

Sampling and Identification of Microplastics in Groundwater

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Citation

Kovač Viršek, M., Bizjak, T., Bizjak, N., Mali, N. Sampling and Identification of Microplastics in Groundwater. *J. Vis. Exp.* (225), e68652, doi:10.3791/68652 (2025).

Date Published

November 7, 2025

DOI

10.3791/68652

URL

jove.com/video/68652

Abstract

Microplastics pollution in groundwater remains significantly underreported within scientific literature. This paper presents a comprehensive protocol outlining the methodology for the sampling of groundwater from boreholes, as well as the steps of microplastics separation and analysis. It provides an extensive description of a filtration sampling system designed specifically for this purpose, along with the detailed sampling procedure. In addition, it presents the laboratory analysis of microplastic particles, including their characterization based on size, shape, color, transparency, and chemical structure using attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) and micro-FTIR spectroscopy. Factors that can influence results are discussed, and special attention is paid to preventing contamination of samples. The methodology described also considers the requirements of *the Annex of Commission Delegated Decision (EU) 2024/1441 of 11 March 2024, supplementing Directive (EU) 2020/2184 of the European Parliament and of the Council*. This comprehensive written protocol, accompanied by video guidance, is intended to support the development of a synchronized methodology for monitoring microplastics in groundwater or drinking water. This resource will be of interest to researchers in the field of microplastics worldwide.

Introduction

In recent years, microplastics (MPs) have been identified as an important environmental pollutant. Due to their potential to enter the atmosphere, MPs are part of the water cycle¹. Atmospheric deposition and surface runoff are the main pathways through which MPs enter surface water². MPs originate from various terrestrial and aquatic sources, with

terrestrial sources contributing to 80% of their total quantity³. Upon entering aquatic ecosystems, the majority of terrestrial MPs are transported to oceans via rivers. The remaining MPs persist in freshwater environments, with surface waters in densely populated and urbanized areas-characterized by long water residence times and significant anthropogenic

influence-exhibiting higher levels of MP pollution. Surface waters can also connect to groundwater in riverbeds, floodplains, wetlands, and springs⁴.

Although research conducted in freshwater habitats made up less than 4% of the available literature in 2018⁵, growing recognition of the need to better understand MP sources in aquatic environments has since driven a notable increase in studies focused on freshwater systems⁶. While the number of studies on groundwater remains limited, evidence of the presence of MPs in groundwater has been well-documented. As groundwater is the most important source of freshwater in the world, providing more than two billion people with access to safe drinking water, water for domestic, agricultural, and industrial use⁷, the presence of MPs in groundwater raises new questions about groundwater safety in the 21st century⁸.

Terrestrial sources of MPs can be very diverse, with fibrous MPs from the washing of textiles accounting for approximately 35% of the MPs detected in aquatic systems⁹. Other important sources of different types of MPs in the environment include personal care and cosmetic products, tires, agricultural plastic films, artificial turf and road coatings, landfills, improperly disposed plastics, packaging, and construction industry pollutants^{9,10}.

Due to their many different sources, MPs can differ substantially in terms of their chemical composition, color, shape, density, size, and other characteristics¹¹. International standard ISO 24187:2023 classifies MPs into two categories: 1) "large microplastics": solid, water-insoluble plastic particles with sizes ranging between 1 mm and 5 mm, and 2) "microplastics": any solid plastic particles insoluble in water with sizes ranging between 1 µm and 1 mm. Particles smaller than 1 µm are considered nanoparticles¹². The polymer materials most frequently found in the form of MPs

are polyethylene (PE) and polypropylene (PP), as the most widely produced polymer materials¹³.

MPs can be transported into groundwater from soils and sediments, via interactions with surface water and seawater, and through recharge/discharge processes. During these processes, MPs can be transported into and from the groundwater¹⁴. The hyporheic zone (HZ) serves as an important interface for the exchange between the river and the shallow groundwater system¹⁵. The transport of MPs through the HZ is influenced by various particle properties (size, shape, material composition), as well as certain hydrological and geochemical factors, including riverbed morphology and flow turbulence¹⁵. The relationship between pore diameter and MP size is another crucial factor in these processes¹⁶, as MPs with smaller dimensions move more easily through the pore space from the surface into the subsurface layers^{15,16}.

MPs can enter groundwater through the unsaturated zone¹⁷. The unsaturated zone represents an important connection between the land surface and the groundwater¹⁸. The processes of transport and retention of MPs in the unsaturated zone depend on particle properties, soil properties, and environmental factors^{17,19,20}. Soil organisms such as earthworms, collembolans, and mites can influence the transport of MPs from the soil surface to deeper layers through various mechanisms such as displacement, ingestion, engulfment, and adherence^{21,22}. Invertebrates can indirectly affect the transport of microplastics by forming macropores in the soil, which act as pathways for microplastic movement through leaching²¹.

