Circular and Bio-Based Solutions for the Ultimate Prevention of Plastics in Rivers Integrated with Elimination And Monitoring Technologies

Deliverable D.1.1
Harmonized protocols for surveying and monitoring litter, plastics and microplastics

Deliverable information

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Executive Summary

Deliverable 1.1 reviews the latest advances in harmonisation and standardisation of monitoring of litter, microplastics and nanoplastics. No single analytical technique can measure and quantify the litter-micro-nanoplastic continuum across its entire diversity. The analytical capabilities across the consortium were therefore mapped and key shared analytical spaces identified. These are critical to understand where direct comparison may be possible across data generated for different demo sites, where different partners are responsible for monitoring. Where there are common analytical "windows" shared across partners, solutions to allow for data comparisons are proposed for further development, such as the idea of data restriction methods, to adjust monitoring data generated to shared analytical spaces across demo sites. This approach focuses on harmonisation, in the absence of agreed and validated international standards across the diverse range of analytical methods for quantifying plastic pollution. The aim of the deliverable is therefore to ensure that the project achieves its aim of effective monitoring of litter, microplastics and leachable compounds through co-ordinating all partners responsible for monitoring these classes of plastic pollution to establish guidance on accepted best practice across the project. To ensure that these best practices are adhered to, a Sample Collection Record Template is established for microplastic monitoring, where the greatest number of partners and methods are used, and so the need for harmonisation across the project is greatest.

Detailed guidance is described for monitoring litter, microplastics and leachable compounds individually. Some key results of this exercise that are generalisable across classes of the plastic pollution continuum:

- A summary of the shared analytical spaces across the project, as well as gaps in this coverage to identify where data from demo sites may be most reliably compared later in the project.
- A summary of internal validation planned between partners to assist in data interpretation across demo sites.
- Proposals for data interpretation across demo sites with shared analytical spaces (e.g. data restriction methods)

A summary of key results of this exercise that are specific to monitoring litter:

- A review of the latest developments in standardisation of litter monitoring
- An agreed definition of "hotspots" for litter in the project, harmonised with current international standards, best practice and evidence.
- SOPs, QA/QC checklists and data templates for unmanned arial vehicle detection of beach litter and beached litter ground surveys

A summary of key results of this exercise that are specific to monitoring microplastics:

- A review of the latest developments in the standardisation of microplastics monitoring.
- A draft Sample Collection Record Template, which takes the principles of quality assurance and control criteria for microplastics and provides a standard template within which to harmonise collection of relevant meta data for any sampling campaign for microplastics in the project.
- The Sample Collection Record also provides a standardised QA/QC scoring system that can be applied to any monitoring of microplastics in the project against agreed principles of best practice.
- Detailed guidance on agreed principles of best practice are summarised.
- A new tool for Representative Sample Volume Predictions (RSVP) developed by UKCEH is presented which was developed to address a significant gap identified – flexible guidance for justifying representative sample volumes that can be specific to individual analytical techniques, which may look at different regions of the microplastic continuum.

- Links to published SOPs used by partners monitoring microplastics are provided.

A summary of key results of this exercise that are specific to monitoring leachable compounds:

- An overview of the analytes currently expected to be quantified/monitored by different partners is summarised, allowing common analytes across demo sites to be identified.

In all, the deliverable is structured around key principles identified for the monitoring of litter, microplastics and leachable compounds and detailed guidance on best practice, harmonised records and quality assurance and control have been developed and agreed across all relevant partners in UPSTREAM to ensure that all monitoring data generated

Deliverable Keywords: *Monitoring, harmonisation, methods, data records, quality assurance and control*.

