Output 2.3

Microplastics in drinking water obtained from surface/river bank filtration water resources in Danube Region

Drafted by Eurofins Environment Testing Hungary

Elaborated with input provided by all project partners

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Project MicroDrink

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1. Introduction

MicroDrink (Capacity building for management and governance of MICROplastics in DRINKing water resources of Danube Region) addresses the growing concern of microplastic (MP) pollution in the Danube River Basin (DRB). Each year, large amounts of microplastics are released into the environment worldwide and in Europe, and international monitoring campaigns – such as the Joint Danube Survey 4 – have revealed widespread MP contamination along the Danube, its major tributaries and aquatic organisms. However, occurrence and impacts of microplastics in surface water and groundwater resources used for drinking water supply in the DRB remain largely unexplored.

The **main objective of MicroDrink is to enhance capacity building and governance at different levels for the management and prevention of microplastic pollution in drinking water resources of the Danube Region**, with the inclusion of project partners from 8 countries (AT, BiH, CZ, DE, HU, HR, SI, RS). This is pursued by strengthening policy- and decision-makers' knowledge and ensuring their collaboration with practitioners and the scientific community, and by jointly collecting, valorising and extending existing knowledge on microplastic sampling, analysis, mitigation and prevention in drinking water environments.

Within this framework, Specific Objective 2 (SO2) – "Occurrence of microplastics in the water environment used for drinking water supply" – focuses on testing, learning and demonstrating a harmonised MP approach in pilot areas. **Pilot sites, grouped into three clusters (karst, intergranular and surface/river bank filtration)**, serve to obtain information on MP occurrence in raw water resources and treated drinking water across the DRB. Results from SO2 may directly feed into the EU MP risk assessment (EU Drinking Water Directive watch list) and build capacity among key actors in both EU and non-EU countries.

This Output provides a concise, cluster-specific overview of MP occurrence in drinking water resources of the Danube Region, based on the results generated within the MicroDrink project. It synthesises the monitoring efforts, pilot-site findings and lessons learned for the **surface/river bank filtration** cluster.

The document is intended to be an accessible summary for water suppliers, regulators, laboratories, researchers, and the broader water-management community. It highlights what levels and types of MPs can be expected in this hydrogeological setting, how environmental characteristics influence MP occurrence, and what the key operational, analytical and governance lessons are. The Output provides a foundation for future monitoring and decision-making under the EU Drinking Water Directive (DWD). Its scope is strictly limited to **MP occurrence in drinking water resources** and the associated pilot supply systems. It does not assess risks to human health but instead generates the methodological and contextual evidence that is needed for future policy and risk-assessment efforts at European and national levels.

2. Brief literature review

2.1. Sampling for microplastics in drinking water and drinking water resources

Sampling is widely recognised as one of the greatest sources of uncertainty in microplastic (MP) studies. Because MP concentrations in drinking water are extremely low, inadequate sampling volumes and non-controlled procedures can greatly distort results. Koelmans et al. (2019) demonstrate that grab samples of a few litres – commonly used in early MP studies (e.g., Kosuth et al., 2017) – are not representative for low-contamination matrices. Small volumes (<10 L) and open-vessel approaches are highly vulnerable to airborne fibres and inconsistent capture efficiency, often generating inflated counts (WHO, 2019).

Modern studies therefore rely on **large-volume, closed-system** sampling. Mintenig et al. (2018) and Johnson et al. (2020) showed that filtering 1000–2500 L through stainless-steel cartridges or other closed inline systems dramatically improves representativity and reduces contamination. Mintenig (2019), working in groundwater environments, demonstrated how rigorous sampling – including stainless-steel equipment, high volumes, and field blanks – can yield consistently near-zero MP detections. Key equipments used so far for large-volume sampling are presented in **Fugure 1.**

These findings are directly utilised by the European Commission's Delegated Act under Directive (EU) 2020/2184, which prescribes ≥1000 L sampling, dual-stage filtration at 100 µm and 20 µm, and strict blank control requirements. Such harmonisation helps address the historical lack of comparability between national studies. This harmonisation directly addresses long-standing problems with cross-study comparability, highlighted repeatedly in reviews by WHO (2019) and Koelmans et al. (2019). Overall, these studies collectively show that **reliable MP sampling requires harmonised volumes, closed systems and contamination control**.

2.2. Sample processing, sample pretreatment steps

Pretreatment removes organic and inorganic matter to allow reliable spectroscopic identification. Hydrogen peroxide (H_2O_2) digestion is the most widely used oxidative step, offering reproducible removal of organic material without significant polymer degradation (Mintenig et al., 2018; WHO, 2019). Acid digestion (HCl) is used more selectively, mainly where carbonate precipitates or iron oxides interfere with spectral clarity. Enzymatic digestion (cellulase, proteinase-K) is increasingly tested to reduce polymer damage and minimise Raman fluorescence (Johnson et al., 2020).

Density separation is applied when samples contain mineral-rich matrices, particularly raw surface water. Zinc chloride (1.6–1.7 g/cm^3) is standard due to its high density and minimal polymer alteration. Sodium iodide is an alternative but is less cost-effective and less stable. In treated drinking water—where suspended solids are minimal—density separation is often unnecessary, allowing samples to proceed directly to filtration.

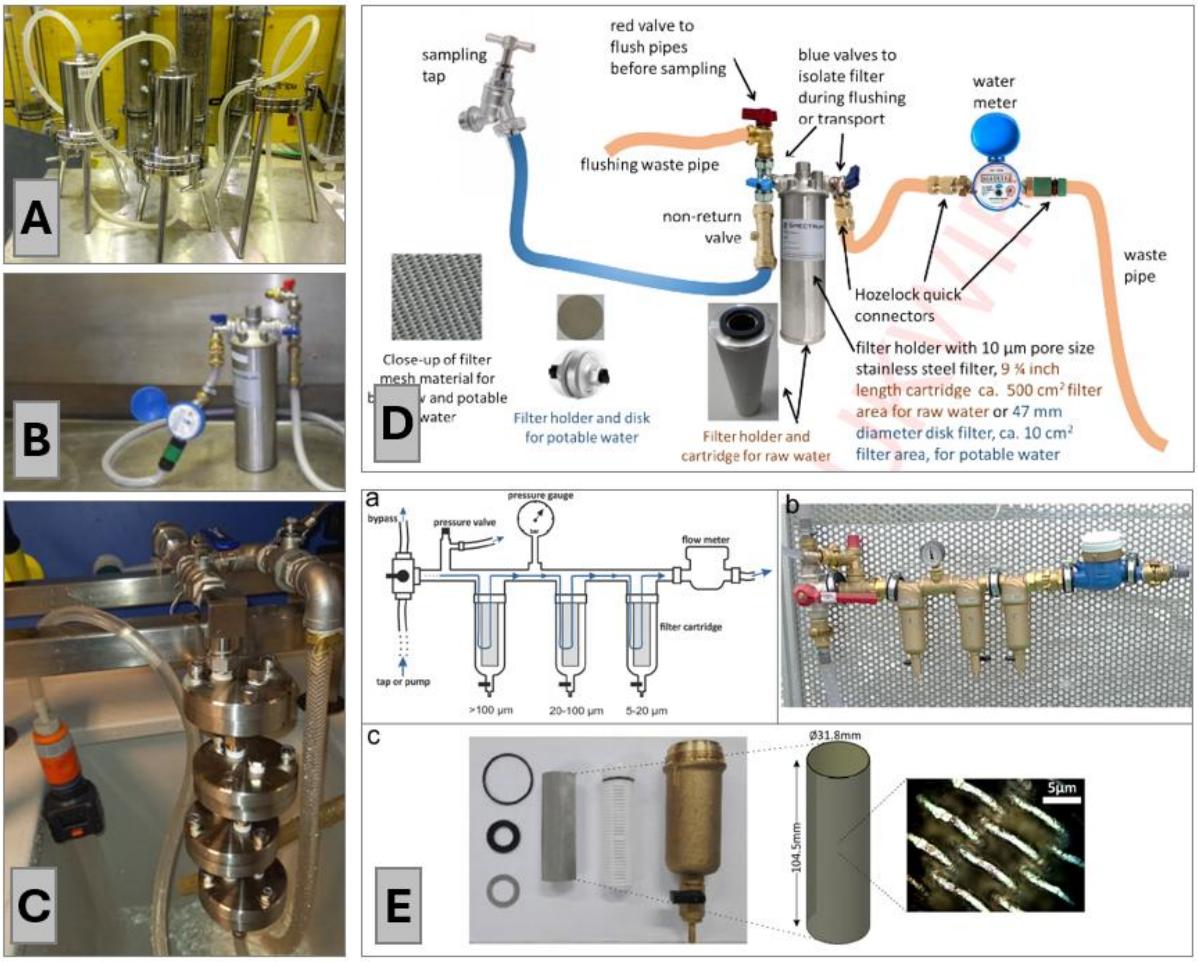

Figure 1: Sampling equipments used for high-volume sample collection (A: Vymetal et al., 2020; B: Johnson et al., 2020; C: JRC, 2025; D: Ball et al., 2020; E: Pitroff et al., 2020)

After pretreatment, samples are transferred onto substrates compatible with FTIR or Raman analysis. Commonly used substrates include aluminium oxide Anodisc filters for FTIR (low background noise, good IR transparency) and gold-coated polycarbonate filters for Raman (minimised fluorescence). Throughout these steps, blank contamination remains a significant concern: several studies note that procedural blanks often contain more fibres than the treated water samples (Johnson et al., 2020; AWI, 2021). Pretreatment choices must align with downstream requirements of analysis (Danish EPA, 2020), the literature therefore emphasises that pretreatment directly affects recovery, polymer integrity, and detection limits.

2.3. Laboratory analysis with different instruments

Laboratory analysis determines the resolution and reliability of MP identification. **FTIR imaging** is the most widely adopted method for particles ≥20 µm, offering automated full-filter scanning and high-quality polymer identification (Mintenig et al., 2018; WHO, 2019). These capabilities correspond with EU regulatory requirements, which designate ~20 µm as the lower size boundary for routine monitoring.

Raman microspectroscopy extends detection into the 1–5 µm range, enabling identification of much smaller particles and dark-coloured MPs (Danish EPA, 2020). Its principal limitation is fluorescence, especially for aged or pigmented particles and samples not fully digested (WHO, 2019). Raman is typically slower than FTIR because particles are analysed individually.