The primary concern regarding the presence of MPs in groundwater is their persistence related to the large surface area, less environmentally friendly degradation processes (leading to the formation of micro- and even nano-sized

particles), and strong hydrophobicity¹⁷. Their persistence causes a potential risk of affecting groundwater quality from a chemical and biological perspective. MPs can chemically pollute groundwater by leaching unbound monomers and additives, as well as sorbed chemicals from the environment (e.g., hydrophobic persistent organic pollutants)²³. MPs can also serve as a substrate for biofilm formation and influence the microbiology of groundwater. Biofilms on MPs may also contain free-living microorganisms and pathogens²³. If ingested, the particles themselves represent a physical hazard. The smaller the particle size, the greater the possibility that they will be absorbed into cells or cross biological barriers of organisms²³.

Research into MPs in groundwater is increasingly recognized as critical due to the potential risk that MPs pose to human health. Consequently, the revised Drinking Water Directive for measuring MPs entered into force in January 2021. EU Member States were obliged to transpose the Directive into national law and ensure compliance with its provisions by 12 January 2023. Nevertheless, the number of studies on MPs conducted to date remains limited. There is currently no standard procedure for sampling and analyzing MPs in groundwater. Studies assessing the occurrence of MPs in groundwater are difficult to compare as they use different sampling and analytical approaches. Therefore, recent studies have highlighted the urgent need to standardize the protocols for MPs sampling and analysis to ensure the collection of high-quality samples and obtain comparable results^{12,17,24,25}.

Filtration-based sampling and grab sampling are two commonly used methods for collecting groundwater samples in MP studies to date. Filtration involves passing water through mesh filters, either stainless steel cartridges²⁶

or sieves²⁷, in the field to capture MP particles. A major drawback of using cartridges and sieves is the difficulty in thoroughly cleaning them of residual particles, which compromises the ability to ensure complete sample analysis and increases the risk of cross-contamination. Grab sampling, frequently used in many studies^{28,29,30}, is a simpler approach where water is collected directly using bottles or containers without pretreatment. While suitable for exploratory studies, grab sampling does not accurately reflect MP concentrations due to small sample volumes.

This study introduces a newly developed system for sampling MPs in groundwater (**Figure 1**), based on field filtration using commercially available filters of customizable pore sizes. The system enables simultaneous filtration of multiple samples and supports cascade filtration. Designed as a fully enclosed setup, it effectively prevents environmental contamination of samples. A detailed sampling protocol is provided, accompanied by video guidelines, along with procedures for subsequent analysis of the chemical composition and other characteristics of the detected MPs. The system aims to enhance the quality, consistency, and comparability of future research in this field.

Protocol

1. Preparing the borehole for sampling

NOTE: To prevent contamination in the field, keep the filtering system closed except when inserting the filters or taking samples. Avoid the use of plastic tools and containers. Avoid synthetic clothing (e.g., fleece); wear a white cotton lab coat.

1. Open the borehole and remove any samplers, if present. Measure the groundwater level using a water level meter.

2. Record the GPS coordinates of the borehole, the date of sampling, the water level, and other sampling details on the data sheet (**Table 1**).

2. Setting up the sampling equipment

1. Assemble and carefully lower the submersible pump into the well to the desired depth. Ensure that the pump intake is positioned approximately 1 m above the top of the filter screen to prevent sediment intake and maintain optimal water flow.

NOTE: During installation, handle the pump with care to prevent damage to its wiring or mechanical components.

2. Set up the filtering system without the filter support screens and filters and place it horizontally.
3. Close the main valve and open the bypass valve.
4. Connect the water supply hose.

3. Cleaning the borehole

1. Start the submersible pump and let the water flow through the bypass to clean the borehole. Pump at least 3x the volume of water present in the borehole or continue pumping until physicochemical parameters stabilize, to ensure fresh groundwater is sampled.

4. Cleaning the filtering system before sampling

1. Open the valves of the sampling branches and the main valve of the filtering system. Afterwards, close the bypass valve.

NOTE: Allow water to flow through the filtering system for a sufficient duration to ensure effective flushing, which may depend on the pumping rate. During the cleaning process, the filter support screens and filters must be removed.

5. Inserting the filters

1. First open the bypass valve and then close the main valve.
2. Open the filter chamber and check that the filter chambers are clean. Rinse with ultrapure water, if necessary.
3. Insert the filter support screen.
4. Rinse the filter of the desired pore size with ultrapure water and place it on the filter support screen.
5. Close the filter chamber.
6. Repeat the process for all branches.

NOTE: Several filters with decreasing pore sizes can be inserted sequentially for cascade filtration. Additional filter chambers with filters of the same pore sizes as the sampling filters can be subsequently installed. This is required by *the Annex of Commission Delegated Decision (EU) 2024/1441 of 11 March 2024, supplementing Directive (EU) 2020/2184 of the European Parliament and of the Council* for sampling MPs in drinking water, which mandates the use of 100 µm and 20 µm filters followed by another set of 100 µm and 20 µm filters (as blanks for quality control purposes).