WP no.	1	Lead beneficiary	VITO
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Objectives

Overall, ensure that the project achieves its aim of effective monitoring of L, P and MP produced from various pollution sources and serve as an input for uptake of innovative solutions to prevent, collect, reuse and treat L, P and MPs in European rivers. Specific objectives are:

- Establish a set of validated, cost-efficient, robust, and easy implementable protocols for surveying and monitoring L, P and MP in the 5 demonstration sites and rivers.
- Conduct mapping of L, P and MP in selected demonstration sites and rivers using established protocols and identify hotspots of plastics and MP and understand their dynamics within these sites.
- Provide data on the effectiveness of different innovative technological solutions for removal of L, P and MP.

Task 1.1 Harmonized protocols for surveying and monitoring litter (L), plastics (P) and MP (MP) (Lead: CEH; partners: NIC, VITO, UNSPMF) (M1-M18 – March 2025) The current state of the art for harmonised approaches to sampling, sample preparation and analysis of L, P and MPs relevant to the demonstration technologies that are the focus of monitoring in the project will be reviewed by **CEH**, **NIC**, **VITO**, and **UNSPMF**. New protocols where required will be established, but our ambition is to utilise existing harmonisation efforts to be most efficient and effective in defining protocols for use within the project. Key existing projects such as EUROqCHARM (Horizon 2020, 101003805) which aims to harmonise at the European level methodologies for the monitoring and assessment of macro and MP in the environment, along with UNEP guidance/recommendations and ongoing standardisation efforts including ISO future norms for MP such as ISO/TR 21960:2020(en) and ISO/AWI 16094-2 will be monitored by **CEH** and **VITO** to make best use of the existing investment in this research area. Key principles will be identified around representative sampling, harmonised reporting and quality assurance and control will be established and recommended that can be easily transferred and applied to all demonstration technologies. Ultimately, a series of protocols that cover the range of sampling, sample preparation and analysis pipelines that constitute the core monitoring program for the project will be delivered (D1.1). In addition to this, guidance on minimum data reporting requirements for L, P and MP in the environment will be developed by **CEH**, to ensure that the data generated during the monitoring phases of the project can be reliably used in exposure assessments for example.

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Abbreviation	Definition
WWTW	Wastewater treatment works
QA/QC	Quality assurance, quality control
MP	Microplastic
SOP	Standard Operating Procedure

1. Introduction

Plastic pollution is a diverse contaminant that represents a continuum of properties, from macro scale litter, down to micro and nanoplastic particles, as well as the leachable compounds associated with such plastics. As such no single analytical technique can monitor “plastics” in the environment, and the region of this continuum targeted for monitoring in UPSTREAM is dependent on the technology under development at each of the demonstration sites in the project and the analytical techniques available across the partners.

A total of five demonstration sites connecting seven rivers, feeding into five sea basins are to be investigated in the UPSTREAM project. Of these demonstration sites, four technologies associated with wastewater treatment works (WWTWs) are investigated whilst the fifth site is the Danube River itself in Serbia. At each demonstration site, either litter, microplastics, leachable compounds associated with plastics, or a combination of these are the target of monitoring. Different institutions are responsible for monitoring efforts, associated with the different demonstration sites. In addition, some of the analytical techniques used to monitor plastics or their associated chemicals require innovations in their own right. This includes the use of remote sensing approaches, higher throughput microplastic analysis through fluorescence staining and mass spectrometry-based methods for the analysis of leachable compounds. Given this diversity of analytes, locations and institutions required to achieve the monitoring goals in the project, it is essential that standards are used where available, and harmonisation of methods is encouraged where such standards do not exist.

There are four critical areas for harmonisation when considering monitoring in the environment:

- Sample collection
- Sample preparation (QA/QC of MP extraction and handling)
- Sample analysis
- Data analysis and interpretation

For each of these areas, we must set out the problem (where harmonisation is needed), where aspects can be unified across the demonstration technologies, case study sites and laboratories performing the analyses, reporting requirements to allow harmonisation where methods necessarily diverge and finally areas where harmonisation is not possible and why.

Taking each of the major analytes for monitoring; litter, microplastics and leachable compounds in turn, these aspects of harmonisation are considered, SOPs collected, and agreement reached for recommendations for harmonisation across methodologies. The ambition is to allow evaluation of demo sites themselves but also between sites.

