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Water quality — Sampling —

Part 27: Guidance on sampling for microplastics in water

Qualité de l'eau — Échantillonnage —

Partie 27: Recommandations pour l'échantillonnage des microplastiques dans l'eau

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Foreword

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This document was prepared by Technical Committee ISO/TC 147 *Water quality*, Subcommittee SC 6, *Sampling (general methods)*.

A list of all parts in the ISO 5667 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Microplastic occurrence in the environment is a prominent concern both to the public and to the scientific community. Determining the amount and distribution of microplastics in water bodies and domestic water is therefore a critical task.[1]-[6] However, the methodology for sampling microplastics in water samples is still lacking in precision. Consistent methodology is only starting to emerge, but still no universal protocol exists for the sampling of these contaminants in water.

The presence of small plastic fragments in the ocean was first reported in 1972,[7] but it was in 2004 that the term “microplastics” was proposed for the first time to describe plastic particles of a few micrometres in diameter.[8] Since then, a wealth of information became available on the abundance and type of microplastics in the marine environment, freshwater and estuarine systems. However, the different studies have used diverse techniques to sample, extract, treat and detect microplastic present in water.

There are many reasons why different studies investigating microplastic occurrence in water and wastewater show different results. The disparity between some of the findings (for microplastic type and abundance) can be partially explained by the fact that differing sampling techniques have been used. Variables pertaining to both time of year and time of day, flow rate and volume of water sampled, grab sampling or sieving the water over an extended period, the use of plastic containers or tubing, selection of a few parts of the sample for analysis, or dissimilar devices to capture the microplastic fragments, can be the causes of variation in study results.

While several standards for water sampling and water quality already exist (e.g. ISO 5667 series and, in particular, ISO 5667-17), microplastics as particular determinands pose a specific challenge which requires a more specific approach. For example, microplastics sampling requires the use of very specific materials for collecting, handling and storing to avoid cross-contamination. Also, microplastic buoyancy can vary depending on their composition, size, shape or colonization by microorganisms, and microplastics are not homogeneously distributed in the water column. Therefore, a more targeted and detailed set of sampling protocols is required to account for these differences. To better understand the fate and impact of microplastics in the environment, a more specific standardized sampling approach should be adopted and applied.

Water quality — Sampling —

Part 27: Guidance on sampling for microplastics in water

WARNING — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is essential that tests conducted according to this document be carried out by suitably trained staff.

1 Scope

This document specifies the basic methods for sampling suspended microplastics in water (domestic water, freshwater, seawater, treated wastewater and untreated wastewater) for their subsequent characterization. Suspended particles can also include synthetic or semi-synthetic polymeric materials (such as rubber). This document does not cover chemical analysis, biological (ecotoxicological) methods or physical methods, nor the pre-treatment or digestion methods intrinsic to such analyses.

This document covers general methodologies:

— for grab sampling, sampling using a set of successive filters of different pore sizes (cascade filtration), for water samples with low, medium and high content of suspended solids, and

— for net sampling using, for example, manta, plankton or neuston nets.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 5667-3, *Water quality — Sampling — Part 3: Preservation and handling of water samples*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
microplastic
solid plastic or synthetic polymer particle insoluble in water with the largest dimension between 1 µm and 5 mm

Note 1 to entry: Microplastics can show various shapes.

Note 2 to entry: This definition encompasses the ISO/TR 21960 definitions of large microplastics and microplastics.

3.2
plastic
material which contains as an essential ingredient a high polymer and which, at some stage in its processing into finished products, can be shaped by flow

[SOURCE: ISO 472:2013, 2.702, modified — Notes 1 and 2 to entry have been deleted.]

3.3
rubber
family of polymeric materials which are flexible and elastic

[SOURCE: ISO 1382:2020, 3.420, modified — the domain and Notes 1 and 2 to entry have been deleted.]

3.4
suspended solid
solid remaining in suspension in water, which can be removed by sedimentation, filtration or centrifugation

[SOURCE: ISO 6107:2021, 3.554]

3.4.1
high suspended solid content
concentration of solids suspended in water above 500 mg/l

3.4.2
medium suspended solid content
concentration of solids suspended in water ranges between 100 mg/l to 500 mg/l

3.4.3
low suspended solid content
concentration of solids suspended in water ranges between 1 mg/l to 100 mg/l

3.5
grab sample
single discrete sample directly collected from a water body at a specific time and location (and depth when relevant)

3.6
blank
sample identical to the sample of interest, but in the absence of the determinand

3.7
field blank
blank (3.6) to verify possible contamination during sampling

Note 1 to entry: A field blank is prepared in the laboratory using water (filtered before use through an inert filter with a pore size smaller than 1 µm) and sent with the sampling personnel for exposure to the sampling environment.

3.8
domestic water
water either in its original state or after treatment

Note 1 to entry: Domestic water is intended for human use, such as cooking, food preparation, washing, drinking or other domestic purposes.

3.9
limit of detection
smallest true value of the measurand which ensures a specified probability of being detectable by the measurement procedure

Note 1 to entry: For *microplastics* (3.1), the limit of detection is defined as the methods' capability of reliably detecting at least one particle or a defined mass.

[SOURCE: ISO/TR 22930-1:2020, 3.6, modified — the term has been changed from "detection limit" to "limit of detection", Note 1 to entry has been replaced and Note 2 to entry has been deleted.]

4 Principles and general considerations

4.1 Methodologies

4.1.1 General

The microplastic sampling approaches described in this document can be grouped mainly as grab sampling and volume-reduced sampling.

Grab sampling (also known as spot sampling) consists of collecting a given volume of water (without reducing it during this procedure). Subsequent separation, pre-treatment (when required) and analysis of the microplastics are done in a laboratory. In volume-reduced sampling, the microplastics are collected while reducing the volume of water during the sampling process, by passing the water through nets or sieves. The collected microplastics are preserved for further processing in a laboratory.

4.1.2 Grab sampling

The methodology for the sampling of microplastics via grab sampling entails filling non-plastic containers (or made of materials which are less present in the environment or not part of the measurement concept) with domestic water, freshwater, treated or untreated wastewater, or surface, near surface or deep-water samples, for the subsequent pre-treatment and analysis in a laboratory. The container material must be suitable for the sample. The sampling date, time, location, depth (when relevant) and collected volume are recorded.

The main advantage of grab sampling is that all the microplastics present in the sampled water are collected without size limitation, in contrast to volume-reduced sampling, where the selected mesh size determines the smallest size of sampled particles. In grab sampling, risks of contamination are reduced when compared to volume-reducing sampling in an open system (e.g. using manta trawls, plankton or neuston nets), because handling of the sample and time of exposure to the surrounding environment are shorter. However, the main disadvantage of grab sampling is the limited volume of sample that can be collected, stored and processed.[9]

4.1.3 Volume-reduced sampling

In volume-reduced sampling, the microplastics are extracted and aggregated from the medium before analysis. This can be done via cascade filtration, where a volume of water passes through a series of filters with decreasing mesh sizes, or by using manta trawls, plankton or neuston nets. While volume-reducing methods allow for a greater area and/or volume of water to be sampled, their main disadvantages are the limits on the minimum microplastic size that can be collected. This is determined by the sizes of the filters, sieves used and mesh selectivity,[10][11] alongside a higher risk of contamination because the time of exposure to the surrounding environment is long.

The methodology of cascade filtration allows for the sampling of domestic, treated and untreated wastewater, surface and subsurface waters with low, medium or high suspended solid content, and consists of passing the water to be sampled through a series of filters of different pore sizes, for the retention of the microplastics in the different filters. The sampling date, time, location, mesh pore size and the flow rate of the water passing through the filters over the sampling time interval are recorded, in order to express the results in terms of collected particles per unit volume, or mass per unit volume.[1)]

1) Cascade filtration systems can be limited by the minimum pore size of the sieves used or volume of water sampled (see Table 1). The use of sedimentation boxes or continuous flow centrifuges have recently been proposed as potential alternatives of volume-reduced sampling of microplastics from large volumes of water, with initial studies showing the capability to capture the smallest microplastics (<30 µm) from river waters. While these techniques are currently outside the scope of this document, References [12] to [16] can be consulted for further information of these recent proposals. General information on sedimentation boxes and continuous flow centrifuges can be found in ISO 5667-17 (not related to microplastic sampling).