Thermoanalytical methods, especially pyrolysis–GC/MS (Py-GC/MS), provide polymer mass information and detect particles below optical size limits, including heavily degraded MPs (Kirstein et al., 2021). However, because particle size, shape and counts cannot be derived, Py-GC/MS is generally used for matrices with higher Mp loads or in combination with spectroscopic methods.

Automated identification tools – such as siMPle and other commercial software packages – improve reproducibility by extracting particle outlines and matching spectra to reference libraries (Primpke et al., 2020). The WHO and JRC recommend stringent spectral thresholds and transparent library documentation to avoid false positives.

2.4. Numerical results, comparision of different sites, temporal and geographical variations

Numerical results across the literature show significant variability, driven largely by methodological differences rather than genuine environmental heterogeneity. When validated spectroscopic methods are applied, **MP concentrations in treated drinking water are consistently very low**, often <1 particle/L (Koelmans et al., 2019; WHO, 2019).

Groundwater-derived systems show the lowest MP occurrence. Mintenig et al. (2018) and Mintenig (2019) report sporadic detections at or below method detection limits; the Norwegian Institute for Water Research (2022) similarly found results dominated by background contamination levels. Danish studies show comparably low concentrations in groundwater-fed distribution systems Danish EPA (2020).

Bank-filtration and surface-water systems exhibit higher variability. Studies of Danube bank filtrate (Vymetal et al., 2020) found moderate MP levels in raw water and bank filtrate, driven by hydrological fluctuations. Pivokonsky et al. (2018) reported seasonal variations in surface waters related to rainfall, runoff and urban pressures. Treatment (coagulation, sedimentation, sand filtration) generally removes >90% of MPs >20–50 µm.

Temporal patterns usually reflect hydrological events: high-flow or rainfall periods increase MP mobilisation in rivers, whereas groundwater systems display minimal temporal variation. Spatially, Northern European groundwater-dominated systems show uniformly low concentrations; Central European surface-water systems show higher variability; and UK surface-water-dominated systems exhibit marked temporal dynamics.

2.5. Uncertainties, QA/QC protocols and questions

Uncertainty remains a key challenge due to extremely low environmental MP concentrations. Airborne fibres, laboratory materials and building ventilation frequently dominate procedural blanks (Mintenig et al., 2018; Johnson et al., 2020). This is especially critical because in many drinking-water studies, blank levels are similar to or greater than true environmental signals.

Laboratories employ contamination prevention measures such as cotton clothing, laminar-flow hoods, filtered ultrapure water and systematic equipment rinsing, but contamination cannot be fully eliminated. Instrumental limitations also contribute: FTIR cannot reliably detect particles <10–30 µm, Raman spectra suffer from fluorescence, and polymer libraries vary in completeness.

Pretreatment steps introduce further variability. Oxidation, density separation and filtration can damage or remove certain particle types (e.g., PA, PU). Recovery tests are not uniformly reported, complicating cross-study comparisons.

A major example of uncertainty evaluation is Pitroff et al. (2020), who used **dual cascades** to differentiate true environmental signals from sampling system contamination. Their laboratory blanks contained no MPs, but **field process blanks contained 2–502 MP/m³**, with uncertainties driven by filter construction materials. Reporting limits (LOQ) ranged from ~480 to 2,700 MP/m³ depending on cascade configuration. These findings illustrate the dominant influence of blank contamination when sampling large volumes in the field.

The WHO (2019) and EU DWD framework emphasise mandatory blanks, transparent reporting limits, defined size/shape categories and multi-method validation as essential QA/QC components. Despite substantial progress, challenges remain regarding small particle detection (<20 µm), fibre discrimination and availability of certified reference materials.

2.5. Development needs

Key development needs include expanding spectral libraries (weathered and pigmented polymers), and establishing certified reference materials to support interlaboratory comparability (Primpke et al., 2020). Further improvement is also needed in blank interpretation, uncertainty reporting, and long-term monitoring strategies, particularly for surface-water-derived supplies. These advancements will be essential for implementing the EU Drinking Water Directive's monitoring requirements and for generating robust, transferable datasets.

3. Legislation on microplastics in drinking water – the EU Drinking Water Directive

Directive (EU) 2020/2184 on the quality of water intended for human consumption modernises the EU framework for emerging contaminants, including microplastics. A key innovation is the **watch list**, which allows the Commission to request coordinated monitoring of substances of concern before deciding whether regulatory limits are needed.

To enable this process for microplastics, the European Commission adopted a **harmonised monitoring methodology** through **Commission Delegated Decision C(2024)1459**. This Decision establishes standardised requirements for sampling, laboratory analysis and data reporting so that all Member States generate comparable, quality-assured MP datasets. The Commission recognises that MPs occur widely in the environment and have been detected in drinking water, yet current knowledge on human-health risks remains insufficient; therefore, the initial policy focus is on **data generation**, not on setting a parametric limit.

The EU's strategy reflects three realities:

1. **Microplastics are present** in raw water, treatment systems and distribution networks, though generally at very low concentrations.
2. **Existing studies lack comparability**, due to varying sampling volumes, analytical methods and reporting units.
3. **Health risks remain uncertain**, requiring a strong evidence base before regulatory limits can be considered.

For this reason, the EU method defines a practical analytical window based on the capabilities of current micro-spectroscopic technologies. MPs are monitored as:

- particles with at least one dimension **20 µm–5 mm**, and
- fibres **20 µm–15 mm**,

with results expressed as number concentrations by **size class, shape and polymer category**. The Annex also defines **priority polymers** to support consistent identification.

The overall strategy is **stepwise**: 1. generate harmonised monitoring data; 2. evaluate the evidence for watch-list inclusion; 3. consider regulatory limits if justified.

Sampling is performed using a **four-stage filter cascade**, operated under positive pressure:

- Filters **a** (100 µm) and **b** (20 µm) collect the sample.
- Filters **c** and **d** are parallel **procedural blanks**.

At least **1,000 L** of water must pass through the cascade, reflecting the very low expected MP concentrations and the need for a sufficient signal above blank contamination. Water should be piped directly to the filtration system to minimise atmospheric and handling contamination. All reagents and rinsing liquids must be filtered at **≤0.45 µm**.

Sample preparation also discussed in details. If metal filters from used for sampling cannot be analysed directly, particles are resuspended and transferred to an FTIR- or Raman-compatible membrane. Optional steps – oxidative digestion, enzymatic digestion, or density separation – may be applied as long as polymer integrity is maintained. Laboratories must conduct **recovery tests** using spiked particles to verify the efficiency of the filtration system (acceptable recovery: **100% ± 40%**).

If full analysis is not possible, **≥10%** of the recovered sample must be analysed, and **≥20%** of any filter surface must be covered when sub-area imaging is used. Procedural blanks must undergo identical treatment to quantify background contamination.

Laboratory work combines **optical imaging** and **µ-FTIR or µ-Raman spectroscopy**. All particles or fibres ≥20 µm are counted and classified. Spectral libraries must include all priority polymers and representative non-plastic materials to avoid misidentification. Automated identification may be used, but matching thresholds must be validated.

Results from both sampling filters and both blanks must be reported separately as **particles/m^3**, broken down by polymer type, size class and shape.

Quality assurance is integral to the methodology. Key requirements include:

- no blank subtraction—**both blank and uncorrected sample results must be reported**;
- validated spectral-matching criteria;
- reliable detection at the **20 µm** lower size boundary;
- thorough documentation of sampling and analytical conditions;
- use of filtered reagents and contamination-minimising laboratory practices.

Comprehensive metadata (sampling date, water type, deviations, treatment context) must be included to support interpretation and future comparison.

4. Harmonised monitoring design and methodology

As MicroDrink project was launched in the beginning of 2024, there had not yet been any accepted regulation for microplastic analysis in drinking water. However, draft methods were present and building on these, considering the need for harmonisation emphasized in the literature review, a harmonised protocoll for sampling and analysis, aiming for future EU DWD compatibility was established, while keeping in mind and rationalising financial approaches. To reach this, sampling and analysis minimum criteria have been described and spread among the project partners.

4.1. Sampling minimum criteria

A harmonised sampling strategy is essential to ensure that microplastic (MP) data generated within the MicroDrink project are comparable across pilot areas. Given the low concentrations of microplastics expected in drinking water, sampling procedures must be standardised, contamination strictly controlled and all teams equipped with consistent tools and documentation practices. The minimum criteria applied in the project are aligned with the EU Drinking Water Directive's methodological requirements and complementary ISO guidance, covering also requirement described in the currently released ISO 16094-2:2025 standard.

4.1.1. General principles

Sampling activities follow a plastic-free, contamination-controlled approach. All equipment that comes into contact with the water sample must be constructed from **stainless steel, glass or rubber**, avoiding synthetic polymer materials wherever possible. Hoses made of rubber or silicon should be used, such as sealing and gaskets made of rubber, silicone or natural materials like wool hemp. Prior to each sampling event, all sampling equipment is thoroughly cleaned using **microplastic-free water**. This water is produced by filtrating deionised water through an **inorganic filter with a mesh size below 1 µm**, ensuring that reagents and rinse water do not introduce artefacts. Special attention is given to preventing atmospheric contamination. All equipment is kept closed or covered whenever it is not actively in use, and operators avoid wearing clothing containing synthetic fibres.

4.1.2. Sampling procedure and minimum volume requirements

To obtain reliable results at environmentally relevant low concentrations, the **minimum sampling volume is set at 1 000 L**, consistent across all project sites. This requirement mirrors both the EU Delegated Act and the ISO 5667-27:2025 guidance. The rationale is twofold: first, concentrations in drinking water are often close to blank levels; second, variability decreases significantly when larger volumes are filtered. Sampling of smaller volumes may result in particle counts too low for meaningful interpretation.

The project applies a **filter-cascade system**, where water is passed directly from the sampling point into a stainless-steel cascade fitted with the required filter sizes. A possible sampling scheme, similarly to previous studies (Mintenig et al., 2018; Johnson et al., 2020; Ball et al., 2020; Pitroff et al., 2020), applied for most of the pilot areas in MicroDrink project is presented in **Figure 2.** Consistent with EU-level methodology, the **smallest mesh size used during sampling is 20 µm**, ensuring that all particles within the regulatory size window are retained. To detect and quantify potential contamination introduced during sampling, the harmonised sampling methodology aims for **blank generation** procedure, implementing subsequent filter of identical mesh size. A **difference compared to EU DWD** during the Microdrink project is that the filter cascade was not built as 100-20-100-20 µm, but 20-20 µm. The rational behind is to **build a cost effective sampling approach, while meeting the minimum particle size and procedural blank preparation requirements**. As no large particle or turbide water was expected during the sampling, the large filter area applied seemed to be a viable option to collect 1000 L of water sample without clogging. The number of filters reduced by 50% the number of separate analysis that significantly eased challenges pertaining to high costs. However, particles still could be divided into the requested size range classes with spectroscopy-driven data post processing.