1.1. Status of international standardization and harmonization efforts

1.1.1. “Standard” definitions of plastic litter of different sizes

There are many different definitions for nano, micro and macroplastic litter that have been adopted within different academic, regulatory or policy contexts. A pragmatic definition of plastic categories based on size was recommended to monitor debris trends in the marine environment by the National Oceanographic and Atmospheric Administration (NOAA, USA) over a decade ago. This defined mega (>1 m), macro (1 m–2.5 cm), meso (2.5 cm–5 mm), micro (5 mm–1 µm), and nanoplastics (<1 µm) (Lippiatt, Opfer and Arthur, 2013). These definitions have informed the MSFD Guidance on monitoring litter in European seas. It should be noted that even in this guidance, whilst an upper limit of 5 mm is used to define microplastic litter, the lower limit is acknowledged to usually be determined by the mesh size used to capture particles from the environment, or on technical limits of the analytical instruments, such as a lower limit of ~20 µm for vibrational spectroscopy Fourier Transform Infra-Red microscopy, µ-FTIR (JRC, 2013). Indeed, Hartmann *et al.*, (2019) critically reviewed how these common terms of nano, micro, meso and macro

plastic debris were not unified or consistent in the literature, with many different size regions defined for each classification in both the academic literature but also institutional reports. Whilst definitions of larger litter are highly standardised (e.g. JRC/MSFD), the definition of smaller microplastic litter is still inconsistent across different institutional reports and has not successfully been harmonised since Hartmann's report in 2019.

Considering the task in UPSTREAM of quantifying microplastics in European rivers and in the technologies designed to eliminate microplastics or prevent them from entering freshwaters, it is perhaps relevant to consider more contemporary references and standards as a starting point for a definition of micro and nanoplastics. ISO 24187:2023, "Principles for the analysis of microplastics in the environment" (ISO, 2023), separately defines large microplastics as any solid plastic particle with any dimension between 1-5 mm in size, and microplastics as those with any dimension between 1 and 1000 µm. This is aligned with the definition from ISO/TR 21960:2020 "Plastics - Environmental aspects - State of knowledge and methodologies" (ISO, 2020).

More recently, the two draft standards relating to vibrational spectroscopy and thermogravimetric techniques to quantify microplastics (ISO, 2024b, 2024c), refer to microplastics as being defined as any solid plastic or synthetic polymer particle insoluble in water with the largest dimension between 1 µm and 5 mm. These draft standards acknowledge that this encompasses both the definition of microplastics and "large microplastics" from the earlier ISO standards 24187 and 21960. These two draft standards are highly relevant in the context of UPSTREAM as they relate to analytical methods employed by the consortium.

We report the above variations and nuances in definitions both between organisations but also across standards, to demonstrate that a formal and universal definition of "microplastics" as compared to other size categories of plastic litter is neither straightforward, nor agreed to date.

Therefore, we take a pragmatic view on these definitions. In UPSTREAM, we use JRC/MSFD (Joint Research Centre/ Marine Strategy Framework Directive) monitoring guidelines to classify litter, whilst for microplastics, we use the latest ISO terms and definitions for microplastics as between 1 µm and 5 mm. We take this broad definition from the draft standards for the analysis of microplastics in water (ISO, 2024b, 2024c) as these are the most relevant standards to the analytical methods available across the consortium. However, it should be noted that the lower size limit of detection does vary between analytical methods, which can result in large differences in reported number concentrations of microplastics if not carefully considered in the interpretation. We discuss this in detail in the following sections 1.1.4 "Standardization of microplastics monitoring" and 1.2 "Mapping analytical capabilities to identify commonalities and gaps in analytical coverage".

