1. Sampling of microplastics in water by an automated microplastic sampling device

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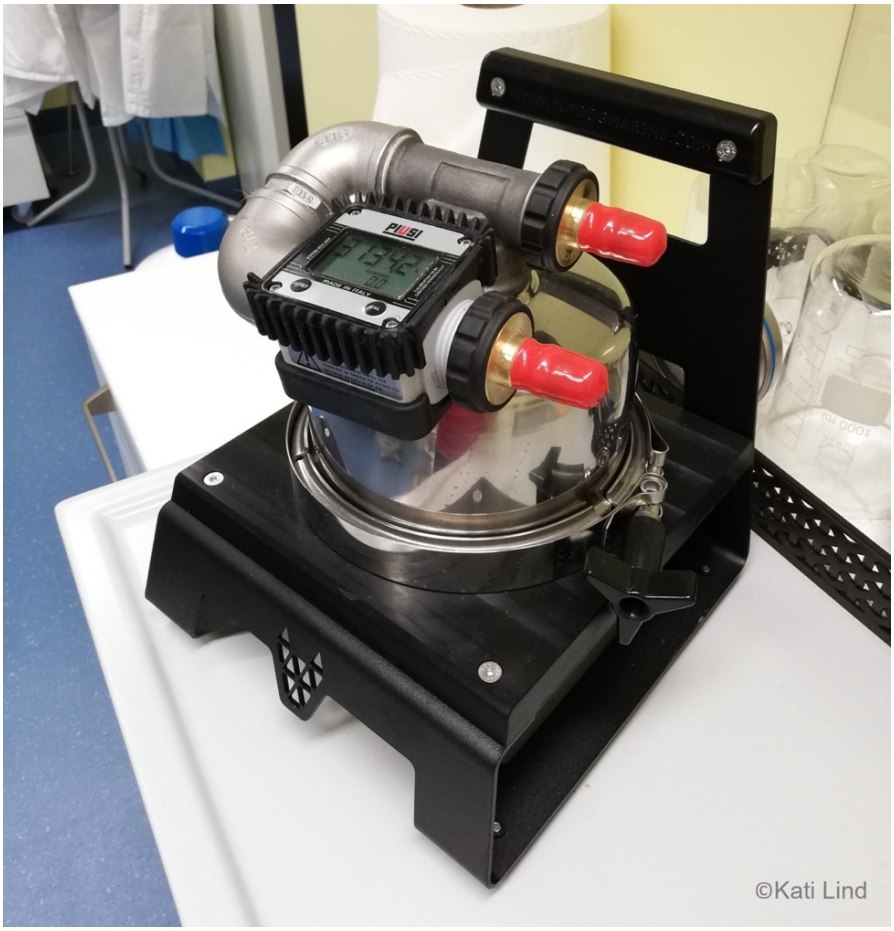
Deliverable 2.1 Analysis techniques for quantifying nano-and microplastic particles and their degradation in the marine environment., as part of the ANDROMEDA project, 2023



1.1 Scope of Protocol

The objective of this protocol is to provide a comprehensive guide for efficient and cost-effective sampling of microplastics (MP) in water using an optimized device attached to flow-through systems on research vessels and ships-of-opportunity (*ferrybox*es). The developed device enables unattended sampling of MP within specific particle size ranges, ranging from 300/100 µm (comparable to Manta and Neuston nets) to 50/100 µm, which are considered environmentally more relevant fractions. By implementing cost-effective sampling and analysis methods, this protocol aims to detect and quantify MPs in environmental samples. The utilisation of *ferrybox* systems can provide the greatest value for money in plastic analysis, offering a promising approach for future monitoring strategies. The developed sampling instrument has the potential to support researchers and policy makers in making informed decisions regarding different MP workflows.

1.2 Materials and Equipment

- Flow-through system or *ferrybox*es on research vessels
- Automated sampling device for MPs
- Sieves with needed size of mesh (recommended 300, 100, 50 µm)
- Collection containers for water samples
- Blank filters to check contamination
- Chemicals for sample preservation
- Sampling recording system or notebook

1.3 Protocol

1.3.1 Construction

The device is a simple flow through add-on for *ferrybox* systems to collect MP samples with commercial sieves (see Figure 1). Its construction consists of outer case which contains inner container (both made from stainless steel) where are, on top of each other, three Retsch 100 mm diameter sieves (top to bottom) – 300 µm, 100 µm and 50 µm mesh. On top of the outer container, there are connectors for seawater inlet and outlet and valves for easy

detachment. Flowmeter is included and connected to the outlet – this can be replaced with different flowmeter if more precise measurement is required.