All operational variables – including sample volume, flow conditions, site characteristics, weather, and deviations from standard procedures – are documented on **harmonised sampling sheets**. This ensures that data from all countries and pilot areas can later be interpreted consistently.

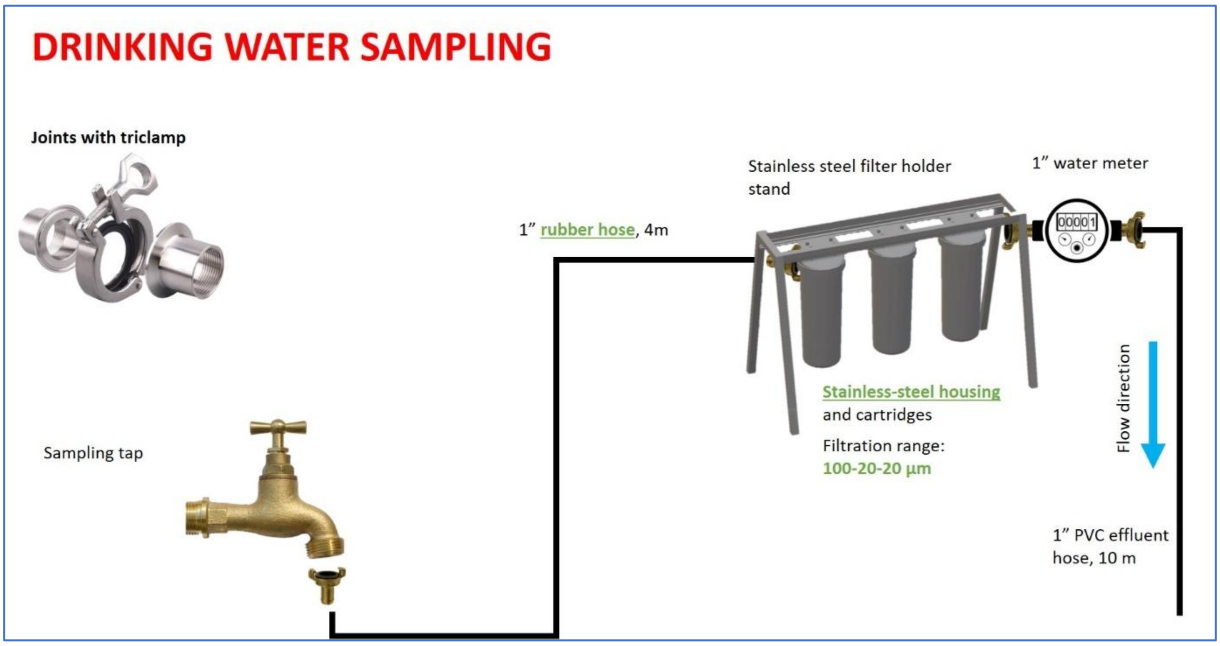

Figure 2: Sampling scheme, similarly to previous studies (Mintenig et al., 2018; Johnson et al., 2020; Ball et al., 2020; Pitroff et al., 2020), applied for most of the pilot areas in MicroDrink project.

4.2. Analysis minimum criteria

To ensure that microplastic (MP) results generated within the MicroDrink project are robust, comparable and aligned with the EU methodology, a common set of analytical minimum criteria is applied across all partner laboratories. The agreed standards harmonise equipment use, sample handling, analytical techniques and reporting practices, aiming for compatibility of results across all pilot sites and resource types

4.2.1. Equipment requirements and preparation

All analytical work must avoid the use of conventional plastic consumables to minimise false positives and reduce the risk of polymer shedding. Sample-contact materials must consist of **glass, stainless steel, ceramic or other non-plastic components**. Before use, all laboratory items – including beakers, filtration units, tweezers and sample supports – are thoroughly cleaned with detergent, rinsed with **microplastic-free water**, and, where appropriate, treated with 96% ethanol. Microplastic-free water is produced by filtering deionised water through an inorganic filter of <1 µm mesh size, ensuring that no polymeric particles are introduced during cleaning or sample transfer. All reagents used during pretreatment or digestion must also be filtered through <1 µm inorganic filters to eliminate background contamination. Throughout the procedure, sample handling steps should be performed under a **laminar flow hood** equipped with HEPA filtration to reduce airborne fibre deposition.

4.2.2. Sample processing and analytical workflow

The MicroDrink project adopts a "**whole sample**" principle: all material recovered from the sampling filters must be processed and analysed. Only in situations where full processing is technically impossible may a subsample be used, and in such cases the decision must be justified and documented. At minimum, **10% of the homogenised total sample** must be analysed if subsampling is unavoidable, ensuring representativity and comparability across laboratories.

All samples are analysed using vibrational spectroscopic methods suitable for low-particle drinking water matrices: **µ-FTIR, µ-Raman or QCL-IR**. These techniques provide the polymer-specific information required by the EU methodology and allow reliable identification down to the 20 µm size threshold. As a general rule, the **entire filter or analytical support** must be scanned. If full-filter imaging is impractical due to high particle counts or time limitations, sub-areas may be analysed but must follow a representative selection strategy that covers **at least 20% of the total filter area** or, alternatively, at least 20% of all detected particles. All subsampling decisions must be transparently reported, including the ratio of analysed to total filter area or particle count.

4.2.3. Reporting requirements

To ensure interoperability with the EU watch-list methodology, the MicroDrink project classifies identified microplastics by:

- **polymer identity**, focusing on the priority polymers:
    - PE – Polyethylene
    - PP – Polypropylene
    - PET – Polyethylene terephthalate
    - PS – Polystyrene
    - PVC – Polyvinyl chloride
    - PA – Polyamide
    - PU – Polyurethane
    - PMMA – Polymethyl methacrylate
    - PTFE – Polytetrafluoroethylene
    - PC – Polycarbonate
- **size categories:** 20–100 µm and >100 µm
- **shape classes:** particles (length:width <3) and fibres (length:width ≥ 3)

All laboratories must report the number of MPs per sample in these categories, along with metadata on the total sample volume, sample treatment steps, spectroscopic methods used, any subsampling performed, and the chemical composition of any polymeric materials present in sampling or analysis equipment. These metadata ensure that results can be compared across countries and integrated into future harmonised European datasets.

4.2.4. Quality control and contamination monitoring

Strict quality-control measures are necessary due to the low expected MP levels in drinking water. Atmospheric contamination is mitigated by covering equipment when not in use, working under laminar flow where possible, and avoiding synthetic clothing that may release fibres. To quantify background contamination, each laboratory processes **at least ten procedural blanks** under identical conditions to the sample workflow. These blanks establish the mean and standard deviation of background MP contamination, forming the basis for defining the **limit of quantification (LOQ)** as: **LOQ = mean blank concentration + 10 × standard deviation.**

This approach ensures that only concentrations significantly above typical blank levels are interpreted as true microplastic occurrence. In addition, laboratories must verify the **recovery efficiency** of the full analytical procedure. Spike experiments are performed using particles between **120–200 µm** and **30–70 µm**, including at least one polymer with density greater than water (e.g., PET) and one with lower density (e.g., PE). A spike count of 50–150 particles is recommended for each size range. Analytical recovery is considered acceptable when it falls within **100% ± 40%**, consistent with the EU methodology.

4.3. Parallel sampling and analysis

Further aim of the project was to get more robust information on microplastic occurrence with analysing the same samples in different laboratories. For this, PP8 Eurofins acted as a central lab and with the same sampling and analysis method samples were always collected and analysed parallel to the local partner's sampling and analysis technique. Precleaned cylindrical filters in closed cartridges were always shipped to the local pilot sites where the previously trained personnel conducted the high-volume on-site filtration. The samples and sampling reports were transported back to the central laboratory in closed cartridges and underwent the same sample preparation and FTIR microscopy analysis procedure. This exercise may help to better identify potential differences between versatile sampling and laboratory analysis practices.

To get an overview and possible comparison on differences in analytical methods deployed in different countries, a joint sampling activity was organised during the project. All partners gathered in Hungary, where raw and treated drinking water samples were taken with the harmonised approach during the same day. These samples meant to represent a quasi-homogeneous set of samples – as they were taken on the same day with the same equipment under stable conditions – to help discover potential variability that may occur in between different laboratories, despite using the harmonised analytical approach.

5. Surface/river bank filtration pilot actions description

Bank-filtration and surface-water sources represent hydrologically dynamic systems where river water, floodplain infiltration and direct runoff interact to shape drinking-water quality. For the MicroDrink project, three representative pilot sites were selected to capture this diversity: the **Malenščica spring in the Planinsko polje (Slovenia)**, the **Ivančice waterworks (Czech Republic)** drawing from river-influenced alluvial sediments, and the **Staničenje reservoir system (Serbia)**. These sites reflect a continuum from natural spring systems with strong surface-water influence to engineered riverbank-filtration. Their characteristics, environmental context, water quality attributes, and potential microplastic sources were comprehensively documented in **Deliverable 2.1.3**.

5.1. Description of pilot sites

A summary table of core features is provided in **Table 1** of this document.

Table 1: Description of bank filtration/surface resources pilot sites.

Parameter	**Malenščica (SI)**	**Ivančice (CZ)**	**Staničenje (RS)**
Catchment area (km²)	726	0.15	n/a
Recharge / influence	Karst-surface interaction	Riverbank filtration	Surface reservoir runoff
Avg. discharge (m³/s)	6.704	0.01	17.4 (river)
Land cover	Forests, settlements	Agriculture, settlements	Mountains, rural areas
Treatment	Standard treatment	Bank-filter + treatment	Reservoir + treatment

5.2. Environmental context

All three pilot sites share a strong dependence on **surface-water dynamics**, but differ in hydrological behaviour.

At Malenščica, the Planinsko polje acts as a natural floodplain infiltration system. Seasonal flooding results in rapid mixing of surface water with shallow subsurface flow. Because the area is predominantly forested and protected, direct anthropogenic pressure is low, but hydraulic variability is high.

The Ivančice system represents a classic bank-filtration aquifer, where gravel and sand layers provide natural filtration of river water. The river corridor passes through agricultural areas, small settlements and transport routes, offering a diverse set of potential surface inputs. Natural attenuation occurs during infiltration, but episodic high-flow events can bypass filtration layers.