1.1.2. Standardization of litter monitoring

Beach litter monitoring using traditional field surveys and litter collection is highly standardized. Three main monitoring and collection protocols exist:

- JRC/MSFD Guidance on the Monitoring of Marine Litter in European Seas (Joint Research Centre (European Commission) and MSFD Technical Group on Marine Litter, 2023).
- OSPAR Commission - Guideline for Monitoring Marine Litter on the Beaches in the OSPAR Maritime Area (Wenneker and Oosterbaan, 2010).
- UNEP/IOC – Guidelines on Survey and Monitoring of Marine Litter (United Nations Environment Programme and Intergovernmental Oceanographic Commission, 2009)

All three protocols follow a very similar format, with key elements being identical across protocols, making them to a great degree interoperable:

- Survey area: Standardised transects, most commonly 100 m long

- Litter categories: Comprehensive and consistent categorization
- Frequency: Usually quarterly or seasonal surveys for trend analysis
- Data collection: Structured field sheets or apps
- Public involvement: Citizen science participation is encouraged

Riverine litter monitoring on riverbanks on the other hand is not standardised to a great extent, but the same protocols with beach monitoring can also be applied here. van Emmerik *et al.*, (2020) follow a River-OSPAR protocol, which is largely based on the OSPAR (Convention for the Protection of the Marine Environment of the North-East Atlantic) guidelines. They collect data on 100 m long stretches of riverbank parallel to the waterline. The survey width is defined as the distance from the waterline and the high-water line, which is recognized by deposited debris, at a maximum of 25 m. All macro litter items (>2.5 cm) that are visible from standing height are collected within the entire sampling area.

In UPSTREAM, we will follow the JRC/MSFD (Joint Research Centre/ Marine Strategy Framework Directive) monitoring guidelines, which are compatible with EU directives and (River)OSPAR guidelines. Details of the protocol methodology are outlined below, in the section Harmonized Methods for Monitoring Litter.

In the context of floating litter, a new monitoring app has been developed through UPSTREAM's HORIZON sister project INSPIRE - Innovative Solutions for Plastic Free European Rivers (JRC, 2025). The JRC Floating Litter Monitoring app is designed for tablet computers to monitor floating macro litter (>2.5 cm) in the sea and rivers. The monitoring method is based on visual observations from vantage points over the water surface (e.g., ships, bridges). Observers fill in metadata and GPS position before starting monitoring, where a full list of litter items is recorded, harmonized with the EU MSFD and specifically the Joint List of Litter Categories for Marine Macrolitter Monitoring (European Commission: Joint Research Centre *et al.*, 2021). The observers register as users of the app via an EU login account and upload the monitoring sessions to the Floating Litter Monitoring data portal. The new version of the app will be available for testing and data collection under the INSPIRE project.

1.1.3. Standardization of hotspot definition

Hotspot definitions vary substantially between reporting bodies and plastic pollution studies. And while most plastic pollution studies focus on the identification of plastic accumulation areas, methodologically they vary greatly in terms of definition criteria and temporal and spatial domains. The UNEP's draft text of the international legally binding instrument on plastic pollution, including in the marine environment, dictates the need for prioritizing hotspots and accumulation zones, stating that a definition for both terms is potentially needed. To date however, no globally applicable definition framework of a hotspot with a concise purpose and boundaries exists (Tasseron *et al.*, 2024).

Marine litter hotspots on beaches are usually identified by the number of litter items per 100 meters of coastline, with any area with a litter count above a set threshold considered a hotspot. The JRC technical report on Threshold Values for Marine Litter states that threshold values for beach litter should be based on data on the abundance of litter recorded during beach surveys, and should be defined by cut-off values, absolute values or percentiles, determined through expert judgement (Werner *et al.*, 2020). The OSPAR commission's current threshold to define a clean beach was set in 2023 at 20 items/100 m stretch of coastline, adopted at the EU level, which is an indicative value of beach litter status in the OSPAR Maritime Area (Lacroix, André and van Loon, 2023). The report noted that, due to limited scientific data on the ecological and socio-economic harm caused by beach litter, the threshold was set based on this percentile to ensure a precautionary approach. However, it is important to recognize that this threshold is a target for environmental quality and does not represent the current state of pollution, as many beaches currently

exceed this number. Specifically, the median total count in the OSPAR Area over the period from 2018 to 2020 is 252 items/100 m.