Connecting the device into existing *ferrybox* system can be done via ¾'' connectors and attaching between existing pipe or hose to the device. It should be connected via separate loop to avoid interfering with other measurements of the *ferrybox* system *i.e.* biological or other environmental measurements that can be affected by the sieves collecting the material before sensors and *vice versa*. The device comes with a flowmeter that must be turned on before the measurement starts as well marking the start and end time. Taking the initial reading and end reading gives the volume in liters passed the system during measurement, knowing the time duration from start to end of measurement time and material collected from the sieves can be then analyzed together with *ferrybox* data.

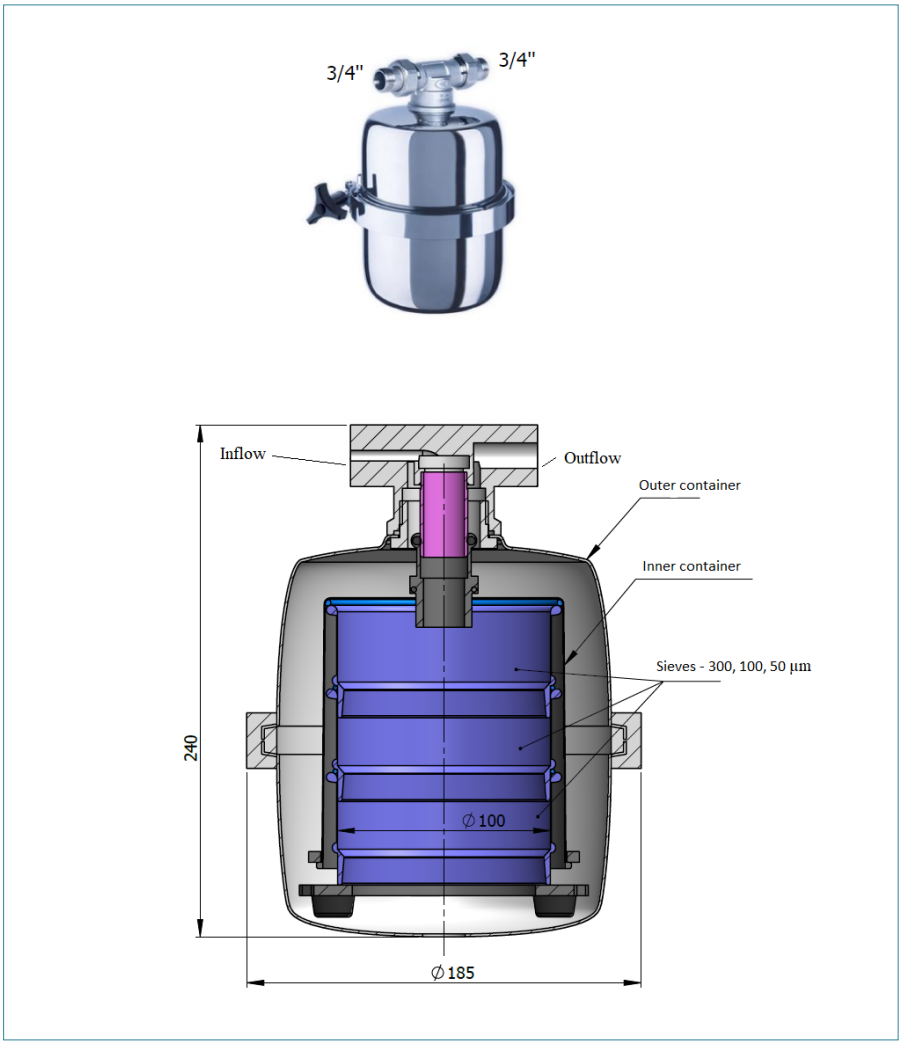


Figure 1: Microsampling device and design by Flydog Solutions LLC

1.3.2 Working Principal

The main aim of this device is to collect micro-litter samples with sieves placed on top of each other and collecting material according to the mesh size. After releasing water to the

device, it goes through a sieve with the biggest mesh size (300 µm), then medium size (100 µm) and finally the smallest mesh size (50 µm). After passing the final sieve, water goes through outflow and registries in flowmeter for volume (l) and speed (l/min).

1.3.3 Assembling the Device

Outer container is hold together with collar that can be tighten or open unscrewing the black thumbscrew (see Figure 2).

Figure 2: Microplastic sampling device outer container with black thumbscrew

After opening the outer container, you might notice some water coming out if additional valve does not have the functionality to extract excess water (depends on the use case of the device). This is expected as the outer container acts as a medium for outflow when water has passed the sieves. Inside the outer case inner container holding the sieves can be found (see Figure 3) by pulling it out.

The inner container is connected to the base with three Hex bolts (Size 4) after unscrewing them, three sieves can be found. Some attention must be paid to the rubber seal and ensuring it stays correctly on top of the sieve when inserting them into the container.