At Staničenje, the environmental setting is defined by mountainous terrain, rural land use and seasonal hydrological fluctuations. The open reservoir receives direct runoff, which may carry organic matter, sediments and polymer residues from the catchment. The absence of large-scale industry keeps pressure low, but low population density does not eliminate diffuse sources.

5.3. Water quality

Across the three pilot sites, water quality reflects a combination of natural surface-water influences and anthropogenic pressures. Water quality parameters are summarised in **Table 2**.

Table 2: Water quality parameters (mean) of bank filtration/surface resources pilot sites.

Site	pH	Conductivity (µS/cm)	Nitrate (mg/L)	DOC (mg/L)
Malenščica (Slovenia)	7.18	350	7,54	1,22
Ivančice (Czech Republic)	7.40	79	3.0	1,21
Staničenje (Serbia)	7.60	651	3.1	1,23

Site	Malenščica (SI)	Ivančice (CZ)	Staničenje (RS)
pH	7.18	7.40	7.60
Conductivity (µS/cm)	350	79	651
Nitrate (mg/L)	7.54	3.0	3.1
DOC (mg/L)	1.22	1.21	1.23

5.4. Potential sources of microplastics

Potential microplastic inputs at these sites arise from a mix of **diffuse and point sources** associated with surface-water exposure:

- **Riverborne particles** transported from upstream catchments (all sites).
- **Agricultural activities**, including tyre wear, mulch fragments and polymer-based fertiliser packaging (Ivančice, Staničenje).
- **Road run-off**, introducing tyre wear particles and road-marking polymer flakes (Ivančice).

- **Tourism and recreation**, particularly during high-use periods (Malenščica).
- **Atmospheric deposition**, an unavoidable source across all surface-water systems.
- **Catchment runoff**, especially during storm events, which can mobilise fibres and fragments from soils and settlements (Staničenje).

6. Results

Within the MicroDrink project, nine pilot sites across the Danube River Basin implemented a largely harmonised but locally adapted approach to sampling and laboratory analysis of microplastics in drinking water. The pilots cover karst (Croatia, Austria, Bosnia and Herzegovina), bank-filtration and surface-water systems (Slovenia, Czech Republic, Serbia – Staničenje) and intergranular aquifers (Hungary, Serbia – Pavliš, Germany).

6.1. Sampling and laboratory practice in the Danube River Basin for microplastic analysis

6.1.1. Sampling and laboratry instrumentation

Across all sites, sampling was designed to be compatible with the EU Commission Delegated Decision 2021/1441 and JRC technical guidance, with a nominal lower size limit of **20 µm** and target sample volumes of around **1000 L** per sample. This large-volume, closed-system strategy reflects experience from earlier drinking-water studies, where small grab samples and open containers were shown to be inadequate in low-concentration matrices.

Most pilot partners used **cylindrical stainless-steel filter elements with 20 µm mesh** as the primary sampling device. This applies to Croatia, Austria, Czech Republic, Hungary, Serbia (Staničenje and Pavliš), Bosnia and Herzegovina, Slovenia and Germany. Austrian, Czech and Slovenian partners additionally implemented **flat filter holders and flat membranes**, allowing easier cleaning and more controlled blanks. The average processed volume per sample was close to 1000 L at every pilot. In general, the cartridge systems were considered effective for capturing sufficient volume in a closed configuration, but several partners reported practical challenges such as variable internal volume (volume of liquid in the cartridges after filtration and sent to lab processing), occasional overflow when opening the cartridges, and suspected particle bypass where mesh quality or seals were imperfect.

Sampling was embedded in a network of specialised microplastic laboratories. Croatian and Bosnian samples were processed by Sample Control d.o.o., Slovenian samples by the National Institute of Biology, Austrian samples by the Environment Agency Austria (EAA), Czech samples by the TGM Water Research Institute (Brno), Hungarian samples by Eurofins Environment Testing Hungary, Serbian samples by the University of Belgrade (Faculty of Chemistry, Proteomics Research Team), and German samples by INAM Forchheim. Analytically, the Danube practice is dominated by **micro-FTIR imaging**, with a single pilot (Germany) using **confocal Raman microscopy** for detailed sub-area mapping. Most FTIR laboratories operated Bruker LUMOS II instruments (4 labs for 6 pilots), occasionally Thermo Scientific Nicolet iN10 Infrared Microscope (1 lab for 2 pilots) or PerkinElmer FTIR imaging systems (1 lab for 1 pilot). Data evaluation relied on specialised software such as Purency Microplastics Finder and OPUS (Bruker LUMOS II), Omnic

Picta and SpectrumIMAGE™ (Thermo Scientific Nicolet iN10), for Raman LabSpec 6. Different sampling and analysis techniques are summarised in **Figure 3.**

All laboratories considered a harmonised set of **major polymer categories**: polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS), polyvinyl chloride (PVC), polyamide (PA), polyurethane (PU), polymethyl methacrylate (PMMA) and polycarbonate (PC). PTFE was included in some but not all workflows, and the German Raman laboratory focused on PC, PET and PVC in the subsamples it mapped. This common polymer spectrum, together with similar-size thresholds and large-volume sampling, creates a coherent basis for cross-comparison between pilot sites.

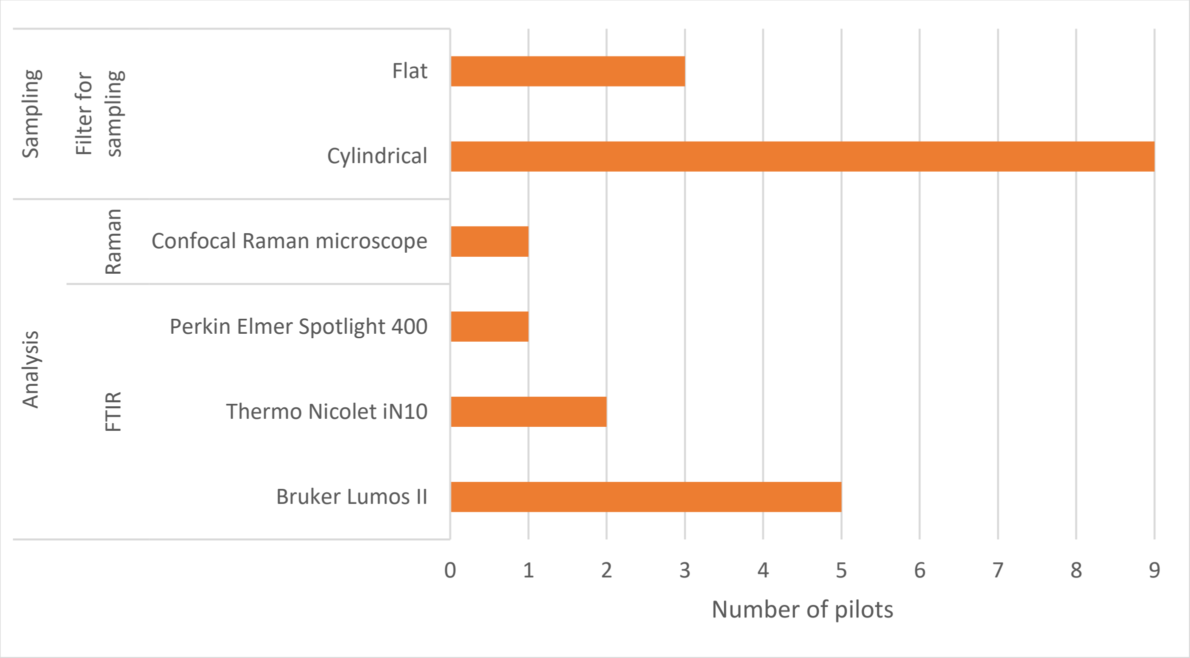

Figure 3: Sampling and analysis techniques in the DRB pilot sites applied in MicroDrink project. All pilots and the central laboratory used cylindrical filters, but additionally Austria, Slovenia and Czech Republic introduced flat filtration devices.

6.1.2. Sample processing and analysis

Sample processing within the DRB pilots followed similar principles but was adapted to local water quality and laboratory capabilities. Several partners (Slovenia, Austria, Czech Republic, Hungary, Serbia–Staničenje, Serbia–Pavliš) processed the **full collected volume** or its entire concentrated residue, whereas others (Croatia, Bosnia and Herzegovina, Germany) processed or analysed only a **defined fraction** of the sample or filter content. In Croatia and Bosnia, around **10 % of the total mixed sample volume** was subjected to digestion and final filtration, while Austria sometimes analysed approximately 20–26 % of the collected sample, based on experience from the first campaigns. On the analytical side, Slovenia examined the full area of 100 µm filters but only **20 % of the 20 µm filter area**, Austria mapped **25 % of the final Anodisc filters**, and Germany mapped a **4 mm² area** (about **6 % of the 20 µm filter**) in each size fraction. Other partners (5 out of 8 pilots) analysed the **entire filter area** after transfer to suitable analytical substrates.

Pretreatment workflows were broadly consistent with international practice. Most pilots applied **oxidative digestion** for organic material removal using hydrogen peroxide (H_2O_2), sometimes combined with **alkaline digestion** and **density separation**. Austria used a sequential pretreatment including 30 % H_2O_2, NaOH and HCl, with optional K_2CO_3 density separation, followed by vacuum filtration onto 0.2 µm Anodisc membranes. The Czech site applied H_2O_2 digestion at 48 °C and $ZnCl_2$ density separation (1.6 g/cm³). Hungary also combined 30 % H_2O_2 with $ZnCl_2$ separation, concentrating samples onto 25 mm Anodisc filters. Croatian and Bosnian pilots used **Fenton's reagent** for oxidation, followed by filtration onto Anodisc filters. The Serbian laboratories extracted particles from stainless steel filters by **ultrasonic treatment**, treated the suspension with **KOH (10 %) and H_2O_2 (15 %)** and transferred residues to **1 µm silicon filters** for micro-FTIR. The German Raman laboratory implemented a **sieve cascade** (500 µm and 200 µm sieves with a bottom pan) and then filtered each fraction at **20 µm and 1 µm**, obtaining four size classes (>500 µm, 500–200 µm, 200–20 µm, 20–1 µm).