6. Sample collection

1. Read and record the water meter or reset it to zero.
2. Open the main valve, close the bypass valve, and mark the start time of the sampling.
3. Monitor the pressure gauge during sampling to ensure the pressure does not exceed four bars to avoid damaging the equipment and minimizing MP fragmentation.

4. Stop sampling when the planned water volume is filtered or when the filters start to clog, indicated by increased pressure or significantly decreased flow.
5. To stop sampling, first open the bypass valve and then close the main valve and the valves on the filtering branches.
6. Turn off the pump.
7. Record the time and final readings from the water meter.

NOTE: To obtain a representative groundwater sample at the sampling point, it is recommended to sample larger volumes of water, e.g., at least 1 m³/replicate.

It is imperative to continuously monitor the entire sampling system, particularly the water meter and pressure gauge, throughout the sampling procedure. An increase in pressure accompanied by a decrease in water flow indicates that the filter is beginning to clog. When this occurs, it is advisable to stop the system and replace the used filters with new ones. By collecting the sample across multiple filters in this manner, the desired volume of filtered water can still be achieved efficiently.

7. Collecting the filters

1. Rinse a glass Petri dish with ultrapure water.
2. Open the filter chamber and carefully transfer the filter maintained in a horizontal position, into a clean Petri dish.
3. Seal the Petri dish with a sealing film and label it with the sample name and the date of sampling.
4. Repeat this procedure for all filter chambers (**Figure 2**).
5. After the sample collection, disassemble the system. Always rinse the system with freshwater and dry it before storage.

NOTE: Alternatively, store the entire filter chamber component of the filtering system for further processing in the laboratory.

8. Separation of microplastics from samples

NOTE: To prevent contamination in the laboratory, before starting the analysis of the samples, make sure the laboratory is cleaned of dust, close the window, and use air conditioning with a HEPA filter. Avoid using plastic tools and containers. Glassware must be rinsed with ultrapure water and checked under the microscope before use. Use non-synthetic clothing. Wear a white cotton lab coat to minimize contamination.

NOTE: The stereomicroscope must be equipped with a camera and image analysis software that enables accurate particle size measurement.

1. Remove the sealing film and open the Petri dish.
2. Transfer the Petri dish under the stereomicroscope with at least 30x magnification and search for potentially plastic particles, focusing on characteristics such as color, shape, and other visible features.
3. Transfer each particle that seems to be plastic, take a photo, and measure its size. Evaluate the following properties for each MP particle: size (particles: feretmax or area-equivalent diameter; fibers: width and length), shape (particles: fragment, film, foam, pellet, granule; fibers), color, and chemical composition (methodology is described below) (**Figure 3**).

NOTE: Keep in mind that some MPs will be easily identifiable by their color and shape, while others may be more challenging.

The main parameters for MP identification are described in the protocol for MP sampling on the sea surface and sample analysis²⁶. Polarizing light can be very helpful

in separating MP from sediment and organic particles. When isolating potential MPs from the samples, opt for a conservative approach by selecting more rather than fewer particles for detailed analysis.

9. Chemical identification of microplastics

NOTE: Chemical analysis of potential MP can be conducted for large MPs (1-5 mm) using ATR-FTIR and for small MP (<1 mm) using micro-FTIR. Alternative methods, such as Raman spectroscopy, are also possible.

The FTIR instrument software should support precise control of measurement parameters and real-time spectral data acquisition, along with advanced processing tools such as background correction and smoothing. It must include a comprehensive polymer library and enable reliable identification of substances through spectral library comparison to support accurate analysis of microplastics in environmental samples, particularly in complex matrices such as groundwater.

1. ATR-FTIR spectroscopy

1. Before beginning the analysis, thoroughly clean the ATR crystal and sample pressor using 70% alcohol and a lint-free cloth.
2. Configure the measurement settings to typically 16 scans with a wavenumber ranging from 4000 to 450 cm^{-1} and a resolution 4 cm^{-1} . Then, collect the background spectrum. Place the particles one by one onto the ATR crystal, apply pressure, and initiate the measurements.
3. Match the acquired infrared spectra with those in reference libraries to confirm the particles as MP. Typically, a 70% correlation with library spectra

is considered sufficient for positive identification (**Figure 4**).

4. Export the obtained data for further analysis and reporting.

2. Micro-FTIR spectroscopy

1. Ensure all relevant parts of the instrument, such as the stage, are cleaned with alcohol and a lint-free cloth, prior to analysis.
2. If sampling has not been conducted directly on a surface suitable for the selected measurement mode, such as ATR or reflection, place the potential plastic particles on an appropriate reflective surface, such as gold- or aluminum-coated membranes or microscope slides.
3. Select the measurement settings for the session, including the number of scans, spectral range, resolution, and session name. Locate the sample and capture a mosaic image of the area where all particles are situated.
4. For reflection measurements, first measure the background before measuring the infrared spectra of the potential plastic particles.
5. Identify and mark the points where the infrared spectra of the selected particles will be measured. If necessary, select multiple points on each particle. After selecting all the points, start the measurement.
NOTE: It is also feasible to obtain infrared images of larger areas of interest, particularly if the sample is collected directly on a suitable infrared measurement surface.
6. Compare the collected infrared spectra with those from reference libraries. Choose a threshold value

for establishing the presence of MPs. Typically, a 70% match is sufficient for positive identification.