For sampling surface waters (up to about 100 cm below surface), the use of manta trawls, plankton and neuston nets in dynamic and stationary methods are described. Other types of nets are also used for subsurface water sampling. The sampling date, time, location, mesh pore size and water passing through the nets over the sampling time should be recorded. Additionally, for dynamic sampling, recording of log speeds of boats or vessels, tow duration, area covered, wind velocities and significant wave heights is recommended.[6]

4.2 Selecting the most appropriate sampling method

Sampling strategies can differ depending on the targeted environmental compartment that needs to be monitored and depending on the target question (see Table 1).

Grab sampling can be used not only to collect microplastics from the water surface but also from the water column, by using a container or a submersible water pump. Grab sampling is applicable to all categories of water samples (domestic water, freshwater, seawater, treated wastewater and untreated wastewater) and is preferred over net tows for smaller microplastics, which typically cannot be collected by the larger mesh sizes. However, given the more limited volume that can practically be collected and stored, the detection limit for grab samples can be higher, particularly when sampling water bodies such as rivers, lakes or sea water. Replicates or combined spot samples can compensate for this deficiency.

Column waters can also be sampled by pumping, followed by a set of sieves to isolate microplastics of different size ranges (cascade filtration). This method is applicable to domestic water, freshwater, treated wastewater and untreated wastewater. Cascade filtration systems are also limited by the minimum pore size of the filters or sieves used. While mesh sizes can be down to 1 µm, samples such as untreated wastewater require larger pore sizes to avoid clogging. Cascade filtration systems allow more significant capture of smaller microplastics when compared to nets.

For sampling the water surface and water column in rivers, lakes and sea, the most common method is the net tow, using neuston or plankton nets or manta trawls.[2][6][17]-[19] Manta trawls and neuston nets are mainly used for microplastics sampling in the ocean, while plankton nets are used for sampling in rivers. Net mesh sizes vary between 50 µm and 500 µm, with 330 µm the most commonly used.[2][6][18][20] Plankton nets have the smallest mesh pore sizes (between 50 µm to 100 µm) and they usually need to be towed at lower speeds in order to reduce clogging.[9] Microplastics smaller than the minimum mesh size are not collected, resulting in a potential underestimation of their total abundance in the sampled water. It is important to note that the frequency of occurrence of dispersed microplastics is likely to vary inversely with size (i.e. there will be fewer larger items)[2][21] and needs to be taken into account when planning the sampling method and sampling strategy.

In net tow, the collection of microplastics can be performed by a dynamic or stationary sampling method.

In dynamic sampling, trawls are towed by a boat with a rope, keeping the nets outside the waves from the vessel to prevent disturbance or dispersion of the particles to be collected. It is recommended to position the net on the side of the boat, by a suitable pole installed.[6]-[9]

For streams, creeks and smaller rivers with variable water regime not entirely navigable, stationary sampling is recommended. For this, the floating nets need to be fixed on the banks. The nets filter water using the stream current with their mouths skimming the surface, with a weight used to maintain a continuous and consistent submersion depth. The nets are collocated in the opposite direction of the water flow.[9] Plankton nets are more commonly used for stationary sampling in rivers.

Table 1 — Comparison of different methodologies for sampling microplastics in water

Method	Overview and application	Advantages	Limitations
Grab sampling	Discrete sample collection with non-plastic containers (or made of materials which are less present in the environment or not part of the measurement concept). Applicable to domestic water, freshwater, treated wastewater and untreated wastewater. Applicable for surface waters and water column.	Relatively quick and straightforward method. Reduced risk of contamination. No limitation of microplastic size. It can sample surface and water column.	Sampling smaller volumes of water. It requires transportation of large or multiple containers to the lab. Less representative. If sampling in deeper waters such as sea, middle of a lake or river, a boat or ship can be needed.
Cascade filtration	Volume-reduced sampling. Applicable to domestic water, freshwater, seawater, treated wastewater and untreated wastewater. Applicable for surface waters and water column. Cascade filters using non-plastic sieves (or made of materials which are less present in the environment or not part of the measurement concept).	Mesh sizes can vary between 1 µm to 5 mm, allowing for a more efficient and wider range of particle sizes when compared to manta or neuston nets. Cascade filtering using finer filters can collect smaller-sized particles more efficiently than the use of manta, plankton or neuston nets. It allows sampling large volumes of water. It permits size fractionation directly in the field. It can sample surface and water column.	The minimum mesh pore size and mesh selectivity determine the minimum particle size to be captured. It requires electric energy (unless sampling from a pressurized system or if a manual or hand-operated pump is used). It involves more equipment than grab sampling. Filters can be clogged, particularly when using smaller mesh sizes or when sampling water with high-suspended solid contents. Higher risk of sample contamination from apparatus and due to manipulation. If sampling in deeper waters such as sea, middle of a lake or river, a boat or ship can be needed.
Net sampling: Dynamic sampling	Volume-reduced sampling. Widely used for sampling water in navigable rivers, lakes and sea. Applicable for surface waters and water column. Meshes are towed by a boat with a rope. The position of the net should be away from the wake from the vessel. Ideally, mesh materials should be made of compounds which are less present in environment or not part of the measurement concept. The mesh pore size determines the minimum particle size to be captured. The mesh opening must be reported. For multiple depth collection at the water subsurface, nets can be tethered at predetermined increments. Multiple opening or closing nets can also be used for water column sampling (e.g. vertical multiple-opening plankton sampler, VMPS or multiple opening and closing net with environmental sensing system, MOCNESS).	It allows sampling large volumes of water and covers large areas.	Most available nets have mesh sizes between 500 and 50 µm, with 330 µm being the most common one. This limits the minimum microplastic size that can be captured. Clogging problems. It requires a boat or ship. Risk of sample loss or contamination due to manipulation and transfer from the nets.
Net sampling: Stationary sampling	Volume-reduced sampling. Widely used for sampling surface waters. Recommended for streams, creeks and smaller rivers with variable water regime not entirely navigable. The nets are collocated in the opposite direction of the water flow, with a weight used to maintain a continuous and consistent submersion depth, and fixed on the banks. Ideally, mesh materials should be made of compounds which are less present in environment or not part of the measurement concept, The mesh pore size determines the minimum particle size to be captured. The mesh opening must be reported.	It allows sampling large volumes of water and sampling over longer periods of time.	Most available nets have mesh sizes between 500 and 50 µm, with 330 µm being the most common one. This can limit the minimum microplastic size that can be captured, with potential underestimation of the real quantity of microplastics in the sampled water. Clogging problems. Anchoring the nets to the riverbed can be difficult. Mostly for surface sampling (up to about 100 cm below surface). Not usually applicable for deep water column. Risk of sample loss or contamination due to manipulation and transfer from the nets.

4.3 Sampling volume

Sampling volume must be adjusted according to the analysis purpose, to the microplastics content and sizes expected, and to the water quality (e.g. a high quantity of suspended solids can cause filter clogging).[22][23]

Different factors should be considered when determining the optimum water volume to be sampled. For microplastics, the limit of detection can be defined as the methods' capability of reliably detecting at least one particle or a defined mass with statistical rigour.[4][21][23]-[26]

Limits of detection also depend on the microplastic size range or concentrations to be studied. Sampling small volumes reduces the chance of detecting microplastics and increases the margin of error. Therefore, the detection limit benefits from large sample volumes.[26] In addition, larger sampling volumes are more representative, as microplastics are not distributed homogeneously in the sampled matrix. Smaller microplastics are more abundant, therefore smaller volumes can be required when targeting exclusively small microplastics (e.g. <50 µm). However, if the objective of the study includes the detection of larger microplastics, larger volumes are needed.[4][21][24][26] This is particularly important if the subsequent analysis will report total mass of microplastics per volume, or per sample. Larger particles will dominate the total mass, with just one or two particles containing more mass than several smaller particles. Therefore, the results of mass per volume or sample can become biased if an insufficient volume is collected.