All FTIR-based laboratories performed **particle-level size and shape classification** in line with the regulatory concept. Size classes included 20–100 µm and >100 µm, and in some cases an additional 10–20 µm category. **Size metrics** were derived from maximum Feret diameter, area-equivalent diameters, or digital ruler tools embedded in the FTIR software. **Shape classification** was generally based on length-to-width ratios, with a threshold of about 3:1 to distinguish fibres from fragments/particles. In practice, both fragments and fibres were observed at most sites, although fragments were more common overall, particularly in intergranular and bank-filtration systems. The German Raman workflow did not classify particle shapes, focusing instead on polymer identification and size fraction. Processing and analysis overview is presented in **Table 3.**

Several pilots reported **matrix-related challenges** that influenced processing and analysis. Bank-filtration and surface-water sites (e.g. Austria, Staničenje) experienced **high total particle loads**, including organic debris and iron oxides, which clogged filters and required robust digestion and density separation. Polyamide was frequently noted as difficult to distinguish from natural organic or proteinaceous residues, and thick or strongly absorbing particles were sometimes problematic in transmission-mode FTIR. The Raman-based workflow in Germany highlighted that multiple transfer steps and high particle loads increase contamination risk and complicate mapping, favouring careful sub-area selection rather than full-filter analysis. These experiences underline the need to balance analytical resolution with practical constraints and uncertainty.

Table 3: Processing and analysis overview

Pilot	**Sampling equipment**	**Pretreatment (short)**				**Subsample in lab**	**Size-classification method**
		Oxidation	**Alkaline digestion**	**Acidic digestion**	**Density separation**		
Croatia	cylindrical	Fenton	no	no	no	10%	not specified
Slovenia	Flat, cylindrical	no	no	no	NaCl	no	Feret max
Austria	Flat, cylindrical	30% H_2O_2	NaOH	HCl	K_2CO_3	20–26%	Feret max
Czech Republic	Flat, cylindrical	H_2O_2 (48°C)	no	no	$ZnCl_2$	no	Longest axis (Purency-derived size classes)
Hungary	cylindrical	Fenton	no	no	$ZnCl_2$	no	Area-equivalent diameter
Serbia–Staničenje	cylindrical	15% H_2O_2	10% KOH	no	no	no	Digital ruler
Bosnia and Herzegovina	cylindrical	Fenton	no	no	no	10%	not specified
Serbia–Pavliš	cylindrical	15% H_2O_2	10% KOH	no	no	no	Digital ruler
Germany	cylindrical	no	no	no	no	no	Stack of sieves

6.1.3. Quality assurance and quality control

Quality assurance and quality control (QA/QC) were central elements of the Danube River Basin practice, reflecting lessons from earlier microplastic studies in drinking water. All pilots implemented a combination of **blank controls**, **contamination-prevention measures** and, in several cases, **spiking experiments** to evaluate recovery. The Croatian, Bosnian and Hungarian laboratories conducted 10 **blanks during method validation**, while the Slovenian partner ran eight blanks (approximately one per sampling site). **Laboratory contamination control blanks** were run as follows: the Austrian laboratory performed at least one blank per 20 samples, plus additional blanks for water, chemicals and air monitoring. The Czech site included two blanks, Hungary and Bosnia combined validation blanks with one blank per four samples, and the Serbian laboratories adopted a similar “one blank per four” approach. The German Raman lab measured two blanks (raw and treated) for each sample set.

Reporting limits (RLs) and detection limits were defined using different but transparent approaches. In Croatia and Bosnia, detection limits were expressed as particle counts in 50 mL blank samples, with specific values for PE and PP in different size ranges. The Austrian and

Hungarian partner explicitly followed the draft ISO/DIS 16094-2 procedure, calculating RLs as the **mean of ten water blanks plus three standard deviations**. This yielded polymer-specific RLs between roughly 1.0 and 1.5 microplastics per litre in Austria and 5 particles per litre in Hungary. Some labs (e.g. Slovenia) were still finalising RL determination, but all reported blank results and discussed their implications for interpreting low particle counts, especially when the difference between raw and treated water was small.

Experimental verification of method performance was carried out by several partners. Croatia and Bosnia spiked samples with **70 µm polystyrene microspheres**, achieving **>60 % recovery** during validation. The Czech laboratory used **PET-G fragments** of <100 µm and >100 µm and obtained recoveries of roughly **80 %** in both raw and treated water. Hungary deployed **fluorescent polyethylene microspheres** in two size ranges (90–106 µm and 300–355 µm), reporting recoveries around **76 %**. These results are consistent with expectations for complex matrices and demonstrate that digestion, density separation and filtration steps are reasonably efficient, though not loss-free.

Contamination-prevention measures were broadly similar across all laboratories. Partners reported the use of **cotton laboratory clothing**, **avoidance of plastic consumables**, and **covering samples** whenever they were not being processed under laminar flow. **Laminar-flow cabinets** or clean working areas were used in most labs, especially during critical steps such as filtration, digestion and transfer to analytical filters. Work surfaces were routinely **cleaned with filtered water and ethanol**, and equipment such as glassware and tweezers was cleaned with detergents, rinsed in ultrapure or filtered water, sometimes burned at high temperature, and stored covered. All solutions and rinsing waters were **filtered prior to use**, with pore sizes ranging from 0.45 µm to 10 µm depending on the filter material (glass fibre, cellulose, stainless steel, ceramic or nylon). Some partners noted that **glass fibre and cellulose filters can themselves shed fibres**, which can complicate analysis, and therefore recommended stainless-steel or ceramic filters wherever possible. Key QA/QC measures across pilot laboratories are shown in **Table 4.**

Despite these efforts, several pilots observed that **blank contamination can approach or even exceed environmental signals**, particularly for small size fractions and fibre-rich matrices. This is consistent with published work such as Pitroff et al. (2020), which showed that process blanks in field-based cascade systems can dominate the uncertainty budget and require careful interpretation of reporting limits. **The Danube River Basin experience confirms that robust QA/QC – including field and laboratory blanks, clearly defined RLs, recovery checks and systematic contamination-prevention protocols – is indispensable** for defensible microplastic monitoring in drinking water. **At the same time, partners highlighted the need for continued improvement of equipment design, filter materials and harmonised reporting frameworks to further reduce variability and support inter-laboratory comparability in future monitoring programmes.** Key uncertainties are presented in **Figure 4.**

Table 4: Implementation of key QA/QC measures across pilot laboratories.

Pilot	**Cotton clothes**	**No plastic consumables**	**Laminar flow**	**Covered samples**	**Nitrile gloves**
Croatia	yes	yes	no	yes	yes
Slovenia	yes	yes	yes	yes	yes
Austria	yes	yes	yes	yes	yes
Czech Republic	yes	yes	yes	yes	no
Hungary	yes	yes	yes	yes	no
Serbia–Staničenje	yes	yes	yes	yes	yes
Bosnia and Herzegovina	yes	yes	yes	yes	yes
Serbia–Pavliš	yes	yes	yes	yes	yes
Germany	yes	yes	yes	yes	yes

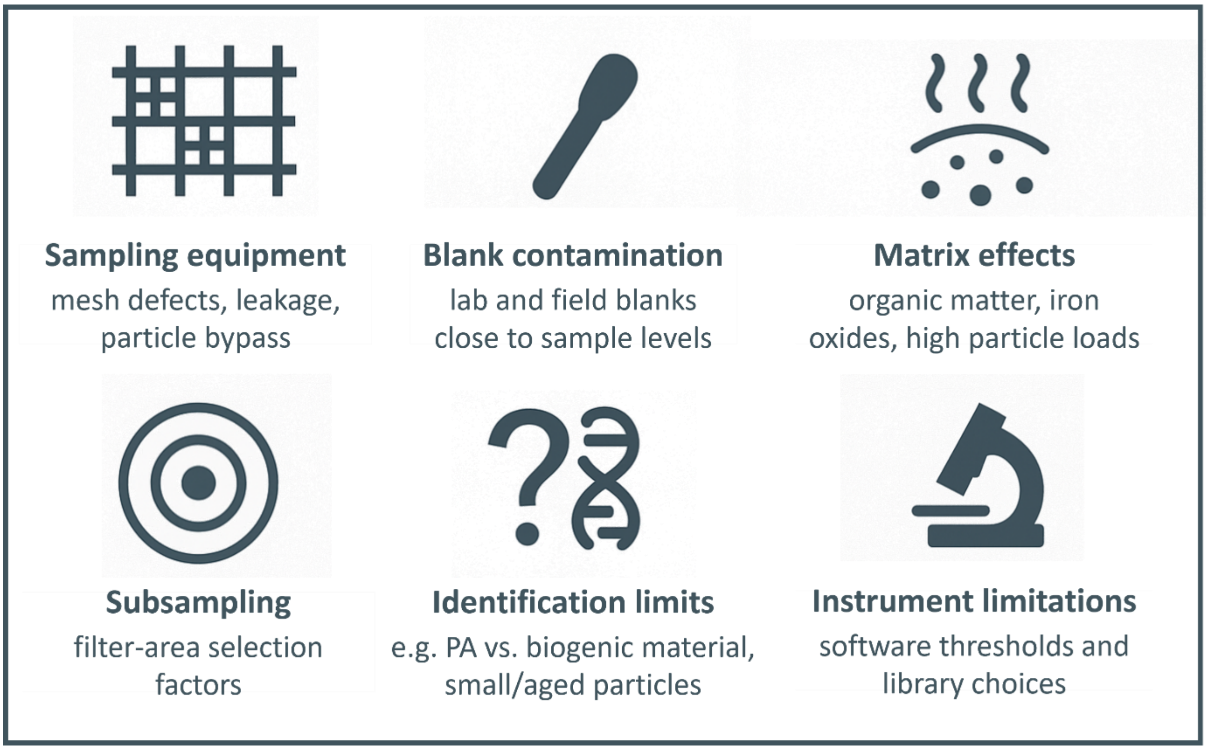

Figure 4: Key uncertainties of sampling and analysis.

6.2. Microplastic occurrence in drinking water obtained from karst water resources

> **Disclaimer:** *The implementation of the requirements for sampling and analysis of microplastics in accordance with the Commission Delegated Decision (EU) 2024/1441 still presents challenges. Specifically, the method for sampling is not yet fully developed, and no validated or certified equipment is available, and applied analysis methods might not fully meet the required specifications and quality criteria. Therefore, the results presented are solely for documentation purposes and should not be used for quantitative assessments or comparisons. The results do not reflect actual microplastic concentrations in water intended for human consumption, however, provide the first comprehensive data collection experience on microplastic occurrence on international level with the harmonized approach of the cited legislation.*

The bank filtration and surface water cluster – Malenščica (Slovenia), Ivančice (Czech Republic) and Staničenje (Serbia) – provides insight into MP behaviour in systems that are more directly connected to surface waters and show stronger temporal dynamics. These sites combine surface water influence with engineered filtration processes and therefore represent settings where MP inputs and removal processes are expected to be most variable.