7. Export the obtained data for further analysis and reporting.

Representative Results

The first results of this protocol is the database of all MPs found in each sample (**Table 2**), which can be used for further analysis of the quantity of MP and its properties (color, size, shape in material composition).

The primary objective of MP sampling and sample analysis is to determine the quantity of MP particles per sample (**Figure 5**). These data can subsequently be normalized per cubic meter (m^3). The normalization formula is as follows:

$$\text{MP particles per m}^3 \text{ per sample} = N / V$$

Where:

N = sum of MP particles per sample

V = sample volume (m^3)

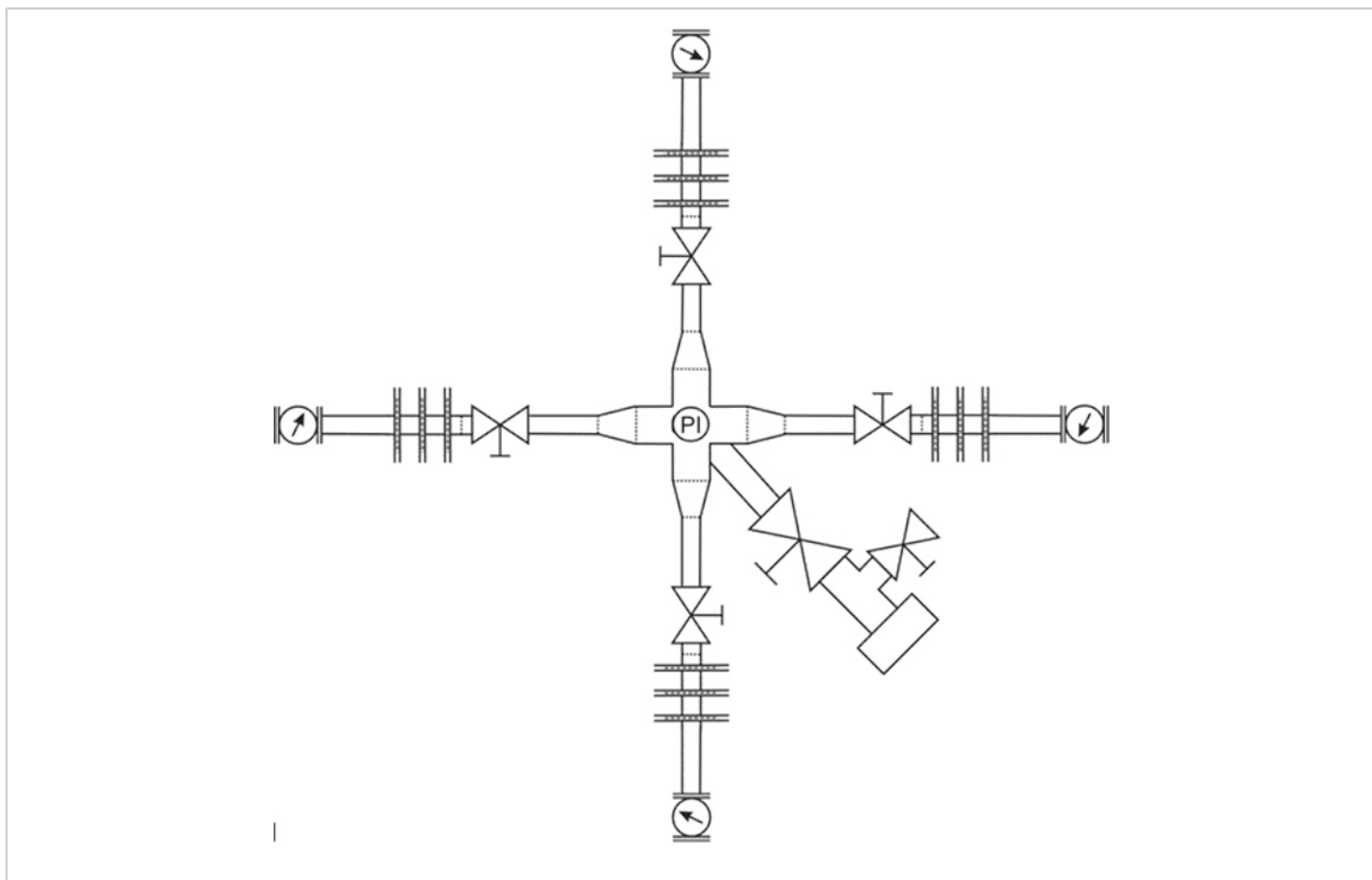


Figure 1: Schematic of the sampling system used in the protocol. The sampling system consists of an inlet pipe with three legs, wherein one leg is arranged for connection to a pump, a second leg is arranged for connection to a distribution unit, and a third leg is arranged to ensure a bypass of the water past the sampling units. The distribution unit has four symmetrically arranged branches for connection to the corresponding sampling unit, wherein in the center of the distribution unit, a pressure measuring device is installed. Each sampling unit is provided with a valve, three filter holders, and a flow meter installed downstream of the filter holder to prevent sample contamination. [Please click here to view a larger version of this figure.](#)