For domestic waters (e.g. drinking water or water from a household tap), the minimum volume for grab sampling is one litre, as reported in several scientific publications.[22][26] This volume is representative for daily human consumption (WHO guidelines) and corresponds to the recommendations of ISO standards for water contaminants analysis. For sampling domestic waters in cascade filtration, the volume can be extended.[27][28] Previous studies have indicated that the presence of large microplastic particles is very low (usually one particle per metre cube). Therefore, sample volumes of 1 000 litres or more are recommended, whenever possible, for a sufficiently representative sample which includes large microplastics.[26][29]

For treated wastewater, previous studies have reported a wide range of microplastic particles (values varying between 10 particles per metre cube to 30 000 particles per metre cube). For untreated wastewater influents, the number of particles are expected to be even higher (but the number of large microplastics is expected to be lower).[29]-[34] The mass loads for wastewater treatment plant effluents have been reported between 200 µg m^{-3} and 3 800 µg m^{-3}.[35] Therefore, volumes of 10 l to 100 l of untreated wastewater are recommended for a sufficiently representative sample. Larger volumes are recommended for treated wastewater or when sizes up to 5 000 µm need to be quantified. Lesser volumes are used when targeting exclusively smaller microplastics or when sampling untreated wastewater influent with cascade filtration (because of high amounts of suspended solids which can clog the filters).

For collecting microplastics in rivers, lakes and sea, the most common method is by using nets. Manta trawls and neuston nets are mainly used for microplastics sampling in the ocean, while plankton nets are mainly used for sampling in rivers. Due to the low concentrations of large microplastics usually reported in previous studies,[21][26] sample volumes of at least 200 m^3 to 500 m^3 have been suggested in the literature, which is equivalent of surveying an area of approximately 1 000 m^2.[6][9][26] However, for remote locations with expected very low particle numbers, greater volumes can be needed.

4.4 Quality control

4.4.1 General

Particular importance should be given to careful sampling performed on-site and to correct recording of the water sampling and handling. Reference should be made to ISO 5667-14 regarding quality assurance of water sampling and handling. This guidance is independent of the quality assurance required for the laboratory analysis of samples.

Sampling quality programmes include documented evidence that:

— the individuals who collect samples are competent and well trained;

— appropriate sample collection and sample handling methods are employed;

— equipment is maintained and calibrated;

— correct practices are followed;

— records are both complete and secure.

Sampling programmes should include field blank samples handled as if genuine samples, to assess contamination of samples and use of appropriate replicate samples to assess precision and repeatability. Addition-recovery tests with standard plastic particles can also be employed to check how accurately the standard plastic particles are sorted and collected in the process of sampling.

Weather, tidal currents and river inflows can also affect the content and composition of microplastics in the water. In addition, the content and composition of microplastics can be affected in areas near land following rainfall.[6] Therefore, in order to ensure that the samples are as representative as possible and to account for seasonal variations or peak releases, it is recommended to repeat the sample collection at different times of either the day or the year, or both. Time-proportional composite sampling is also recommended, particularly when sampling untreated wastewater (i.e. combining discrete volume aliquots collected at different time intervals).

4.4.2 Sources of sampling errors

Sources of sampling errors include the following:

a) contamination by cross-contamination between samples;

b) incorrect storage; the choice of sampling vessels, containers and the options for preservation can affect the integrity of the microplastics, particularly with samples such as untreated wastewater. Sampling vessels and containers can be a source of contamination. The choice of preservation agents largely depends on the research question being considered; care should be taken when selecting the preserving agent (and the concentration used) as they can damage some polymers;

c) incorrect sampling; deviation from the sampling procedure can be a source of error;

d) losses of materials due to incorrectly applied sampling or filling technology, (e.g. incorrect use of suction pumps, incorrect transfer method, multiple transfer or turbulent filling of sample).

e) use of unsuitable or insufficiently purified facilities (e.g. flasks, filtration equipment, tubing, nets); this can cause sampling errors and contamination;

f) contamination of samples from air or vessel owing to not covering equipment and samples during sampling, processing and storage;

g) unsuitable clothing (i.e. synthetic fibres) during sampling;

h) confusion of sampling collection location by, for example, inadequate documentation. Correct documentation should contain the position coordinates and photos (taken in different seasons if necessary). GPS navigation handsets have proved useful;

i) confusion of samples due to inadequate labelling or incomplete or incorrectly completed sampling reports;

j) sampling in non-representative, non-homogeneous or other inappropriate sampling points.

4.4.3 Blanks

This technique can be used to identify any errors relating to contamination of sampling containers and the sampling process.

For grab sampling, field blank samples are laboratory blank samples which are taken into the field, treated as samples and analysed as a check on sampling procedures (see Figure 1).

At the laboratory, divide a blank sample of pure water (filtered through inert filter with a pore size smaller than 1 µm) into two parts: part A and part B. Part A is retained in the laboratory. Part B is the "field blank" transported into the field.

Divide part B into two portions b1 and b2; portion b1 should be processed, as far as is practical, with the same technique as real samples, using the same sampling equipment and containers as the real samples.

For grab sampling, portion b1 should be transferred with the same technique as real samples, as far as is practical, to the same type of sampling containers as real samples.

For sampling using filtration equipment, portion b1 should be processed using equivalent sampling containers and filtration equipment, as far as is practical, with the same technique as the real samples.

Portion b2 should be retained and returned to the laboratory without any further processing in the field.

The comparison of results of part A and portion b1 identifies errors due to handling, processing and transportation.

The comparison of results of part A and portion b2 identifies errors due to container and sample transportation only.

The comparison of results of portion b1 and portion b2 identifies errors due to contamination of sampling containers or sampling processes.

Refer to ISO 5667-14:2014, 11.3 for additional guidance.

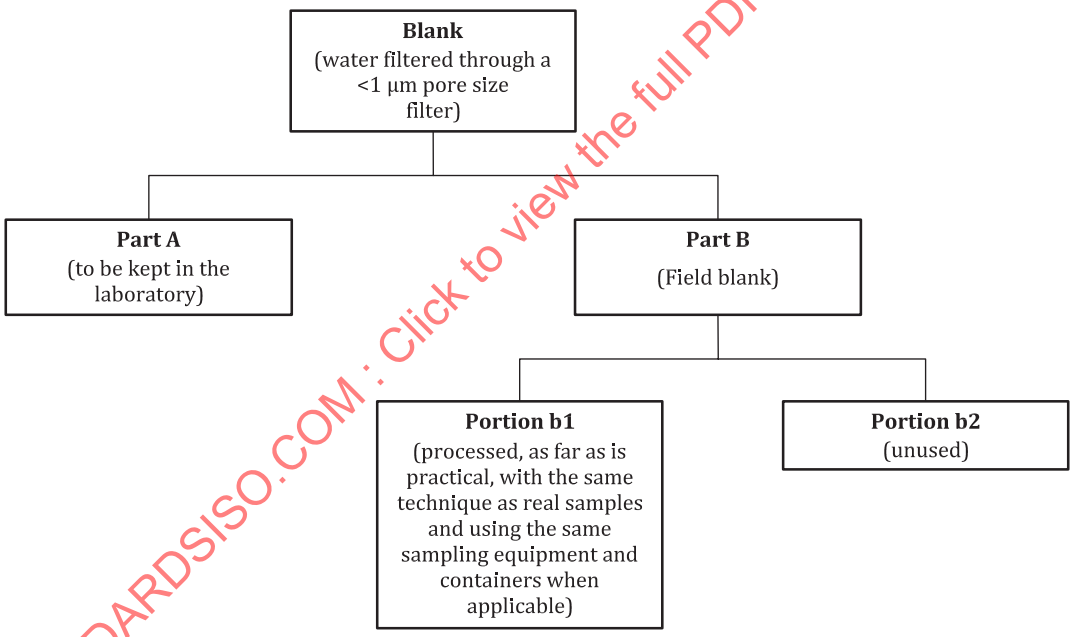

Figure 1 — Flow chart of the the field blank sampling technique for grab sampling

For the cascade filtration, initial validation of the system should be performed. For this, the entire sampling system shall be characterized prior to the sampling phase, in order to determine systematic microplastics contamination which can be coming from this system. This can be done by repeating the sampling protocol at least three times (using different volumes of pre-filtered ultrapure water) and analysing the resulting samples. The laboratory analyses should demonstrate the effectiveness of the device cleaning protocol in preventing cross contamination of the samples.

When using net sampling (such as manta, plankton or neuston nets), it is impractical to bring additional laboratory water or plastic-free seawater to pass through the nets in the field. When using these methods, a "blank" sampling can be conducted by washing unused nets (prepared, stored and transported in the same way as nets used for sampling) in the same manner as the nets used for sampling. The sample and the blank

sample shall be stored and transported back to the laboratory in identical containers and shall be processed in the same way.

4.5 Sampling plan

4.5.1 General

Sampling strategy includes identification of the area under investigation, choice of sampling procedure, choice of location and number of sampling sites, seasonality and input patterns.