6.2.1. General descriptive patterns

The Czech Ivančice site provides one of the most robust datasets for this cluster. Here, total MP counts in raw water typically range between 250 and 500 particles per cubic metre, while treated water samples contain around 100 particles per cubic metre. These values are appreciably higher than those observed in intergranular systems but still relatively low compared to many surface water studies. The polymer spectrum in Ivančice is dominated by **PP, PET and PE**, with **PC and PMMA** detected only occasionally. This mix reflects a blend of diffuse catchment sources and treatment-plant influences. Size-class information shows that MPs are present in all size ranges considered, but that the **most abundant particles are within the 20–100 µm size class** that dominates the MicroDrink dataset. Numerical results are shown on **Figure 5.**

The Staničenje surface water site shows a somewhat different profile, partly because of lower counts and partly because of the detailed size and shape analyses carried out. On average, about seven MPs per sample were recorded in both raw and treated water. The most common polymers are **PS and PP**, with PU the least common. Shapes are almost exclusively **fragments**, with irregular dimensions and both flat and angular forms.

In terms of overall shape patterns across the bank filtration/surface water cluster, the **fragment signal clearly dominates**, with fibres either secondary or confined to specific polymer types (for example, PET fibres at Ivančice). This pattern reflects both the treatment processes and the nature of the input: bank filtration attenuates larger and more buoyant particles, while repeated physical and chemical stresses promote fragmentation.

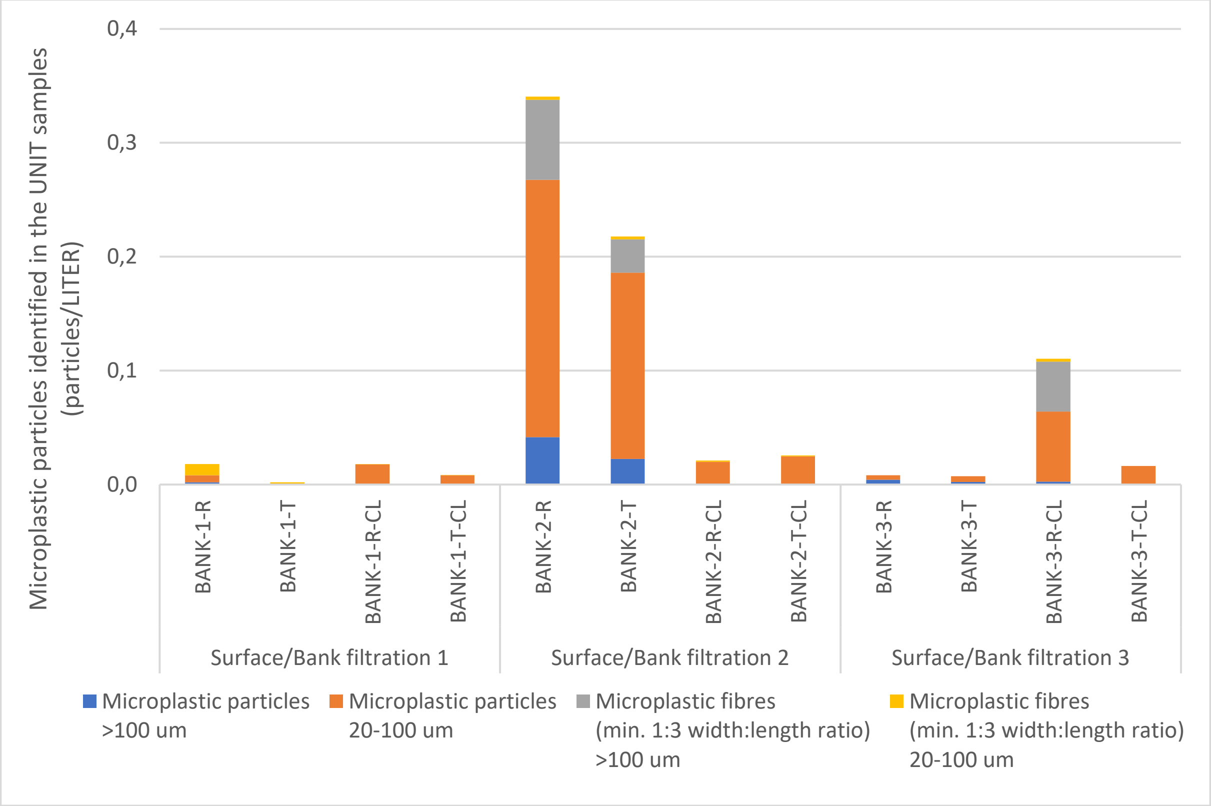

Figure 5: Microplastic occurrance in bank filtration pilot sites. (Legend: XX: Country code 2 digit; MO: monitoring campaign; R: Raw; T: Treated; CL: Central lab).

6.2.2. Temporal patterns

Temporal trends in this cluster are more visible than in the intergranular sites but still moderated by analytical limitations. One partner notes that results are “more or less stable,” with **raw-water counts consistently in the several-hundred particles per cubic metre range** and **treated water consistently near ~100 particles per cubic metre**. This suggests a relatively steady surface-water MP input over the monitoring year, with limited seasonality discernible at the current level of resolution.

At Staničenje, temporal behaviour is more complex. All campaigns show the presence of MPs, but **polymer composition varies**: PS and PP are present in all evaluated campaigns, while some polymers (e.g., PE, PVC, PET, PU) are absent in specific quarters. This may reflect changing upstream pressures, hydrological events, or stochastic sampling variation given the low absolute particle numbers. Despite these limitations, the data collectively suggest that **MP presence in bank-filtration and surface-derived systems is more consistent over time than in karst, and significantly higher than in intergranular aquifers**, but still generally at low absolute concentrations.

6.2.3. Raw water and treated water comparison

In this cluster, the **contrast between raw and treated water is clearest at Ivančice**. Here, raw water systematically contains several times more MPs than treated water, and specific shapes and polymers (notably PET fibres and PA particles) appear to be removed efficiently by the multi-barrier treatment train. The Czech partner notes that treated water counts are roughly half those of raw water and that the polymer composition remains similar but with reduced contributions from certain polymers. This indicates meaningful MP removal by sand filtration, Fe/Mn precipitation, activated carbon and disinfection.

At Staničenje, results are **less straightforward**. The comparison between raw and treated water is described as “inconclusive” with respect to total particle counts. The partners emphasise that, given the low counts and the inconsistencies between campaigns, it is difficult to derive clear treatment-removal efficiencies. Polymer types and shapes are present in both raw and treated water across campaigns, underscoring the stochastic nature of the dataset.

For the Slovenian bank-filtration site, preliminary comparisons from the first two campaigns suggest that **treated water generally contains fewer particles than raw water**, and that ultrafiltration performs as an effective barrier.

Across the bank-filtration and surface water sites, the overall picture is that **raw water generally contains more MPs than treated water**, especially at Ivančice, but that the magnitude of this difference varies and may at times be masked by sampling and analytical uncertainty. Given that filtration cartridges do not achieve perfect retention and that second filters cannot be treated as blanks, apparent increases or small decreases in treated water at very low concentration levels should not be overinterpreted.

6.3. Joint sampling

The results of the joint sampling activity might help to have an insight in the possible variability occurring between different labs across the Danube region. Quasi-homogeneous set of samples (taken on the same day with the same equipment under stable conditions) were distributed among national labs to get analysed along the harmonised analytical approach.

Six out of the nine laboratories reported less or around 0.01 MP particles per litre, while 3 laboratories seem to present outlier data – with an order of magnitude higher results – with MPs between 0.1-0.3 particles per litre. However, this is unclear if this difference is origination from the heterogeneity of the samples or deviations in laboratory protocol (e.g. subsampling). Fragments were dominating over fibres and mostly identified between 20-100 µm. results are presented on **Figure 6**.

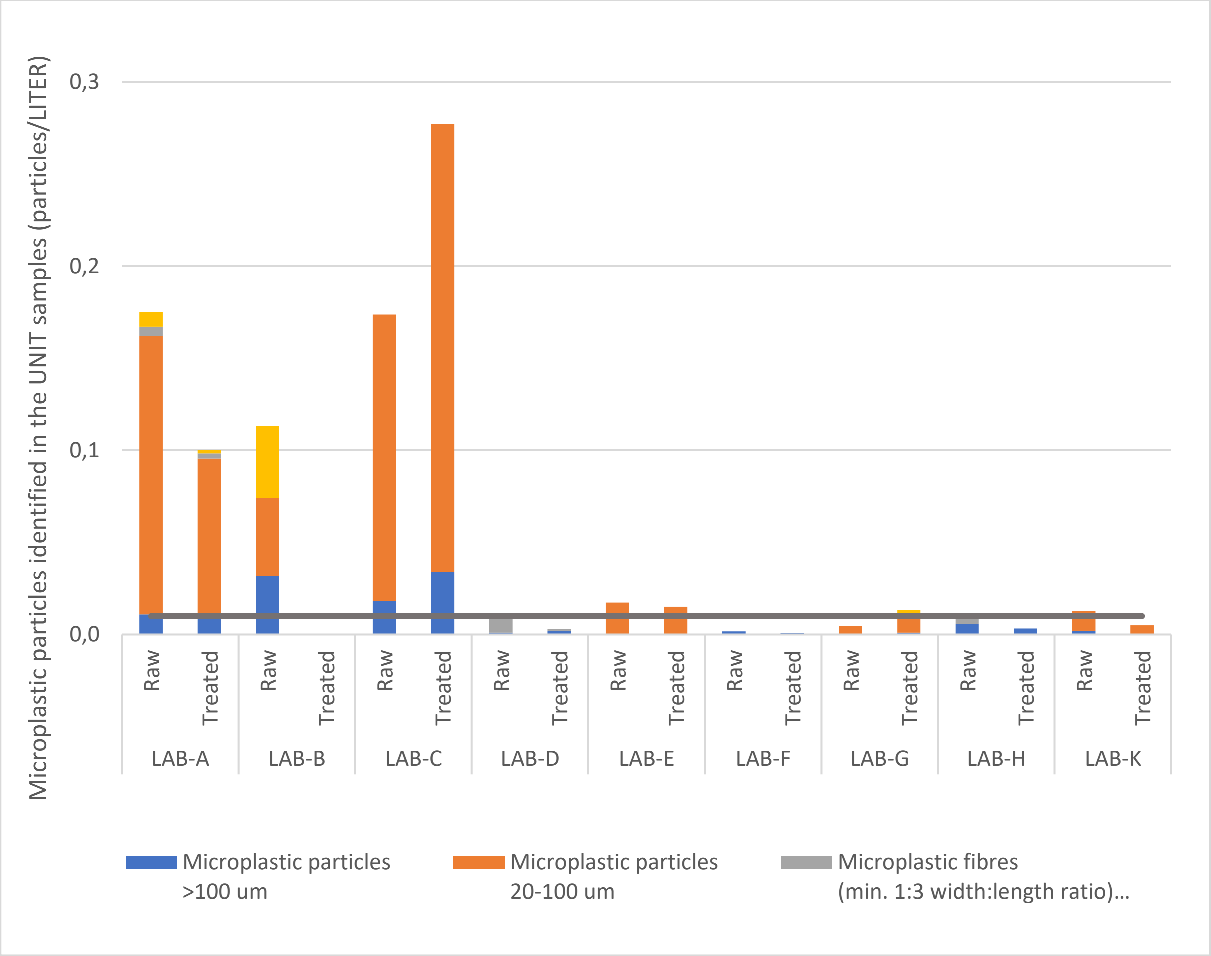

Figure 6: Microplastic occurrence in quasi-homogeneous samples obtained during the joint sampling event and analysed in different national laboratories.

