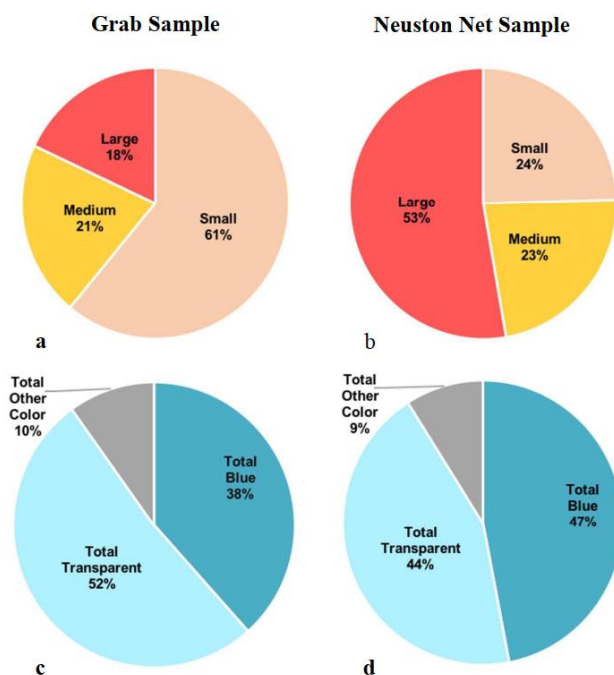


Figure 8. Microplastic composition proportions by size (a,b) and color (c,d). Microplastic size categories: small (100µm-1.5µm), medium (1.6mm-3.2mm), large (3.3mm-9.6mm). Total plastic particles: grab samples (N=117), neuston tow sample (N=1128). From Barrows et al. (2017)

7.1.4 Annex D: MP polymer density properties and sample processing methods

Table 9. Polymer density properties. Data from Hidalgo-Ruz et al. (2012)

Polymer type (abbreviation if applicable)	Density range [g cm ⁻³]
Polyethylene (PE)	0.92-0.97
Polypropylene (PP)	0.90-0.91
Polystyrene (PS)	1.04-1.1
Polyamide (nylon)	1.02-1.05
Polyester (including Polyethylene terephthalate (PET))	1.24-2.3 (PET: 1.37-1.45)
Acrylic	1.09-1.20
Polyoximethylene (POM)	1.41-1.61
Polyvinyl alcohol (PVA)	1.19-1.31
Polyvinylchloride (PVC)	1.16-1.58
Poly methyl acrylate (PMA)	1.17-1.20
Alkyd	1.24-2.10
Polyurethane (PU)	1.20

Table 10. Specification and examples of commonly applied sample processing steps for MP sample purification. Non-exhaustive.

	Processing step	Specifications / examples
Density separation (separating matrix/inorganic matter from MPs)	Floatation in separatory funnel	Sodium chloride (NaCl): 1.2 g cm ⁻³ Sodium bromide (NaBr), sodium iodide (NaI): 1.4-1.6 g cm ⁻³ Zinc chloride (ZnCl ₂), zinc bromide (ZnBr ₂): 1.6-1.7 g cm ⁻³ Calcium chloride (CaCl ₂): 1.3 g cm ⁻³ Sodium tungstate dihydrate (STD) (Na ₂ WO ₄ · 2H ₂ O): 1.4 g cm ⁻³ Sodium polytungstate (SPT) (Na ₂ WO ₄): 1.4 g cm ⁻³ (Mai et al. 2018)
	Elutriation	Vertical column with water or air inflow from below, supernatant recovery by overflow of MP particles at the top, flow rate ~300 L h ⁻¹ for 15 min (Claessens et al. 2013)
	MPSS (or similar)	Different sizes (up to several kg sediments can be treated), use of zinc chloride solution (ZnCl ₂): 1.6-1.7 g cm ⁻³ , recovery rate: 95.5-100 % for MPs <1 mm, better for organic-poor sediments. (Imhof et al. 2012)
Digestion (removal of organic matter)	Enzymatic	Cellulase (>30 U mL ⁻¹) Lipase (>15 000 U mL ⁻¹) Chitinase (>40 U mL ⁻¹) and Protease (1 100 U mL ⁻¹), Proteinase-K (500 mg mL ⁻¹) 97% (For more details see also Stock et al. 2019) Cellulose degradation in plankton sample: 10 mL cellulase TXL (EC 3.2.14, ASA Spezialenzyme), pH: 5 time: 96 h, temperature: 50 °C (Löder et al. 2017) Cellulose degradation in 200 mL wastewater sample: cellulase (Aspergillus sp., CAS no. 9012-54-8), time: 48 h, temperature: 40 °C (Simon et al. 2018)
	Oxidation	10 % H ₂ O ₂ , time: 18 h (Frias et al. 2018) H ₂ O ₂ efficiency not clarified
		Highly recommended: Fenton reaction: 35 % H ₂ O ₂ , + Fe ²⁺ at 60 °C (Mai et al. 2018) Time: 4 days, H ₂ O ₂ 250 g cm ⁻³ and Fe ²⁺ SO ₄ to 2.5 g cm ⁻³ , pH ~3 (adjusting with sodium hydroxide NaOH), exothermic reaction in ice-bath to keep temperature between 15-30 °C, rinsing residue (H ₂ O or 80 % ethanol)
	Alkaline	1 M NaOH, recovery rate: 90 % (Cole et al. 2011) Elevated temperature increases recovery rate (Stock et al. 2019) KOH recommended for MP (Dehaut et al. 2016)
	Acidic	HNO ₃ or HCl, recovery rate: 94 % and 98 %, respectively; dissolution of PS and PE (Claessens et al. 2013; Stock et al. 2019)