Aspects such as the sample identification method, the number of sampling points, locations and the number of samples to be taken at each site shall be defined in the sampling plan. Necessary adjustments can then be performed in the field, in which case the sampling record should be updated to include a reasonable justification for the changes.

The types of local substrates, hydrographic features in the area to study, information on nearby sources of discharge and (where available) knowledge from past measurements should be considered during sampling, in order to ensure that results are as accurate as possible.

For further guidance on how to devise sampling programmes, see ISO 5667-1, ISO 5667-4, ISO 5667-6 and ISO 5667-9.

4.5.2 Sampling points

Sampling points should be chosen so that the samples are representative of the wider environment or area under consideration. When identifying sources of microplastic discharge, the sampling points should be appropriately placed in relation to the source of emissions. There are often practical considerations to account for, such as access to the sampling points, suitable locations for the sampling equipment or protecting the sampling equipment from vandalism or theft.

The sampling locations should be documented using its latitude and longitude coordinates as well as the precise location of each measuring point. The sampling points can also be recorded with 1:5 000 and 1:25 000 scale maps and images, as well as a description of the access route. Please refer to ISO 5667-17:2008, 4.4 for additional guidance.

4.5.3 Sampling frequency, duration and timing

The sampling frequency, duration and timing depend on the main purpose of the investigation. A single sampling exercise can be sufficient depending on the problem being investigated. However monthly or weekly sampling can be necessary for estimating loads or establishing long-term projections, especially when the analyses reveal widely spread values. See ISO 5667-1 for details on statistical analyses to assess whether fluctuations are random (i.e. exhibit a normal distribution) or systematic (i.e. trends, cyclic variations).

The amount of microplastics in the water and the volume of water needed for sample collection are key factors that determine how long the collection period is set (see 4.3).

In order to ensure that the samples are representative, specific environmental phenomena, such as rainfall, snowmelt or high and low tides, should also be accounted for during sampling. Microplastics such as those linked to roadways and tyres can be transported during rainfall. Quantities of these particles will therefore vary when collecting water samples close to roads during dry or wet conditions. To make sure that loadings of microplastics in waters are not overestimated or underestimated, it is recommended to account for these events when deciding sampling frequency.

5 Reagents

5.1 All reagents and waters used shall be of at least analytical grade. Water for blanks and reagent preparation should be filtered before use through an inert filter with a pore size smaller than 1 µm.

5.2 Neutral-buffered formalin (the final concentration in the sampled water should be approximately a volume fraction of 4 % with formaldehyde solution diluted using phosphate buffer at neutral pH).

This reagent is only needed when it is required to prevent proliferation of microorganisms (such as plankton, bacteria) and no refrigeration is possible during storage. For the preparation of this solution, ultrapure water (as per 5.1) should be used.

5.3 Mild liquid detergent for glassware and laboratory items (e.g. Decon-90®[2], Aquet®[3], RBS™ T230[4]).

All reagents should be kept in glass or other suitable non-plastic containers whenever possible (or made of materials which are less present in the environment or not part of the measurement concept).

6 Apparatus

6.1 Grab sampling method

6.1.1 Several glass or other suitable non-plastic containers (or made of materials which are less present in the environment or not part of the measurement concept), with corresponding non-plastic or foil-lined lids, in sufficient quantities to store the required volume of sampled water between them.

6.1.2 Grab sampling equipment can consist of surface and deep-water samplers, and can include telescopic rods, mechanical or hand-driven pumps, immersion cylinders and dipping bottles (e.g. non-plastic Niskin bottles).

6.1.3 Tubing or hoses should be plastic-free when possible or made of materials which are less present in environment or not part of the measurement concept [e.g. platinum-cured silicone braided hoses, ethylene propylene diene monomer (EPDM), silicon].

6.1.4 If a mechanical pump is used, it should be a stainless-steel pump or other plastic-free pump able to handle particles of all examined sizes. The composition of all the pump parts that are in contact with the water (and the composition of the hosing that carries the water) should not be made of plastic or contain plastic parts when possible (or made of materials which are less present in the environment or not part of the measurement concept).

Submersible pumps should be magnetically driven so that there is no chance of leakage of lubricating or cooling oil from the submersible electric motor into the impeller housing. Alternatively, a peristaltic pump can be used, as it does not need to be immersed and the risk of contamination is lower. This latter approach also avoids the risk of microplastics breaking down to smaller particles due to mechanical friction. If using a peristaltic pump, it is recommended to use new rather than worn peristaltic tubing before sampling. Refer to ISO 5667-17:2008, 6.6 for further guidance on pumping requirements.

6.1.5 For hand-driven pumps, all the pump parts that are in contact with the water should not be made of plastic or contain plastic parts.

6.1.6 Stainless-steel immersion rod, connected to the water pump, for the collection of sub-surface water samples (optional).

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6.1.7 Scoops attached to telescopic rods, as well as immersion cylinders and dipping bottles should be made of stainless-steel or other plastic-free materials when possible, or materials which are less present in environment or not part of the measurement concept.

6.2 Cascade filtration methods

For cascade filtration, non-pressurized systems (see Figure 2 and Figure 3) and pressurized systems (see Figure 4) can be distinguished. Depending on the setup, the use of the elements described in 6.2.2 to 6.2.10 can be used.

6.2.1 A series of stainless steel or other plastic-free sieves or filters (or made of materials which are less present in the environment or not part of the measurement concept).

The filters can have mesh sizes varying between 1 µm to 5 mm and should be able to be connected or fixed one after another from a larger to a smaller pore size (e.g. with the use of non-plastic spacers) for the retention of microplastics of different sizes. Filters can be stacked sieves or tubular cartridges.

The number of filters and pore sizes needed depend on the minimum particle size to be captured and the content of organic and inorganic matter in the water. Two to four sieves should be used, which can combine a selection of mesh sizes such as 1 µm, 5 µm, 10 µm, 20 µm, 50 µm, 100 µm, 250 µm, 330 µm, 500 µm, 1 mm and 5 mm. The minimum mesh pore size determines the minimum particle size captured quantitatively.

6.2.2 For stacked sieves, a metal or other plastic-free container (or made of materials which are less present in the environment or not part of the measurement concept) able to hold the filters or sieves in place and with an aperture or valve to allow the sampled water to flow out, after passing through the filters or sieves can be used. See the schematic setup with filter arrangements for pressurized and non-pressurized systems in Figures 2, 3 and 4.

6.2.3 Tubing or hoses made of stainless steel or other materials which are less present in the environment or not part of the measurement concept (e.g. platinum-cured silicone braided hoses, EPDM, silicon).

6.2.4 Non-return valve (recommended).

6.2.5 Ball or screw stainless-steel valves.

6.2.6 Water meter with a maximum permitted error from the minimum flow rate (Q_{min}) to the maximum flow rate (Q_{max}) of ±5 %.

6.2.7 Stainless-steel or other plastic-free hydraulic pressure gauge (water pressure gauge meter) with metal and glass manometer dial plate.

6.2.8 For non-pressurized systems (e.g. water tanks, reservoirs or small lakes), a stainless-steel pump or other plastic-free pump able to deliver at least one litre of water per minute and to handle particles of all examined sizes. The pump parts that are in contact with the water (and the composition of the hosing that carries the water) should not be made of plastic or contain plastic parts when possible, or should be made of materials which are less present in the environment or not part of the measurement concept.

Submersible pumps should be magnetically driven so that there is no chance of leakage of lubricating or cooling oil from the submersible electric motor into the impeller housing (see the schematic setup in Figure 3). Alternatively, a suction pump (e.g. peristaltic pump or vacuum sampling system) can be used after the filter assembly (see Figure 4), as it does not need to be immersed and thus the risk of contamination is lower. This latter approach also avoids the risk of microplastics breaking down to smaller particles due to mechanical friction. In this option, the pump should have an adequate pressure to displace the liquid from the sampling point, throughout the filters, and to the drain. It is recommended that, in this arrangement, two pressure gauges are used (before and after the filters) to monitor the pressure difference and detect any filter clogging. When using a peristaltic pump, it is recommended to use new rather than worn peristaltic tubing before sampling. Refer to ISO 5667-17:2008, 6.6 for further guidance on pumping requirements.

For some non-pressurized systems such as water tanks and reservoirs, the water height can provide the recommended flow rate at a lower altitude sampling point, by gravity.