7. Conclusions and recommendations

7.1. Experiences and suggestions on sampling and analysis

7.1.1. Experiences and suggestions on sampling

The implementation of harmonised microplastic sampling across the nine pilot sites of the MicroDrink project has generated an extensive field evidence on the practicality, robustness, and limitations of the sampling equipment and procedures applied. While partners were able to collect the required water volumes at all sites, the experiences reported show substantial variation between sites, highlight several systemic issues with the used filtration hardware, and provide a rich basis for methodological improvement during subsequent project phases.

Across pilots, the **cylindrical stainless-steel filter cartridges, with similar design reported in previous studies (**Johnson et al., 2020; D: Ball et al., 2020; Pitroff et al., 2020) formed the backbone of field sampling, used in Czech Republic, Slovenia, Croatia, Austria, Hungary, Serbia (Pirot and Pavliš), Bosnia and Herzegovina, and Germany. Flat filter holders were additionally applied in Austria, Slovenia and the Czech Republic. Partners generally considered the equipment functional, and many highlighted its ability to collect the intended 1000 L volumes even under variable raw water conditions. However, nearly all pilots using cylindrical cartridges reported notable operational challenges.

A recurring observation across pilots was that the **internal water volume was inconsistent**, with several partners reporting that cartridges overflowed when opened, resulting in immediate sample loss. This variability had direct implications for sample representativity and made estimation of exact processed volumes difficult. Several pilots expressed uncertainty about whether the entire contents of the cartridge could be retrieved consistently for laboratory processing.

The **mechanical robustness and manufacturing consistency of the metal mesh** emerged as a central problem. Multiple pilots noted that mesh layers allowed particles significantly larger than the nominal 20 µm cutoff to pass through. These field observations are corroborated in detail by the **filtration system functionality tests** conducted by the Austrian partner, which documented particle bypass in a controlled, spiked setting. The tests demonstrated that particles up to 200–300 µm were present in second filters (designated as blanks), and that even particles exceeding 500 µm passed through the primary filter during the simulated sampling. These results substantiate the field-level feedback and confirm that the cylindrical cartridge's sieves do not meet their specified retention performance, undermining the validity of MP counts generated with this system.

In parallel, several pilots noted **issues with gasket sealing and mechanical alignment**. The Czech pilot observed that attention "should be payed to the appropriate sealings of the joints & gaskets," while Bosnia and Herzegovina reported that "occasional water leakage occurred despite efforts to ensure all joints were properly assembled.” The functionality test also identified minor leakage during flow verification, requiring repeated resealing before operation. Such alignment problems are exacerbated by the multi-component design of the cartridges, which require precise positioning to maintain full integrity during 1000 L sampling. These challenges were also raised in the internal sampling-lessons presentations, noting the need for “appropriate filters, double O-ring or flat gaskets,” and improved finishing of metal edges to avoid bypass paths.

Another frequently reported practical difficulty concerns **cleaning and reusability of the multi-layer stainless-steel mesh filters**. Partners in Slovenia provided a particularly detailed critique, emphasising that the triple-layer mesh “traps various particles between the layers and prevents effective cleaning through ordinary washing methods,” implying that built-in contamination from manufacturing or previous sampling cycles may never be fully removed. This problem is not merely theoretical: the functionality tests demonstrate that even after dishwasher cleaning, ultrasonic treatment, and rinsing with particle-free water, the second filters (designated as blanks) still contained numerous particles >20 µm. Concerns were raised, that without a validated cleaning protocol or pre-use filter verification, uncertainties in filter-origin contamination remain high. It was also reinforced that future projects may need to “move away from reusable filters to exclude cross-contamination” and consider muffled or pre-burned mesh alternatives as used in other national systems.

The **designation of the second filter in the cascade as a “blank”** became a major methodological question. Multiple partners observed that the so-called blank filters frequently contained more particles than the sample filters. **It is worth to consider, not to call the second filters as blanks as these are also exposed to the analyte**. **The purpose of the second filter is to control filtration efficiency which probably will never be 100%.** The functionality report strengthens this interpretation by showing that large particles intentionally introduced in the spiking test appeared in the blank filters, demonstrating that the second filter is not protected from exposure but is instead an integral part of the filtration pathway. Consequently, partners agreed during 2025 planning to discontinue field blanks using the second filter and to treat both filters as sequential sampling units, while reserving true blanks for laboratory controls.

Experiences also diverged between equipment types. Pilots using **flat filter membranes**, notably Slovenia and the Czech Republic, expressed markedly more positive assessments. The Slovenian team reported that flat holders provide “better particle retention, easy cleaning… and easy preparation of blanks by filling the device with pre-filtered water.” They also stressed the advantage of simpler deconstruction and reduced risk of sample loss. The Czech team characterised flat holders as preferable when “comparing both sampling devices,” especially in contexts with dense organic matter, because the flat design avoids entrapment between mesh layers. These advantages were also echoed in the internal recommendations, which propose that the project “move towards smaller diameter, flat filters” to improve retention reliability and cleaning efficiency.

The **impact of water quality** on equipment performance was highly variable across sites. Several pilots working with raw bank-filtration or karst springs reported that organic matter loads were manageable, whereas Serbia (Pirot) encountered "excessive amounts of organic matter," preventing proper microplastic detection during the second campaign. Austria reported that raw surface-water-derived inflows were heavily enriched in organic particles, requiring extensive digestion steps and causing clogging during sampling. These observations underline the need for flexible sampling approaches that respond to local water matrix conditions.

Another theme across pilots concerns **personnel training and operational clarity**. Several partners requested "more clear instructions on how to operate the equipment… for sampling and cleaning." Slovenia raised methodological ambiguity about how to retrieve the entire sample when the cartridge's internal configuration makes complete rinsing challenging. A standardised tutorial is recommended to be developed in the future to explain opening, rinsing, transferring, and drying filters.

Finally, experiences highlight the broader need for methodological harmonisation and equipment validation. **Despite challenges identified, MicroDrink is "the first in the EU to test the 2024/1441 Delegated Act harmonised filtration concept across many sampling locations**. The project's role is not only to generate data but also to compile a comprehensive feedback package for the Joint Research Centre (JRC) and the European Commission. This includes recognising that "we don't know to what standard we should compare the MicroDrink system", as this has not been done prviously, and other national filtration systems with other alternative designs (from Austria, the Czech Republic, and Slovenia) have not yet been benchmarked in terms of filtration efficiency.

In summary, the experiences gathered during the MicroDrink sampling campaigns indicate that while large-volume sampling was successfully implemented at all sites, the current cylindrical cartridge system suffers from design and performance limitations that affect data reliability. These include particle bypass, inconsistent internal water retention, cleaning challenges, leakage at seals, and the misinterpretation of second filters as blanks. At the same time, the experiences gained across heterogeneous hydrogeological and operational environments have produced a detailed understanding of equipment behaviour and requirements for future optimisation.

7.1.2. Experiences and suggestions on analysis

The analytical work carried out by the MicroDrink pilot laboratories demonstrated the feasibility of applying harmonised microplastic analysis protocols across diverse hydrogeological settings, but also exposed systematic challenges affecting sample representativity, data reliability, and uncertainty reporting. Although each laboratory followed the overarching guidelines, their experiences varied depending on water quality, equipment performance, and laboratory infrastructure.

A primary difference among laboratories concerned **full sample versus subsample processing**. Several partners processed the entire recovered sample volume, enabling maximum representativity. In contrast, some were unable to process whole samples due to high suspended-solid loads or filter clogging. These experiences reflect an inherent challenge: when total particulate loads are large or sample recovery is incomplete, **subsampling becomes unavoidable**, yet it introduces additional uncertainty related to particle heterogeneity, clustering on filter surfaces, and incomplete transfer.

Relatedly, pilots differed in how subsampling was performed **on analytical filters themselves**. Slovenia, Hungary, and Bosnia analysed entire filter surfaces, whereas Austria analysed 25% and Slovenia 20% of the 20-μm membrane, and Germany analysed a mapped sub-area only. While sub-area mapping was efficient for high-resolution spectroscopy, several laboratories noted that this approach risks missing rare or low-abundance MPs, particularly when particle distribution on filters is uneven. These findings underline the need for clearer guidance on how to define representative analytical sub-areas and how to upscale subsample results.

A major determinant of analytical feasibility was the **total particle load**, which in most cases was dominated by natural organic matter, mineral particles, or biofilm fragments. Even at sites with low natural turbidity (e.g., Germany), total loads influenced measurement quality: non-plastic particles generated fluorescence or spectral overlap in Raman images. These observations show that MPs are analytical minorities, and accurate detection depends heavily on the ability to reduce matrix interference without altering polymer integrity.

These matrix effects directly influenced the **reliability of particle identification**. Multiple pilots reported persistent difficulties distinguishing polyamide (PA) from biological residues, and in identifying very thick, weathered, or pigmented particles. Hungary noted that thick particles could not be reliably analysed in transmission mode. Croatia and Bosnia highlighted misclassification risks for small particles near the fibre/fragment threshold. Spectral ambiguity was also amplified by the performance limits of automated classification software, which occasionally misidentified natural organic debris as polymeric materials. Collectively, these experiences confirm that **size, morphology, and chemical state strongly influence spectral clarity**, and that manual verification remains essential – especially for particles below 50–70 μm or for irregular shapes.