Tasseron *et al.*, (2024) propose a hotspot definition framework using quantitative statistical methods to define hotspot thresholds. It can be seen how this would prove useful if the purpose is to identify locally the most significant region of contamination to co-ordinate and plan clean-up efforts for example. On the other hand, defined thresholds are most commonly used in other approaches and are usually based on a combination of scientific judgment, data availability and policy objectives (Bank *et al.*, 2021). Both have advantages and disadvantages depending on the context. Contrary to using statistical thresholds, using arbitrary thresholds across different scales of studies can potentially hinder meaningful comparisons between hotspots, if non-standardised, local thresholds are used. Nevertheless, the use of universal arbitrary thresholds that are based on empirical observations and scientific experience are relevant for a more direct comparison across larger geographical scales and provide a baseline for a more practical definition of a hotspot, which takes into account a de facto absolute threshold, below which a location cannot be considered a hotspot.

In UPSTREAM we will follow the 5-step framework proposed by Tasseron *et al.*, (2024), employing both a statistical and a fixed threshold for setting the hotspot thresholds. This approach adheres to standards and requirements set out in both JRC and OSPAR publications and is harmonized with the EU MSFD. Details of the framework are presented in section 2.3 below.

In addition to the riverbank hotspot identification, high resolution satellite imagery will be used to assess the possibility of identifying floating litter accumulation zones, in areas of low river flow or flow obstructions, in the greater area of the Novi Sad demo site. These floating hotspots can serve as locations for litter collection before it reaches the riverbanks, and as a guideline for WP3 demonstration area selection.

1.1.4. Standardization of microplastics monitoring

Significant efforts are ongoing into the standardization of protocols for quantification of microplastics in the environment. Two International Standards Organization work items are currently under development relating to the quantification of microplastics in water using vibrational microscopy (ISO, 2024a) and thermogravimetric techniques (ISO, 2024c), whilst a third standard is close to publication concerning guidance for sample collection in waters (ISO, 2024d). The ASTM (formerly American Society for Testing and Materials) is also undertaking a new work item on spectroscopic identification of microplastics in water using infrared spectroscopy (ASTM, 2023), and in 2020, published guidance on standard practice for collecting water samples for microplastic quantification (ASTM, 2020). More local or regional efforts have also made significant progress in this area of research, notably the Southern California Coastal Water Research Project (SCCWRP). This group has published two standards for the quantification of microplastics in drinking water, one for infrared (De Frond and Wong, 2021) and one for Raman spectrometry (Wong and De Frond, 2021). These have been adopted in the Californian Statewide Microplastics Strategy. Links to the available standards have been shared across WP1 partners. Whilst UPSTREAM does not investigate drinking waters, the key principles and general considerations for QA/QC are relevant across all environmental matrices. Indeed, these resources formed the starting point for the key summaries presented in this deliverable. Thus, for microplastics, the sample collection, preparation and analysis are taken in turn during this deliverable and key considerations described for which all partners involved in monitoring microplastics must be aware.

As well as the generation of standard protocols, there are numerous efforts to understand the performance and consistency of quantification of microplastics using common analytical techniques. This has led to the undertaking of several large international interlaboratory testing exercises in recent years. These have all highlighted the challenges still faced in consistency and repeatability of measurements across laboratories.

Good performance in polymer identification was found in the first WEPAL QUASIMEME/NORMAN interlaboratory study, however, quantification is still highly variable (van Mourik *et al.*, 2021). Likewise, a similar conclusion was reached during a separate study organized by the JRC/BAM (Belz *et al.*, 2021). Such work has reinforced the importance of understanding performance between measurements and has catalysed the generation of new reference materials or representative test materials (e.g. Martínez-Francés et al., 2023). For this reason, it is important that guidance on the use of positive and negative controls (recovery and blank assessment) was included in the deliverable, so as to provide an internal benchmarking of performance for each analytical technique and each participating laboratory. No shared test material was designed for use in UPSTREAM due to the different requirements for each analytical technique, however, the use of existing in-house standards is encouraged across laboratories.