6.2.9 Stainless-steel immersion rod, connected to the water pump, for the collection of sub-surface water samples in non-pressurized systems.

6.2.10 Glass Petri dishes or glass flasks with their corresponding sealing lids for storage of microplastic samples after collection, covered with aluminium foil.

6.3 Net sampling

6.3.1 Neuston nets with a side length between 45 cm to 100 cm or manta nets with a width between 60 cm to 100 cm, and a height between 15 cm to 40 cm are generally used. Mesh openings of approximately 330 µm are the most common, as this permits the filtration of large amounts of water and are better suited for plankton abundance. Plankton nets have the smallest dimensions of the mesh size (50 µm to 100 µm). They are also more commonly used for stationary sampling in rivers. Nets with mesh openings of 200 µm or 100 µm can also been used.[36] Multiple opening or closing nets have also been used for water column sampling.

Ideally, mesh materials should be made of compounds which are less present in environment or not part of the measurement concept. Common materials usually include polyamide and polypropylene, but when these materials are used, care should be exercised if fibres of the same composition are found; see 4.4.3 and 8.4.2 for the use of field blanks. It is important to consider that the mesh pore size and mesh selectivity determine the minimum particle size to be captured.[11] Therefore, when using this methodology, the mesh opening must be clearly reported.

6.3.2 Net case structure and supporting cables made of stainless steel, and with corresponding resin floats for surface water collection (usually, resin floats are made of PVC, but contamination risk from this material is very low, as they are located at the sides of the nets).

6.3.3 Flowmeter, attached to the net mouth, for measuring the flow of water that enters the net.

6.3.4 For dynamic sampling, research vessel or adequate boat able to sustain speeds of 1 m/s ± 0,5 m/s (1 to 3 knots) against water (e.g. small fishing boat can be considered to be adequate depending on water conditions and after installation of appropriate simple equipment for towing).

6.3.5 For dynamic sampling, nets should be held on the sides of the boat using outfitting (pipes) set at an appropriate length to keep the nets away from the hull. Clamps and ropes can be used for fixing and supporting the pipes to the boat, with the nets mounted as far as possible from the boat to avoid the disturbed water driving plastics downward or below the net, resulting in inaccurate sampling (this also helps avoiding contamination).[2] The outfitting should be fixed to a place on the vessel that is strong, secure and stable, such as a boat bollard. To prevent damage to the outfitting, the ends of the pipes can be stabilized with tension using a support rope (see Figure 5).[6]

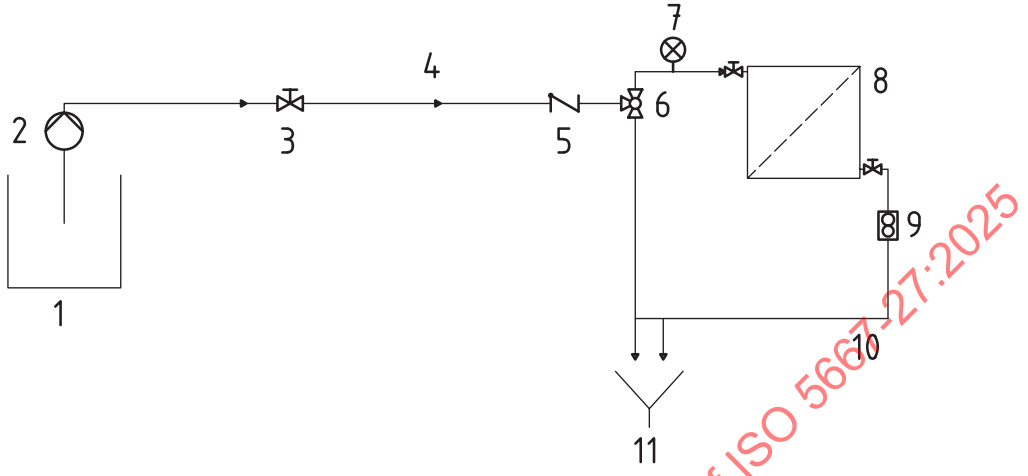
6.3.6 Water subsurface samples can be collected by securing the nets, at the desired depth, to a steel rod anchored to a place on the vessel that is strong, secure and stable. For multiple depth collection at the water subsurface, nets can be tethered at predetermined increments to a cable lowered and secured using outfitting (pipes), with each net targeting a different depth below the water surface (see Figure 5).[37][38] Multiple opening or closing nets are also used for water column sampling.

6.3.7 For stationary sampling, nets should be fixed with appropriate ropes or resistant cables to the banks, in a position so that the net mouth is skimming the surface (see Figure 6). A weight should be used to maintain the nets submerged to a stable distance in the water.

6.3.8 For stationary sampling, the nets can either be held with appropriate ropes or resistant cables to the banks using adequate poles firmly fixed into the river sediments or tied to suitable trees on each side

of the river if possible. Alternatively, in urban areas, stationary sampling has also been performed taking advantage of bridges or man-made structures to which the ropes can be tied (see Figure 6).

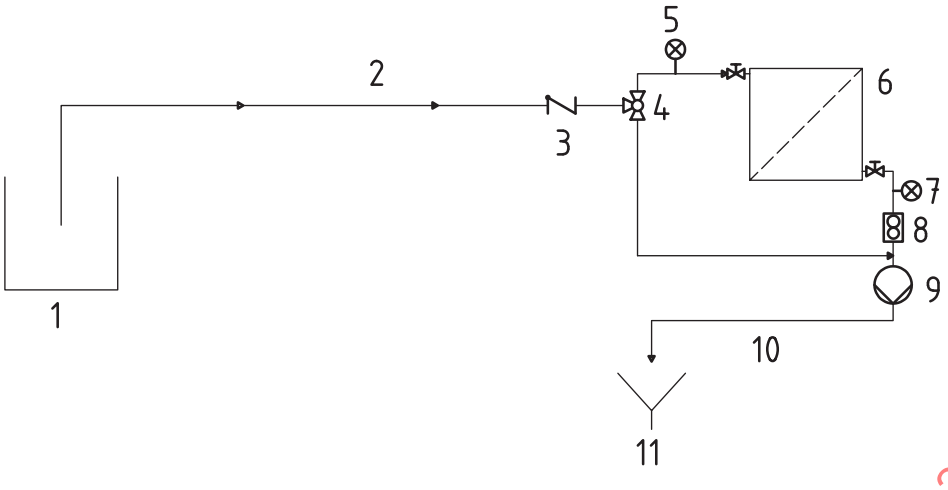
6.3.9 Flasks or containers with corresponding sealing lids for storage of microplastic samples after collection, made of stainless steel or other materials which are less present in the environment or not part of the measurement concept. Additional details are described in 8.4.7 and 8.4.8.



Key

1 water tank, deposit, small lake or reservoir
2 submersible pump or pump with no plastic parts and stainless-steel immersion rod for sub-surface sampling collection
3 screw valve
4 tubing or hoses made of stainless steel or other materials which are less present in the environment or not part of the measuring concept
5 non-return valve
6 3-way L-port valve
7 pressure gauge
8 container with non-plastic sieves or filters of different mesh sizes
9 flowmeter
10 waste pipe
11 drain

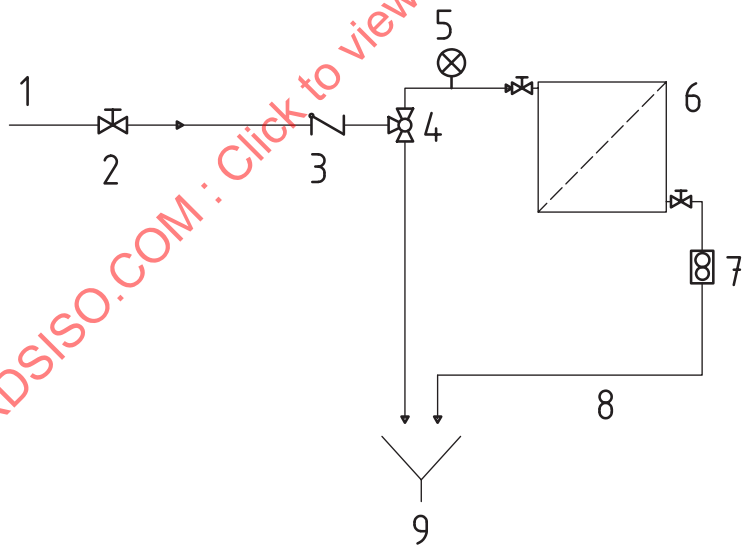
Figure 2 — Schematic setup for sampling non-pressurized systems (e.g. water tanks, reservoirs, small lakes) — Option 1: Submersible pump or positive displacement pump with no plastic parts