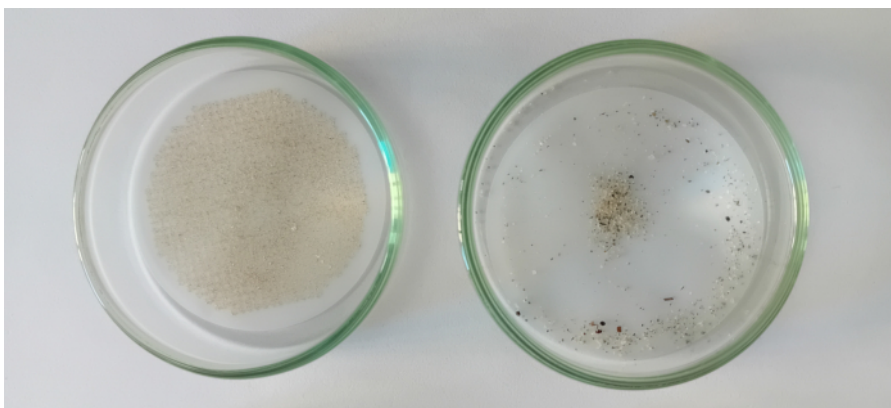


Figure 2: Example of filters after 1 m³ of sampled groundwater. Left: nylon net filter with a pore size of 100 µm, right: nylon net filter with a pore size of 20 µm. Filters can differ in the amount of sediment and organic particles, depending on the sampling location. [Please click here to view a larger version of this figure.](#)

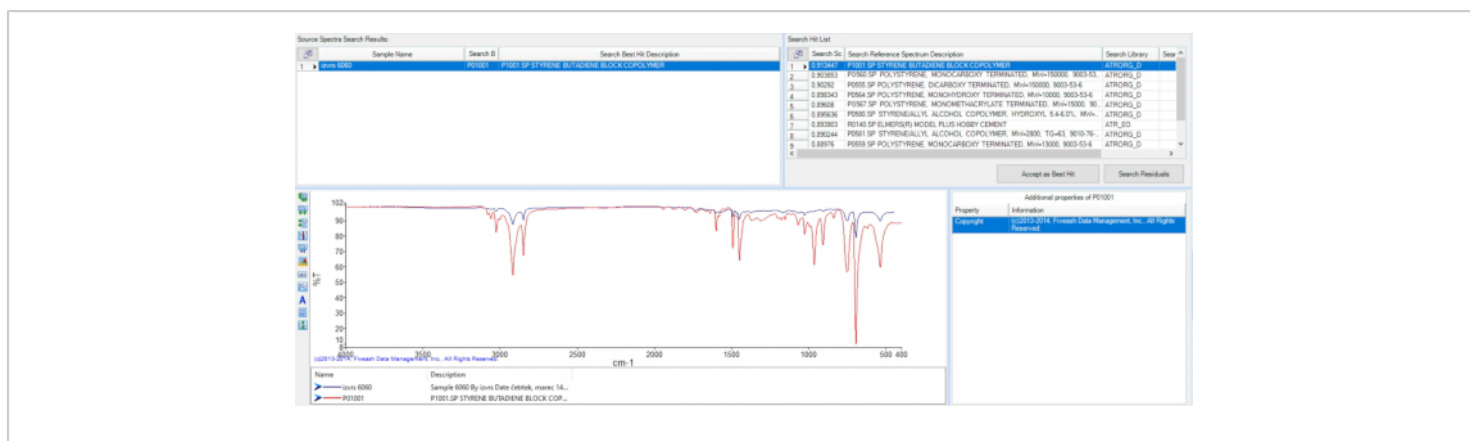


Figure 4: Example spectra measured on a selected particle with marked peaks and their wavelengths [cm^{-1}], compared with a spectral library. The sample spectrum should show at least 70% correlation with the reference spectra in the library. [Please click here to view a larger version of this figure.](#)