Figure 3: Inner container with three Retsch sieves

1.3.4 Transporting the Device

After collecting the device from *ferrybox* system, the inflow is be covered with aluminum foil to minimize the contamination. To prevent any potential presence of seawater, it is recommended to transport the device in a waterproof box, such as a thermal box.

1.3.5 Sample Collecting

When the device arrives in the laboratory, it is recommended to collect the MP samples as soon as possible to avoid the sieves inside the device from drying. If it is not possible, the collection device must be kept in a tightly sealed waterproof thermal box. Otherwise, when the device is completely dry, the sieves are very difficult to clean and most of the collected material will stick to the sieve.

To collect the samples, unscrew the device and remove the lid covering the sieves, do not remove the aluminum foil from inflow. Remove the rubber sealing on top of the first sieve, and make sure it does not fall into the sieve. Take out the top sieve (size class of 300 µm) and leave the other two (100 and 50 µm) inside the device. Cover the remaining sieves with the covering lid. Make sure they are covered properly so the contamination would be minimized.

Place the sieve (300 µm) upside down on a glass funnel that is on top of a glass jar and rinse it with ultrapure milli-Q water. Repeat the rinsing procedure with the other two sieves. Use

a separate jar for each sieve, and mark the jars with date, sampling event name, device no. and sieve no. For longer preservation time, fix the samples with 37% formaldehyde solution.

The smallest sieve (50 µm) may contain seawater due to a vacuum or clogging by organic material and sediments. Carefully remove the sieve on top of it (100 µm) and let the seawater filter through the 50 µm sieve. When the sieve is empty, repeat the rinsing procedure as described above.

1.3.6 Sample Processing

Before further processing the samples, let them settle to the bottom of the jar. Use a glass pipette to remove the water on top of the settled material and filter it through a glass fiber filter. Use a separate filter for each sieve. After filtering, place the filter in a glass petri dish for future analysis. All petri dishes should be marked the same as the glass jars, where the samples were pipetted from.

Dry the filters in the oven at 60 °C for 15 minutes without the lid of the petri dish. The filters should be placed in the oven while it is still heating up. After drying, cover the filters with the lid and analyze them under stereomicroscope.

Organic material and different types of sediments, such as sand and mud, can potentially influence the analysis of samples. MPs can be hidden or mistaken while identifying them under a microscope. In addition, the analyzing process can also be prolonged. If it is not clear, if the sample contains a lot of organic material or sediments, homogenize the sample by shaking it and filter a small amount of the sample through the glass fiber filter. Dry the sample and analyze it under a stereomicroscope.

If the sample contains a lot of organic material, use hydrogen peroxide (H_2O_2) to process the sample. Depending on the amount of organic material, add 1:1 H_2O_2 solution (~35%) into each sample. Let the samples process at least 7 days at room temperature, then filtrate the solution, dry in the oven and analyze under stereomicroscope.

If the sample contains a lot of sediments, use sodium iodide (NaI) to process the sample. Depending on the amount of sediment, add ~25-50 ml of NaI solution (1,8 g/cm^3) to the settled material. Use orbital shaker to mix the samples at 200 rpm for 10 minutes. After mixing, let the samples settle. Settling time depends on the amount and type of sediment. If the material has settled, use an automatic pipette to collect the surface solution. Pipette the solution through the sieve with the same size class where it was initially taken from. Rinse the sieve similarly as described above, using a glass funnel and ultrapure milli-Q water.

Filtrate the solution through glass fiber filters, dry them in the oven at 60 °C for 15 minutes and analyze under stereomicroscope.

1.3.7 Cleaning the Collection Device

After collecting the samples, the device must be cleaned thoroughly. Wash the device cover, the sieves and the base of the device with tap water, use the sponge if necessary. Afterwards rinse with milli-Q water. Lastly, use the ultrasonic bath to maximize the cleaning process of sieves after every sample collection.

1.4 Quality Control Measures

The samples may be contaminated very easily throughout the transportation and preparation process. To minimize the contamination, the device inflow must be covered with aluminum foil throughout the time when the sieves with collected material are inside. To minimize the contamination from clothes, all the researchers preparing the samples must wear 100% cotton laboratory coats. The sample collecting, processing and analyzing must be carried out in secluded space with minimal traversal (none if possible) by other personnel.

Before opening the device, place one clean filter near the sample preparation as a dry blank sample. A dry blank sample will be used to assess potential contamination from the laboratory air during the sample preparation, processing, and analysis.

To check the contamination during filtering process and in the milli-Q water, filter 100 ml of ultrapure milli-Q water through one clean filter. This filter will be a wet blank sample and treated the same as regular samples.

Note: This protocol provides a general framework for water sampling of MPs using an optimized device. Specific adjustments and considerations may be required based on the study objectives, sampling environment, and available resources. It is crucial to consult relevant guidelines, literature, and expert advice when implementing this protocol to ensure best practices and adherence to specific research requirements.

Citation

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