Key

1	water tank, deposit, small lake or reservoir	6	container with non-plastic sieves or filters of different mesh sizes
2	tubing or hoses made of stainless steel or other materials which are less present in the environment or not part of the measuring concept	7	pressure gauge 2
		8	flowmeter
3	non-return valve	9	positive displacement pump (e.g. peristaltic pump)
4	3-way L-port valve	10	waste pipe
5	pressure gauge 1	11	drain

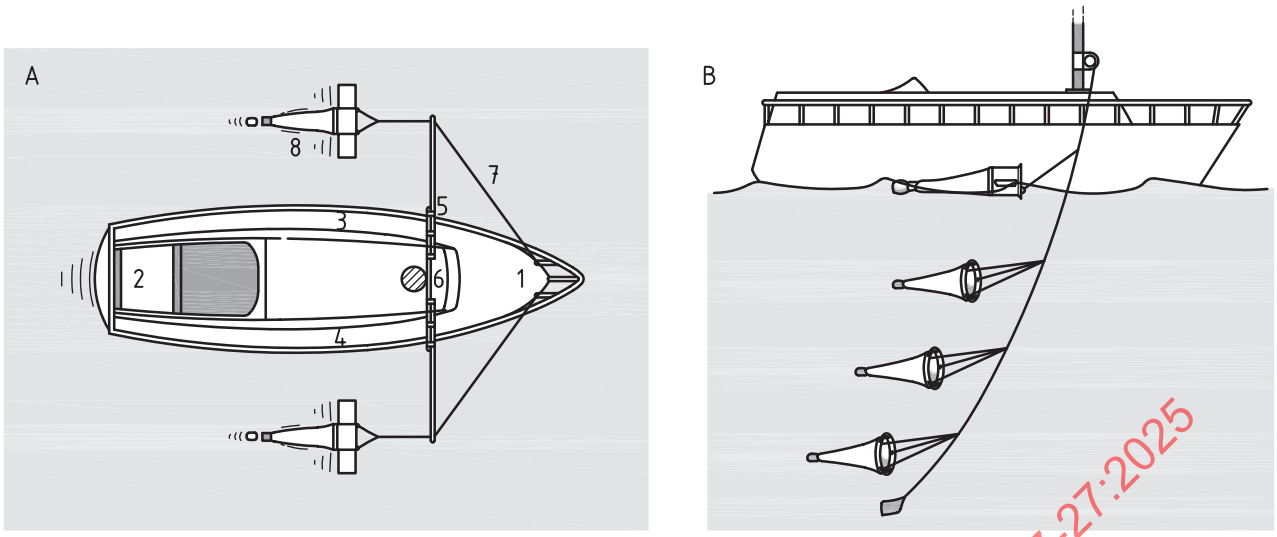
Figure 3 — Schematic setup for sampling non-pressurized systems (e.g. water tanks, reservoirs, small lakes) — Option 2: Using a positive displacement pump (e.g. peristaltic pump) after the filters



Key

1	pressurized line	6	container with non-plastic sieves or filters of different mesh sizes
2	screw valve	7	flowmeter
3	non-return valve	8	waste pipe
4	3-way L-port valve	9	drain
5	pressure gauge		

Figure 4 — Schematic setup for sampling pressurized systems (e.g. tap water from pipes)



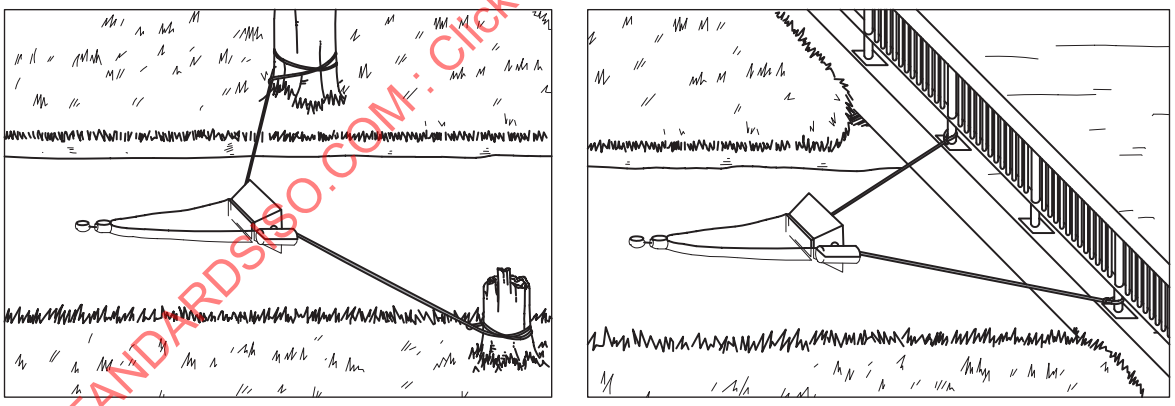
a) Schematic setup for dynamic sampling of surface waters using manta or neuston nets

b) Schematic setup for dynamic sampling of sub-surface waters using vertical hauls, with each net targeting a different depth below the water surface

Key

1 bow
2 stern
3 port
4 starboard
5 clamp
6 boat bollard
7 support rope
8 net

Figure 5 — Schematic setup for dynamic sampling



a) Schematic setup for stationary sampling of surface at a river bank

b) Schematic setup for stationary sampling of surface at a bridge

Floating nets can be fixed with appropriate ropes or resistant cables to the river banks or to structures such as bridges in Figure 6 b).

Figure 6 — Examples of schematic setup for stationary sampling of surface waters using manta, plankton or neuston nets (rivers with variable water regime or potentially unnavigable streams)

7 Sample handling

7.1 General

Working clothes made of natural fabrics (e.g. cotton, linen, wool) should be used whenever possible during sampling, sample transportation and sample handling, to avoid contamination. The use of coloured fabrics is recommended for easy identification in case of contamination.

7.2 Grab sampling method

7.2.1 Grab sampling can capture plastics at the micro- and nano-scales, and can be more suitable in situations where cascade filtration or manta/neuston nets are less practical or difficult to assemble or deploy. This approach also allows better sample collection in intertidal and shallow areas, and targeted sampling of point source pollution.[9][39][40] However, the smaller volume of water usually sampled this way can result in higher variability among samples.

7.2.2 To minimize contamination from airborne dust, it is recommended to prepare all equipment prior to the sampling in a laminar flow cabinet or in a clean room ISO class equal to or greater than 5 (according to ISO 14644-1) where possible.

7.2.3 All glassware and equipment should be washed thoroughly prior to use, first with a diluted suitable cleaning solution (see 5.3) with a natural or non-plastic bristle brush, and then three times with ultrapure water (see 5.1).

7.2.4 The minimum sample volume normally depends on the number and size of microplastics present and on the expected particle quantity.[4] See 4.3 for recommended sampling volumes.

7.2.5 Field blanks should be run according to 4.4.3. Refer to ISO 5667-17:2008, Clause 9 and ISO 5667-14:2014, 11.3 for additional guidance on quality assurance of field samples.

7.2.6 In order to ensure that the samples are as representative as possible and to account for seasonal variations or peak releases, it is recommended to repeat the sample collection at different times of day and/or different times of year (see 4.4.1).

7.3 Cascade filtration methods

7.3.1 Materials should be carefully cleaned and covered directly with aluminium foil when not in use.

7.3.2 To minimize contamination from airborne dust, it is recommended to prepare all equipment prior to the sampling in a laminar flow cabinet or in a clean room ISO class equal to or greater than 5 (as specified in ISO 14644-1) where possible. The filtering sampling rigs should be assembled and disassembled within the laminar flow cabinet or clean room where possible.[41]

7.3.3 All glassware, filters and equipment should be washed thoroughly prior to use, first with a diluted suitable cleaning solution and natural bristle brush, and then three times with ultrapure water (see 5.1 and 5.3). Samples and glassware should be kept covered with aluminium foil.

7.3.4 Field blanks should be run according to 4.4.3. Checks for contamination by running blanks is essential, alongside good field practice that emphasizes cleanliness of apparatus, correct use of materials, correct assembly of pipes/valves/apparatus, use of working clothes made of natural fabrics (e.g. 100 % cotton) whenever possible. See ISO 5667-17:2008, Clause 9 and ISO 5667-14:2014, 11.5.2 for additional guidance on quality assurance of field samples.