Given the diversity of analytical methods available across the partners in UPSTREAM and the lack of available standards, no single standardized method can be adopted across all laboratories and demo sites. Rather, agreed common principles that allow for different methods to be judged against the same criteria are considered the focus of this deliverable. A critical aspect to assist in this is the standardization of reporting requirements when collecting, preparing and analysing samples. For microplastics in water, a set of quality assurance criteria have been proposed that can be distinguished into sample collection, preparation and analysis). These form the basis of the harmonized reporting principles which are the core output of D1.1. At the time of reporting, the Koelmans et al., quality assurance criteria have been translated into an editable table format as the basis for a harmonized “Sample Collection Record Template”. This will be further developed in T1.5 to allow adoption of the core principles of good practice which are needed to be implemented across partners in the project monitoring microplastics and integration of a record of these critical parameters following FAIR principles in the database under development in T1.5. This draft Sample Collection Record Template is provided in Annex .

1.1.5. Standardization of nanoplastic monitoring

There are no existing standards for monitoring nanoplastics in environmental samples. Numerous analytical techniques have been proposed as relevant for the detection and quantification of sub-micrometer scale plastic, or nanoplastics (Schwaferts *et al.*, 2019). A more recent review of published methods identified various techniques that have been reported as relevant, including scanning electron microscopy (SEM), μ-Raman, dye staining, pyr-GC-MS and other techniques based on mass spectrometry, particle tracking analysis or dynamic light scattering (Primpke *et al.*, 2023). Many proposed approaches have been tested primarily with known introduced materials as proof of principle studies, rather than having been applied to quantify unknown nanoplastics in environmental samples (e.g. for fluorescent and staining based approaches, Morgana *et al.*, 2024). Indeed, reproducible analytical pipelines for nanoplastic quantification in waters were not possible to construct in the EUROqCHARM project (Primpke *et al.*, 2023).

Significant gaps have been identified not only concerning the analysis and quantification, but also how to sample, concentrate and isolate nanoplastics from complex environmental matrices. This challenge is not only faced for nanoplastics, but has been highlighted as an urgent priority for standardization at the OECD more broadly for any carbon based engineered nanoparticle (Bleeker *et al.*, 2023). It is important to learn not only from the microplastic community but also the significant expertise that has built up over the last decade or more in the field of engineered nanomaterial research, where international standardization is more progressed. Working groups and test guideline programs relevant to monitor for progress that may be relevant to nanoplastic characterization quantification in environmental samples include:

- TGP Project 1.10, Guidance Document on the determination of concentrations of nanoparticles in biological samples for (eco)toxicity studies

- Study Report No. 340, Study Report on MNS Removal in Wastewater Treatment Plants: Activated Sludge Sorption Isotherm
- TGP Project 3.12, Guidance Document on assessing the apparent accumulation potential for nanomaterials TG 305
- TGP Project 3.16, Guidance Document and Test Guideline on Aquatic (Environmental) Transformation of Nanomaterials

The status of these projects is described in (Heunisch *et al.*, 2022), although some progress is expected to have been made since publication of this update.

1.2. Mapping analytical capabilities to identify commonalities and gaps in analytical coverage

First of all, it is important to understand the range of analytical techniques that will be employed in the project. No single method can identify and quantify the diverse contaminant that is the "plastic universe", that is, across the whole range of polymers, sizes and forms that are encompassed within this term. Rather, to describe all plastic contamination, from litter to nanoplastics, complimentary techniques are required, each of which has its own specific window into this continuum. This "analytical window" is the operational space within the multiple dimensions that can describe microplastic material and must be defined every time we report on microplastic concentrations from the field.