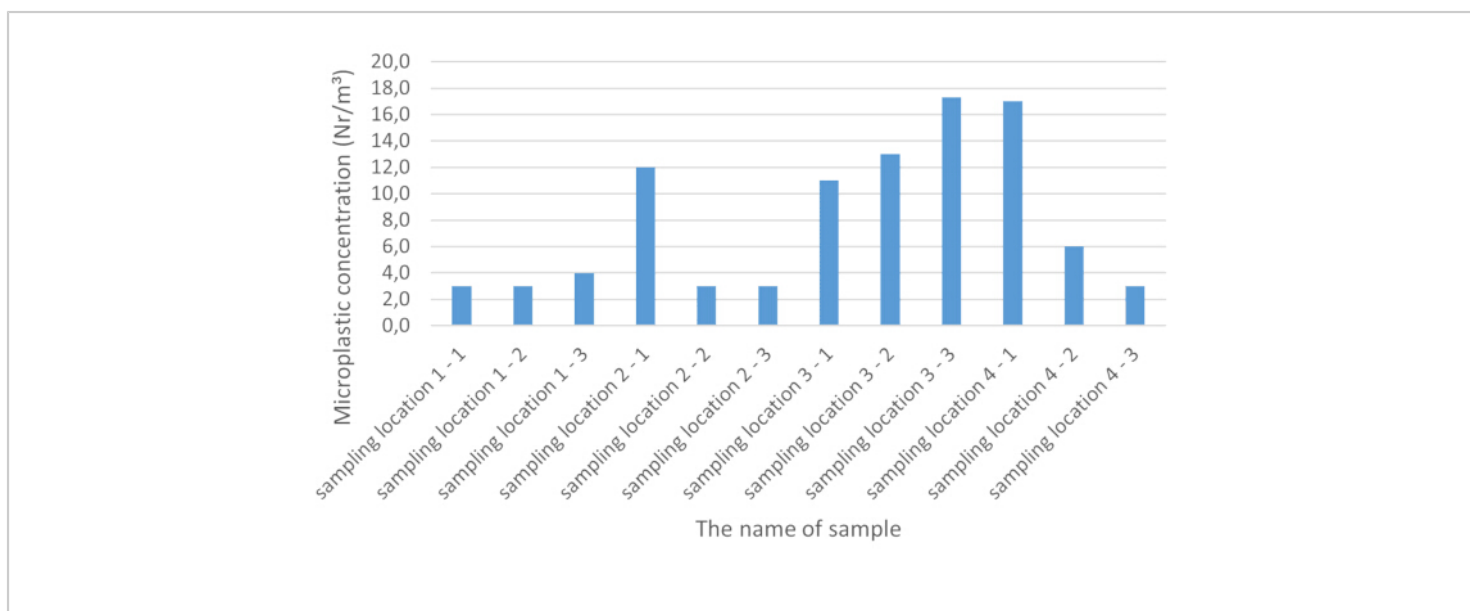


Figure 5: Example results of the number of microplastics per m^3 per sampling location. [Please click here to view a larger version of this figure.](#)

Location: Borehole 1		Date: April 1		Researcher(s): Researcher 1, researcher 2, researcher 3	
GPS coordinates: 46.056946 N 14.505751 E					
Project: Groundwater sampling					
Weather conditions: Sunny					
Temperature (°C): 20					
Samples					
Sample ID	GW1.1	GW1.2	GW1.3	GW1.4	
Filtration position	1	2	3	4	
Filter type	Nylon - 100 µm, 20 µm	Nylon - 100 µm, 20 µm	Nylon - 100 µm, 20 µm	Nylon - 100 µm, 20 µm	
Sampling depth (m)	25	25	25	25	
Start time	10.00	10.00	10.00	10.00	
Water meter START (m³)	437.4199				
End time	10.45				
Water meter END (m³)	438.421				
Sampled water volume (m³)	1.001				
Other					

Table 1: Example sampling data sheet, including parameters such as location, date, environmental conditions, and data related to water filtration.

Location	Sample	Particle ID	Shape	Size (mm)	Color	Chemical composition	% match	Priority polymers	Instrument	Spectrum	Other
Borehole 1	Borehole1_1_20242204	1	particle	0.54	black	Polytetrafluoroethylene	80.2	PTFE	Spectrum two	abc1111	
Borehole 1	Borehole1_1_20242204	2	fiber	0.98	blue	Polyethylene terephthalate	91.9	PET	RapitIR	abc1112	
Borehole 1	Borehole1_2_20242204	1	fiber	1.54	blue	Polyamide	75.0	PA	RapitIR	abc1113	
Borehole 1	Borehole1_2_20242204	2	fiber	2.87	red	Polyethylene terephthalate	98.0	PET	RapitIR	abc1114	
Borehole 1	Borehole1_2_20242204	3	fiber	3.04	red	Polyethylene terephthalate	71.3	PET	RapitIR	abc1115	
Borehole 1	Borehole1_2_20242204	4	fiber	1.27	blue	Polyethylene terephthalate	83.6	PET	RapitIR	abc1116	
Borehole 1	Borehole1_3_20242204	1	particle	1.93	white	Polyethylene	85.3	PE	Spectrum two	abc1117	
Borehole 1	Borehole1_3_20242204	2	particle	0.96	blue	Polyethylene	89.4	PE	Spectrum two	abc1118	
Borehole 1	Borehole1_3_20242204	3	fiber	0.54	blue	Polyamide	73.2	PA	RapitIR	abc1119	

Table 2: Example database of all isolated microplastic particles per sampling, including parameters such as shape, size, color, and material for each particle.