7.3.5 The minimum sample volume normally depends on the number and size of microplastics present and on the expected particle quantity.[4] See 4.3 for recommended sampling volumes. The sampling procedure should reliably collect samples at a known flow rate over the desired time interval.

7.4 Net sampling

7.4.1 It is recommended to collect samples when water conditions are as calm as possible. For instance, wind speed: less than 5 m/s, wave height: less than 0,5 m, Beaufort scale: less than 3. This is not always practical in areas prone to high wind. In such situations, metadata such as wind speeds and significant wave heights should be recorded to allow comparisons with other surveys. Additionally, under rough water conditions, it can be difficult to maintain the immersion depth of the nets as constant, and the flowmeter can be unable to measure the filtered water volume correctly because it can sometimes be above the water surface.[6]

7.4.2 It is recommended to avoid sampling when unfavourable conditions such as high densities of natural particles or organisms occur (i.e. algae and plankton blooms). If this is unavoidable (e.g. due to intrinsic characteristics of the water body), it is recommended to consider shortening the tow duration accompanied with repeated towing, alongside frequent washing of the towing nets.[6]

7.4.3 Tidal currents and river inflows can affect the content and composition of microplastics in the water. In addition, care should be taken when sampling in areas near land following rainfall.[6]

8 Procedures

8.1 Grab sampling method

8.1.1 Water column samples can be collected by immersing the collection container (immersion cylinder or dip bottle) to a specified depth and collecting a sample by opening and closing the sampler (not applicable to pressurized tap water systems). Most deep-water collection devices have valves that open when lowering the container or reach a predetermined depth, and close when the rope is pulled back, trapping the sample inside.

8.1.2 When sampling using a telescopic rod and attached scoop, or sampling surface waters using a bottle, the open container should face upstream in relation to the position of the person collecting the sample.[42]

8.1.3 It is important to avoid disturbing the lakebed or riverbed during sample collection to avoid contamination.

8.1.4 If using a mechanical or hand-driven pump, allow a minimum of five litres of sample water to run through the pump and pipes before commencing sampling.

8.1.5 When transferring the collected sample to larger sample containers, extreme care must be exercised to avoid any loss or contamination. Keep the containers closed at all other times with the corresponding lid or sealing cap. If the sealing cap is made of plastic, aluminium foil should be used as additional sealing between the container and the lid. The large sample containers do not have to be rinsed with site water.[42]

8.1.6 Label the sealed containers with the corresponding sample location and volume of water sampled in each container. If possible, cover the glass containers with aluminium foil or other material to protect the collected particles from light exposure.

8.1.7 When possible, store the containers with the collected particles at 3 °C ± 2 °C and away from light until subsequent analyses to minimize microalgae and bacterial growth, as specified in ISO 5667-3. If refrigeration is not possible, add neutral-buffered formalin to the samples in order to prevent the proliferation of microorganisms such as plankton, bacteria, etc. (see 5.2).

8.2 Cascade filtration method 1: Sampling of domestic water, treated wastewater and water samples with low suspended solid content

8.2.1 The filtering sampling rigs should be assembled and disassembled within the laminar flow cabinet or clean room where possible. Sieves should be placed inside the metal container, in decreasing aperture mesh size as water passes through. Two to four sieves should be used. Recommended mesh sizes are between 1 µm to 250 µm for domestic water, or successive filters between 1 µm and 5 mm for treated wastewater. The minimum mesh size will determine the minimum particle size to be captured quantitatively.

8.2.2 Before commencing sampling, allow sample water to run to waste, circumventing the filters/sieves for at least five minutes, to flush the tubing (flush valve to drain, see Figures 2, 3 and 4).

8.2.3 For non-pressurized systems, adjust the delivery of pumped water using the water pump and/or adjusting the screw or ball valve to deliver a flow rate of at least one litre per minute.

8.2.4 For pressurized systems, adjust the flow using the screw or ball valve to deliver a flow rate of at least one litre per minute. See 4.3 for recommended sampling volumes. Repeat sampling collection considering either various times of the day or peak releases, or both are recommended when possible.

8.2.5 Register the flow rate during the sample collection using a flowmeter connected after the container with the filters or sieves. The flowmeter should be able to measure accurately the water flow rate with a maximum error of ±5 %.

8.2.6 When the desired sampling time has been completed, stop the flow of sample and record the total sample volume.

8.2.7 Transport the filter assembly to laboratory. During transportation, the filter assembly shall be stored in a cooling device capable of maintaining a temperature of 5 °C ± 3 °C whenever possible. The time between sampling and start of transport, and transportation time should be recorded.

8.2.8 In the laboratory, in a laminar flow cabinet or clean room where possible, transfer the solid contents of each sieve or filter to glass Petri dishes or glass flasks and cover them with the corresponding lid or sealing cap. If necessary, use a sufficient amount of ultrapure water or mild detergent solution, each filtered through an inert filter with a pore size smaller than 1 µm (see 5.1 and 5.3), to help transferring the particles to the collection glass vessels.

Ultrasound extraction in ultrapure water is also a suitable method to extract the particles from the sieves or filters. If necessary, use a brush with natural or non-plastic bristles. Check that there are no remaining particles on the filters by visual inspection or with the aid of a magnifying tool or stereomicroscope if possible.

Label the sealed glass Petri dishes or flasks with the corresponding mesh size, sample location and total volume of water sampled. Alternatively, and depending on the required analysis, all particles can be transferred to a single glass Petri dish or flask and covered with the corresponding lid or sealing cap. If the sealing cap is made of plastic, aluminium foil should be used as additional sealing between the vessel and the lid.

Fully cover the glass containers with aluminium foil to protect the collected particles from light exposure (amber glass containers can also be used).

8.2.9 Store the covered Petri dishes or glass flasks with the collected particles in a fridge at 3 °C ± 2 °C until subsequent analyses, as specified in ISO 5667-3. If refrigeration is not possible, add neutral-buffered formalin to the samples in order to prevent the proliferation of microorganisms such as plankton, bacteria, etc. (see 5.2).

8.3 Cascade filtration method 2: Sampling of untreated wastewater or water samples with medium or high suspended solid content

8.3.1 The filtering sampling rigs should be assembled and disassembled within the laminar flow cabinet or clean room where possible. Sieves should be placed inside the metal container, in decreasing aperture mesh size as water passes through. Recommended mesh sizes are between 20 µm to 5 mm, but can be adapted depending on the concentration and size of suspended solids in the samples. The minimum mesh size will determine the minimum particle size to be captured quantitatively, which can result in underestimation of the real quantity of microplastics in the sampled water.

8.3.2 Adjust the delivery of water using the water pump and/or adjusting the screw or ball valve to deliver a flow rate of at least one litre per minute (see Figures 3 and 4). See 4.3 for recommended sampling volumes. Sampling at different times and during peak releases is recommended when possible. Taking multiple samples provides a more accurate picture of the heterogeneity of the sampled body of water as well as of variations in the abundance of suspended solids, reducing the standard error.

8.3.3 Register the flow rate during the sample collection using a flowmeter connected after the container with the filters or sieves. The flowmeter should be able to measure the water flow rate accurately with a maximum error of ±5 %.

8.3.4 During the sample collection period, it is recommended to monitor the water pressure continuously [using the pressure gauge(s)] to monitor if the sieves or filters are becoming clogged. If this is the case, sampling should be stopped or an entirely new cascade/set of filter rigs should be replaced to continue sampling. Therefore, having several pre-assembled filter rigs available and ready to be exchanged during sampling is recommended.

8.3.5 When the desired sampling time has been completed, stop the flow of sample and record the total sample volume. Do not include any period in which the sampling was stopped.

8.3.6 Transport the filter assembly and any additional collection vessel with sample (if used) to the laboratory. During transportation, the filters and containers with sample shall be stored in a cooling device capable of maintaining a temperature of 5 °C ± 3 °C whenever possible. The time between sampling and start of transport, and transportation time should be recorded.

8.3.7 In the laboratory, in a laminar flow cabinet or clean room where possible, transfer the solid contents of each sieve or filter to glass Petri dishes or glass flasks and cover them with the corresponding lid or sealing cap. If necessary, use a sufficient amount of ultrapure water or mild detergent solution, each filtered through an inert filter with a pore size smaller than 1 µm (see 5.1 and 5.3), to help transferring the particles to the collection glass vessels.