A recurring technical theme across all laboratories was the role of **background contamination control**. Most partners employed strict measures – cotton laboratory clothes, exclusion of plastic consumables, ethanol cleaning, filtered solutions, and the use of laminar-flow cabinets where available. Slovenia and the Czech Republic operated dedicated MP analysis spaces with continuous air monitoring. Nonetheless, several sites (notably Germany and Austria) detected polymer types in laboratory blanks (e.g., PC, PVC), highlighting that airborne contamination and laboratory cross-transfer remain unavoidable. Importantly, **all laboratories converged on the same conclusion**: the second filter in the sampling cascade **cannot** be considered a blank because it is exposed to the analyte stream. This is strongly supported by the filtration-system functionality tests, where intentionally added particles appeared not only on the primary filter but also on the second filter, demonstrating that it functions as a sequential sampling element rather than a control. Probably

100% filtration efficiency cannot be expected, and therefore blank interpretation must be based solely on laboratory-prepared controls rather than in-line filters.

Experiences with **experimental verification through spiking** also varied. Croatia and Bosnia achieved recoveries above 60% using 70-µm polystyrene microspheres. The Czech laboratory reported recoveries near 80% using PET-G particles, while Hungary achieved 76% using fluorescent polyethylene beads. These results demonstrate that laboratories can achieve moderately high recoveries with controlled, well-defined particles. However, several partners noted limitations in obtaining representative reference materials. The Czech partner, in particular, pointed out the **need for surrogate particles that are traceable:** chemically stable, and spectrally robust. Internal recommendations also emphasised that harmonised spiking protocols will be required to ensure comparability and to detect systematic biases in subsampling or digestion.

Similarly important were experiences related to **contamination prevention and cleaning**, which influenced analytical uncertainty even in laboratories with rigorous protocols. Partners generally performed thorough cleaning of tools with filtered water and ethanol, used aluminium foil to protect surfaces, and ensured that glassware was rinsed immediately before use. Nevertheless, as noted in internal discussions, contamination “must be prevented but also accepted as partially unavoidable,” and the purpose of quality control is to quantify and bound contamination rather than eliminate it entirely. The filtration-system functionality tests demonstrated that even when filters are cleaned with particle-free water and lab-grade detergents, residual material may remain trapped between mesh layers or become dislodged during sample processing, reinforcing that both false positives and false negatives are possible in low-abundance MP datasets.

In summary, the pilot laboratories demonstrated strong analytical capability but also revealed a consistent set of challenges affecting measurement reliability. High total particle loads, variability in subsampling needs, inconsistent filter cleanliness, the complexity of distinguishing synthetic from natural particles, and the unavoidable presence of low-level contamination all underscore the need for transparent uncertainty reporting. Despite these constraints, the analytical work across the Danube region demonstrates meaningful convergence in laboratory practice and provides a solid basis for refining MP analysis workflows. The collective experiences provide the evidence base for improving analytical protocols, harmonising subsampling strategies, advancing QA/QC design, and providing recommendations to the European Commission and the JRC.

7.2. Key findings on microplastic occurrence by water resource type

7.2.1 Implications for water facility operators

The MicroDrink project demonstrated that bank-filtration and surface-water systems are generally associated with the highest microplastic concentrations and the greatest variability among the investigated resource types. Direct hydraulic connection to rivers and surface-water bodies increases exposure to urban activities, wastewater discharges, stormwater inputs and other anthropogenic pressures. Water suppliers operating such systems should therefore implement robust monitoring programmes and regularly verify the effectiveness of treatment barriers. Particular attention should be given to periods of high flow, flooding and other hydrological events that may increase microplastic transport.

7.2.2 Implications for regulators and policy makers

The findings support prioritising bank-filtration and surface-water systems within future monitoring programmes under the EU Drinking Water Directive. Due to the elevated occurrence and variability of microplastics, these systems are particularly suitable for risk-based monitoring approaches and may require more frequent surveillance than protected groundwater resources. Regulatory frameworks should encourage harmonised methodologies and consistent quality assurance procedures to ensure reliable assessment of temporal and spatial trends.

7.2.3 Implications for laboratories and the technical community

Samples collected from bank-filtration and surface-water systems often contain higher overall particle loads, resulting in increased analytical complexity. Laboratories should ensure that sample preparation procedures are capable of handling elevated concentrations of organic matter and inorganic particles while maintaining reliable polymer identification. The project further highlights the need for continued methodological improvements, interlaboratory comparisons and validation of analytical approaches across different matrix types.

7.2.4 Implications for EU DWD implementation and water managers

The results confirm that resource type is a major determinant of microplastic exposure risk and that bank-filtration and surface-water systems should receive particular attention during implementation of the EU Drinking Water Directive. Water managers should incorporate microplastic occurrence into source-risk assessments and treatment-performance evaluations. Transparent documentation of sampling volumes, analytical procedures and quality-control measures remains essential for interpreting results and supporting regulatory compliance.

7.2.5 Recommendations for monitoring design and further research

Future monitoring programmes should prioritise higher-frequency sampling and seasonal assessments in bank-filtration and surface-water systems. Research should further investigate the influence of wastewater discharges, stormwater inputs, river hydrology and catchment characteristics on microplastic occurrence. Improved understanding of treatment removal efficiencies and environmental drivers will support the development of effective management strategies and strengthen future monitoring programmes.

7.3. Transferability of findings beyond DRB

The microplastic occurrence patterns observed across the Danube River Basin provide insight not only into regional conditions but also into the broader behaviour of MPs in comparable hydrogeological environments across Europe and globally. Because the project applied harmonised sampling and analytical protocols aligned with the emerging EU Drinking Water Directive framework, its findings carry high potential for transferability. The DRB encompasses a wide spectrum of drinking-water resources – intergranular aquifers, karst systems and bank-filtration/surface-water sources – representing hydrogeological conditions that recur on a broader scale. Thus, the lessons learned here can inform risk assessments, monitoring designs and regulatory implementation in other regions confronting similar water-resource challenges.

7.3.1. Applicability to similar hydrogeological settings worldwide

The most robust and widely transferable conclusion is the gradient in MP occurrence across water-resource types. Intergranular aquifers, characterised by thick sedimentary layers and long residence times, show low MP concentrations in the DRB, frequently at or near detection limits. This finding can be confidently extended to other regions where groundwater is drawn from well-confined porous aquifers. In such settings, natural filtration processes remove larger and many smaller particles, while hydrological isolation limits MP introduction from surface sources.

Karst aquifers share hydrological traits such as rapid infiltration, conduit-driven flow and strong responsiveness to rainfall. The DRB data show that karst systems contain slightly higher but still relatively low MP levels, dominated by small PE and PP fragments linked to diffuse surface inputs. Thus, for karst regions beyond the DRB, the MicroDrink results provide a realistic expectation of low but hydrologically sensitive MP presence, suggesting the need for event-based monitoring.

Bank filtration and surface-water-influenced systems in the DRB display the highest MP concentrations and greatest polymer diversity. The DRB pilots indicate that MP levels in bank filtered systems reflect upstream pressures – urbanisation, wastewater effluent, stormwater drainage and industrial sources. These relationships are highly transferable to any setting where drinking water production relies on river infiltration, especially where catchments contain mixed land uses, urban concentrations or industrial corridors. Internationally, this insight reinforces the need for catchment-focused MP management, linking water-resource protection to wastewater treatment, stormwater infrastructure and urban planning.

7.3.2. Role of harmonised methods and the monitoring database in enabling transferability

A key strength of the MicroDrink project is its harmonised methodology, which follows the specifications introduced by the EU DWD and the Joint Research Centre. This methodological consistency allows MP results from nine pilot sites – using multiple laboratories, instruments and hydrogeological conditions – to be compared on a common basis. Beyond the DRB, such standardisation enables two major pathways of transferability.

First, harmonised **large-volume sampling** (around 1,000 L), defined **size classes**, common **polymer-identification protocols**, and agreed **QA/QC criteria** form an operational framework that can be adopted directly by other regions. The fact that partners representing karst, porous aquifers and surface water systems successfully applied the same core methodology demonstrates that the approach is robust across resource types. For countries preparing to implement microplastic monitoring, the DRB experience shows that consistent detection thresholds and unified reporting fields (polymer type, size class, shape) create results that are comparable not only within one region but internationally.

Second, the MicroDrink monitoring database – built from harmonised field and laboratory inputs – serves as a **reference dataset** for environmental baselines. Because many regions worldwide have limited microplastic data for drinking water sources, the DRB database provides a template for what to expect. The structure of the database also supports interoperability with future EU-level repositories, enabling cross-country and multi-basin comparisons. This interoperability is crucial for global research communities aiming to develop models of MP transport, identify transboundary pollution patterns, and benchmark treatment performance.

7.3.3. Lessons learned for implementing EU DWD MP monitoring

The DRB experience provides several clear lessons for the upcoming EU-wide implementation of mandatory microplastic monitoring under the DWD.

First, **resource-type-specific monitoring strategies** are essential. The DRB shows that surface-water-influenced systems warrant higher sampling frequency and more detailed analyses, whereas deep intergranular aquifers may require only periodic confirmatory monitoring. Karst systems, which sit between these, benefit from targeted sampling after high-recharge events. This resource-based differentiation is directly aligned with the DWD’s risk-based approach and can be applied broadly across the EU.

Second, the DRB pilots demonstrate the crucial importance of **validated sampling equipment**. Several sites encountered sampling-system performance issues, leading to unreliable data. As EU Member States begin applying the DWD’s microplastic requirements, equipment validation – including mesh integrity checks, flow-characterisation tests and blank stability assessments – must be considered a prerequisite rather than an optional step.

Third, the harmonised MicroDrink approach demonstrates how **cross-laboratory consistency** can be achieved even when using different FTIR and Raman systems. This reinforces the DWD’s expectation that laboratories follow common data-evaluation principles and interoperable spectral libraries. The DRB experience supports the development of EU-wide training, interlaboratory comparisons and shared QA/QC documentation.

7.3.4. Overall transferability

Taken together, the DRB findings are widely transferable to other European and global regions with similar hydrogeological and catchment characteristics. The natural filtering capacity of intergranular aquifers, the responsive but generally low-MP nature of karst systems, and the higher

MP loads typical of bank-filtration and surface-water supplies are consistent with international observations. The project further demonstrates how harmonised sampling and analysis enable robust cross-country comparisons – an essential feature for future EU DWD implementation. As other regions adopt similar monitoring systems, the DRB experience provides a strong operational model, showing how environmental context, methodological harmonisation and structured data reporting together form the foundation for meaningful and transferable microplastic assessments, while thoroughly considering technical challenges identified via the whole sampling and analysys workflow and detailed during the project.

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