Discussion

The laboratory analysis of MPs in this protocol follows the Annex of Commission Delegated Decision (EU) 2024/1441 of 11 March 2024, supplementing Directive (EU) 2020/2184 of the European Parliament and of the Council by establishing a methodology to measure MPs in water intended for human consumption (Annex of Commission Delegated Decision (EU) 2024/1441) and Guidance on the Monitoring of Marine Litter in European Seas³¹ developed for the implementation of Marine Strategy Framework Directive (MSFD). Upon

completion of this protocol, each MP particle or fiber with its own ID is documented with an image, spectra, and all acquired properties. Each MP particle is described in terms of its shape, size, color, transparency, and chemical composition (polymer type).

According to the Annex of Commission Delegated Decision (EU) 2024/1441, MPs are divided into two types of shapes: particles and fibers. Particles can be further classified into fragments, films, foams, pellets, and granules. This classification is used in monitoring MPs for the MSFD.

The differentiation between different kinds of MPs shapes is described in MSFD guidelines³¹ and video protocol for sampling MP on the sea surface³². The size of each particle is measured as the maximum Feret diameter³¹ or area-equivalent diameter (Annex of Commission Delegated Decision (EU) 2024/1441), while the dimensions of fibers are recorded in terms of their length and width³¹. Although color and transparency are not included in the data acquired per the Annex of Commission Delegated Decision (EU) 2024/1441, they are included in the list of MPs properties collected for MSFD. The classifications are outlined in Tables 7.3 and 7.4 of the MSFD guidelines³¹, respectively.

Based on the result of the spectrum search in the spectrum library, MP particles are categorized into one of the priority polymer groups listed in the Annex of Commission Delegated Decision (EU) 2024/1441 Section 1, points (14) and (15), or classified as other materials under Section 1, point (15). The chemical composition of fibers should be analyzed only if their dimensions and the instrument capabilities allow for positive identification of polymer type; otherwise, they shall be indicated as unidentified fibers.

Monitoring MPs in groundwater is limited to a small number of research studies with differing methodologies that may affect the final results¹⁴. Key factors include: 1) flushing before sampling, 2) pump type, 3) filtration system, 4) filter material and pore size, 5) sampling volume, 6) sample treatment, 7) MPs detection and quantification, and 8) quality control. These factors and their characteristics are discussed in more detail below.

In most existing studies, prepumping (flushing) before sampling is not mentioned. But information about it should also be provided³³, since purging has a significant impact on the concentration of MPs²⁸. Our protocol includes

prepumping, as this process is crucial for sampling fresh groundwater from the aquifer rather than stagnant water accumulated in the borehole. To effectively clean the borehole, it is necessary to pump out 2-3x the volume of the borehole or continue until the physicochemical parameters of water stabilize¹⁴. The water sample should be collected approximately 1 m above the top of the filter screen to prevent sediment intake and ensure the homogeneous sample.

Collecting groundwater samples from boreholes requires the use of a pump. The flow rate is strongly influenced by the sampling depth, the type of pump used, and the condition of the borehole, which is closely linked to the location-specific water characteristics-such as sediment content-as well as the prevailing and recent weather conditions at the time of sampling.

Typically, most pumps contain plastic parts, which can potentially contaminate samples, yet most studies do not specify the type of pump used. Pumps with all components made of stainless steel are recommended. If this is not possible, the materials should be specified in terms of their chemical composition using FTIR analysis. The spectra of materials used must be included in the library, and the particles from the samples must be compared against them to account for potential contamination.

For pumps equipped with impellers, larger MP particles may be damaged or even fragmented as they pass through the pump. To avoid overestimating the presence of MPs, it is essential to assess whether the sampling pump contributes to the fragmentation of plastic particles. This can be tested by introducing a known number of well-characterized MP particles into previously cleaned water and pumping it through the filtering system. After pumping, the particles should be re-characterized to determine if any physical changes-

such as fragmentation or surface alteration-have occurred. This validation step helps ensure that the sampling process does not artificially increase MP counts due to mechanical degradation.

To provide a large volume of sampled water, a filtering system suitable for use in the field is necessary. In our protocol, to avoid contamination, the filtering system (the sampling system is patented under SLO-P-202300155 and is patent pending EPO-EP24217168.4) is made of stainless steel with all its components welded and does not include Teflon (PTFE) or any other insulating tape made of plastic materials. As filters can clog very quickly, cascade filtration is included as an option, which can also allow direct distribution of particles into desired size classes. Typically, filters with pore sizes of 100 μm and 20 μm or 10 μm are used. Additional filters can be added to address possible clogging issues (e.g., an additional filter with pores between 100 μm and 20 μm). The Annex of Commission Delegated Decision (EU) 2024/1441 recommends cascade filtration with four stages: the first filter has pores of 100 μm , the second 20 μm , the third 100 μm , and the fourth 20 μm . The first two filters are used for MPs analysis, and third and fourth are used for the assessment of MPs contamination levels (sampling quality control) and to ensure that all particles have been filtered by the first two.