Ultrasound extraction in ultrapure water is also a suitable method to extract the particles from the sieves or filters). If necessary, use a brush with natural or non-plastic bristles.

Check that there are no remaining particles on the filters by visual inspection or with the aid of a magnifying tool or stereomicroscope if possible.

Label the sealed glass Petri dishes or flasks with the corresponding mesh size, sample location and total volume of water sampled. Alternatively, and depending on the required analysis, all particles can be transferred to a single glass Petri dish or flask and covered with the corresponding lid or sealing cap (if the sealing cap is made of plastic, aluminium foil should be used as additional sealing between the vessel and the lid).

Fully cover the glass containers with aluminium foil to protect the collected particles from light exposure (amber glass containers can also be used).

8.3.8 Store the covered Petri dishes or glass flasks with the collected particles in a fridge at 3 °C ± 2 °C until subsequent analyses, as specified in ISO 5667-3. If refrigeration is not possible, add neutral-buffered

formalin, to the samples in order to prevent the proliferation of microorganisms such as plankton, bacteria, etc. (see 5.2).

8.4 Net sampling

8.4.1 It is important to highlight that, while nets enable the sampling very large volumes of water, they can easily become clogged if the water body contains a high abundance of plankton. When this is unavoidable, it is recommended to consider shortening the sampling duration (see 7.4.2). In the case of dynamic sampling, lower mesh size nets are easily damaged at high towing speed. Moreover, an observer should check the condition of the nets while towing. If clogging of the nets is deemed likely to happen, a reduction of the tow duration is recommended, accompanied with repeated towing.

8.4.2 Nets should be washed thoroughly before each sampling run by using water collected by the water pump system of vessels or boats. If the vessels or boats have no such facility, bringing a water pump and hose to the survey is recommended. The seawater used for cleaning should be filtered (e.g. with a handy plankton net) using a mesh size smaller than the nets used for sampling. The net is cleaned thoroughly from its outside before the start of a sampling run to ensure no plastic particles are left inside the net. A "blank" sampling should be conducted by washing unused nets in the same manner as the nets used for sampling (see 4.4.3) and verifying if microparticles are found in the "blank" nets.

8.4.3 Net immersion depths between 10 cm and 100 cm have been reported in previous studies. Manta net immersion depth is measured as the height of the nets mouth, whereas neuston net is set at about 1/2 to 3/4 of the height of the nets mouth.[6] Immersion depth of the nets can be corrected by adjusting the appropriate floats on each side.

8.4.4 For dynamic sampling, the tow duration can be between 10 min and 30 min at 1 m/s ± 0,5 m/s (1 to 3 knots) speed through water. Sampling duration is affected by the abundance of plankton or suspended matter in the water, but it is recommended to sweep at least 1 000 m^2 per sample. This is equivalent to approximately 200 m^3 to 500 m^3 of filtered water when towed with a typical net.

8.4.5 Smaller rivers with variable water regime or streams which can be unnavigable require stationary sampling. In these cases, floating nets should be fixed with appropriate ropes or resistant cables to the banks, positioned in such a way that the net mouth is skimming the surface (see Figure 6). Nets should be positioned in the opposite direction of the water flow, and weights are used to maintain the nets submerged to a stable depth.[9] Floating nets can be fixed with appropriate ropes or resistant cables to the river banks or to structures such as bridges.

8.4.6 In cases with high waves or currents that can cause the flowmeter to pop above the water surface, it is recommended to manoeuvre the vessel during towing (for dynamic sampling) or adjust the weights of the nets (for stationary sampling) to ensure that the flowmeter remains submerged allowing the filtered water volume to be measured correctly.[6]

8.4.7 After towing, the rotation number of the flowmeter is recorded if it is installed. Then the nets should be washed from the outside by using seawater filtered using a mesh size smaller than the nets used for sampling (see 8.4.2). To avoid contamination of samples and additional rotation of the flowmeter installed in the net mouth, the seawater used for washing should not get inside the net. After washing, all sampled microplastics gathered at the cod end of the nets are collected into a flask. The collecting flask should be rinsed beforehand repeatedly with seawater. An alternative to this approach is to filter the contents of the cod-end over a mesh of corresponding pore size to the net and to wrap the mesh in aluminium foil or in a vessel for storage.[43] Flasks, buckets and every other equipment should be made of stainless steel or other materials which are less present in the environment or not part of the measurement concept. After collecting samples, flasks should be covered with the corresponding lid or sealing cap (if the sealing cap is made of plastic, aluminium foil should be used as additional sealing between the vessel and the lid).

8.4.8 Store flasks with the collected particles in a fridge at 3 °C ± 2 °C until subsequent analyses, as specified in ISO 5667-3. If refrigeration is impossible, add neutral-buffered formalin to the samples in order

to prevent the proliferation of microorganisms, such as plankton, bacteria, etc., and store the sample in a room temperature and dark room.

8.4.9 Microplastics can be reported as the number of particles or weight per unit water volume (/m^3). Tow immersion should also be reported. It is also recommended to report the swept area of the net tow. The swept area of the net tow is calculated as follows:

$$A = W_n \cdot D \quad (1)$$

where

A is the swept area;

W_n is the net width (horizontal);

D is the tow distance.

The use of maps and GPS have proven to be useful to calculate tow distances. See also 4.4.2.

8.4.10 If fibres are observed, it is recommended that they are also recorded separately (alongside the mesh size used) because fibres can usually pass through the nets and the resulting values can cause an underestimation of their total count.[6]

8.4.11 It is recommended to perform at least three replicates for each sample (covering the same swept area in dynamic sampling). The sampling should be repeated at various times of the day or different times of the year, if possible. This is intended to ensure more representative data and to account for seasonal changes that cause variation in the density of microplastics (in principle, applicable to rivers).[9]

9 Sampling report

9.1 For grab sampling and cascade filtration, the sampling report shall contain at least the following information:

a) the sampling method used in accordance with Clause 8 (e.g. 8.1, 8.2, 8.3), together with a reference to this document (i.e. ISO 5667-27:2025);

b) the details necessary to identify the sample;

c) the sample name;

d) the sampling date;

e) the sampling time (start or end);

f) the sampling location;

g) the GPS log input if used (sexagesimal (base 60) notation or decimal notation to input coordinates) – include the start and end positions, if different (see 4.5.1);

h) the sampling equipment, including information on components made of plastic, if any;

i) the filters used or the type of collection containers; include the model number and manufacturer, if known;

j) the mesh aperture sizes and shape of apertures;

k) the flow rate, depth and total volume filtered or sampled;

l) the field blanks (and results from blank tests when available);

m) the weather conditions at the time of sampling (including air temperature) or immediately prior to sampling (e.g. amount of rainfall, cloud, sunshine, snow);

n) the state of floating debris on the water surface (possible obstruction);

o) any specifics noticed during the sampling;

p) any actions not described in this document which can affect the sample collection.

An example of a report for grab sampling and cascade filtration is given in Table 2.

9.2 For dynamic sampling of waters (especially in the ocean) using tow nets, the following information shall be recorded in the sampling report:

a) the sampling method used according to Clause 8, together with a reference to this document (i.e. ISO 5667-27:2025);

b) the sampling date and time (start or end of trawl);

c) the sampling equipment (including information of components made of plastic, if any);

d) the weather conditions during sampling (e.g. amount of rainfall, cloud, sunshine, snow);

e) the sampling location;

f) the GPS log, if used (input style, coordinate at the start or end of trawl);

g) the classification of net frame (type of net frame, model number and manufacturer);

h) the net apertures (shape of net apertures, width of net apertures and height of net apertures);

i) the length of the nets;

j) the mesh (openings, model number and manufacturer, if known);

k) the tow distance (calculation method);

l) the trawl sweep area;

m) the filtered water volume;

n) the tow duration;

o) the vessel speed;

p) the tow position in the boat;

q) the tow distance from vessel;

r) the net immersion depths and whether there were any changes in the immersion depth during tow;

s) the tow direction (N, S, E, W, SE, SW, NE, NW);

t) the blank tests (whether blank tests were conducted, results when available);

u) the direction of the wind;

v) the speed of the wind (Beaufort scale);

w) the vessel movements (heave, pitch and roll);

x) the sea surface temperature;

y) the sea surface salinity;