The flowmeter (particularly if it is a mechanical one) should be installed after the cascade of filters for sampling to accurately measure the volume of sampled water. During sampling, it is essential to monitor system pressure. If the pressure exceeds 4 bars, this indicates filter clogging, and the system must be shut down immediately to prevent damage to the filter or the system. To avoid additional fragmentation of MPs, sampling should be conducted at the lowest possible pressure.

As mentioned, the Commission Delegated Decision (EU) 2024/1441 proposes using 100 μm and 20 μm filters. In this study, nylon filters were used. However, in this case, it is necessary to collect the MP particles from the filters and transfer them directly to ATR-FTIR or reflective slides suitable for working in reflection mode with FTIR or Raman microscopy. Nylon net filters are effective for filtering through filters with pore sizes of 100 μm , as particles of this size can be manually handled. However, when using 20 μm filters, silicone or aluminum-coated filters are recommended to facilitate measurements in transmission or reflection mode using FTIR or Raman microscopy.

The literature suggests sampling at least 500 L of water to avoid underestimating the presence of MPs³⁴. According to the Annex of Commission Delegated Decision (EU) 2024/1441, 1 m^3 of water should be sampled. The recommended volume needs to be considered to avoid overestimating the MP concentration in the sample. This large volume of water increases the risk of filter clogging, making cascade filtration highly recommended.

The samples from the presented system are immediately prepared for MPs analysis using a stereomicroscope. This is a very time- and cost-efficient method. Many studies detail pretreatment steps to remove sand and organic matter; however, these procedures are time-consuming and may involve the use of toxic chemicals. Such pretreatment steps may also negatively affect the results as they lead to additional fragmentation or loss of particles, especially smaller ones (< 100 μm)³⁵. Therefore, it is preferable to avoid these steps unless necessary. Such instructions are also described in the Commission Delegated Decision (EU) 2024/1441, where it is proposed that sample analysis by FTIR micro-spectroscopy may be done directly on the original

collection filters, if they are compatible with the analytical method used, and a flotation step is used if necessary.

First, filter samples are analyzed with stereomicroscopes, whereby each particle is photo documented, ID-labeled, measured for size, and assessed for shape. The determination of shape is important as it can provide information about the source of the MPs. Fibers typically come from textile washing and originate from wastewater treatment plants, while fragments typically come from the degradation of large plastic particles in the environment. In the second part of the analysis, the chemical composition of each particle is determined using ATR-FTIR or FTIR/ Raman microscopy. Generally, all particles from the filter to reflective slides are transferred to perform analysis in reflective mode with FTIR microscopy. If the sample is collected on a 20 μm silicone filter, direct analysis with the Raman or FTIR microscope can be performed, provided the filter is not excessively covered with sand particles. If the particles are embedded in sand, density separation and/or chemical or enzymatic treatments may be applied to reduce the presence of non-plastic materials such as minerals, metal oxides, and natural organic matter. MP particles must be dried before measurement, as water strongly absorbs IR radiation.

To ensure the credibility of the results, the following quality control elements must be considered: First, air pollution prevention: to prevent atmospheric contamination during sampling, a closed filter system is essential. To avoid airborne contamination in the process of analysis, a white cotton lab coat and air filtration through a HEPA filter are required. Second, prevention of contamination by the filter system: the only component of the system where polymers are unavoidable are the hoses. Silicone hoses are recommended and should be replaced in case of visible damage. It is

necessary to know the components of the pump and test the pump for potential contamination of samples if polymer presence is established. Third, prevention of contamination during laboratory work: only laboratory equipment made of glass and washed with ultrapure water is suitable for MPs analysis. Fourth, parallel samples to ensure reproducibility and statistical significance of the results: this filtration system allows simultaneous sampling from four branches. A minimum of three parallel samples should be obtained to ensure the same conditions for all samples and to save time. Fifth, blank samples must be included in all steps of sampling and laboratory processes. During the field sampling, blank filters 100 μm and 20 μm are installed after 100 μm and 20 μm sample filters, as proposed in the Annex of Commission Delegated Decision (EU) 2024/1441. Blank samples obtained in the sampling procedure must be considered in all analytical steps by following the same procedure.

The protocol outlined in this paper meets all the requirements set forth by the Commission Delegated Decision (EU) 2024/1441 and, therefore, provides an excellent resource to all researchers studying or monitoring MPs in groundwater. The filtration system described can also be used for freshwater or marine waters, as it enables sampling of MPs at different depths. The sampling method is simple, user-friendly, and provides fast and accurate results in most cases without needing pretreatment of the sample.

Disclosures

The authors have no conflicts of interest to disclose.

Acknowledgments

The development of this protocol was funded by the Slovenian Research and Innovation Agency under the research project "Improved methods for determination of transport

processes of MPs in groundwater resources" (GWMicroPlast) (J1-50030) and the research program Groundwater and Geochemistry (P1-0020), as well as by the Research Science Funds project "Microplastic in the geosphere" (Manca).

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