There are several key principles around which we can start to define our analytical windows for each technique. This includes both considerations around sampling and technical constraints and the performance of different instruments. The most important factor is the size region in which microplastics are quantified, as this has the greatest implications for particle count based quantification. To assist in understanding where natural overlap in analytical windows exist across partners in UPSTREAM, a mapping exercise was conducted to review the analytical techniques available across all partners and to establish the size region for which each technique is quantitative and the metrics which can be reported from the analysis (e.g. counts, size, polymer identity and mass).

Twelve unique analytical techniques were identified as available across the 9 participating laboratories involved in monitoring activities in the project (Figure 1).

Demo site	Institute	Analytical technique	Lead contact	Nanoplastics nm			Microplastics µm				Meso- and macroplastics cm			Metric			
				1	10	100	1	10	100	1000	1	10	100	Counts	Mass	Size	Polymer
Demo 1: Severn Trent WWTW Cellulose recovery	UKCEH	µ-FTIR	Rich Cross												Estimated		
		ATR-FTIR	Rich Cross														
Demo 1: Daphnia water solutions	UoB	µ-FTIR	Luisa Orsini												Estimated		
Demo 2: Zaragoza WWTW	LEITAT	NIBS	Xialei You Chen														
		NTA	Xialei You Chen														
		Mastersizer	Ruben Rodriguez														
		FTIR	Ruben Rodriguez														
		Optical microscope	Ruben Rodriguez														
		Fluorescence staining (Rhodamine -B)	Ruben Rodriguez														
Demo 3: CAP WWTW	NVMT	NIR	Michele Pognani													Pre defined*	
	VITO	Pyr-GC-MS	Milica Velimirovic														
		ATR-FTIR	Milica Velimirovic														
		Atomic Force Microscopy	Milica Velimirovic														
		DART-MS	Milica Velimirovic														
	NIC	µ-FTIR	Tamara Bizjak														
Demo 4: EW Landau WWTP	W30	Fluorescence staining (abcr W30 MP-1)	Katrin Schuhen													In theory	
	VITO	Pyr-GC-MS	Milica Velimirovic														
Demo 5: Novi Sad Danube River	UNSMF	FTIR	Aleksandra Tubic														
		Pyr-GC-MS	Aleksandra Tubic														
	UoA	Drone-borne RGB camera	Dimitri Papageorgiou														

Figure 1: Analytical window for particle size of the different techniques employed by participating laboratories. Size is reported across a logarithmic scale from 1 nm to 100 cm, with broad classifications of nano, micro, meso and macroplastics overlayed for reference.

Note: The cells represent different typical size ranges which are possible to be quantitative for each technique, dark green shaded cells represent optimal size ranges for quantification, whilst light green cells represent regions closer to the limits of detection. To the right the gold shaded cells represent the different metrics which are possible to record for each analytical technique, whether count- or mass-based concentrations, size distributions or if the technique is chemically specific i.e. can determine the polymer identity.

1.2.1. Key shared analytical spaces across the demonstration sites

Finding common analytical windows across demonstration sites is an important step in identifying where possible evaluation of different technologies and demonstration sites will be possible within the project, as quantitative data generated for the same operational definition of microplastics is more easily compared. A rough log-log relationship between particle size and number-based concentrations in the environment is generally accepted (Kooi and Koelmans, 2019). Therefore, it is critical that data for **methods which are**

not quantitative of the same size region are not compared directly, as this may lead to orders of magnitude difference even with only small differences in the minimum quantified particle size.

The mapping exercise, summarised in Figure 1, helps identify where such shared operational definition of microplastics is most easily achieved across demonstration sites and so where harmonisation to allow future evaluation and comparison of data can be prioritised. Broadly, most techniques used to measure microplastics across the laboratories are applicable between 100 – 5000 µm, providing an opportunity in the project to compare data within these restricted ranges across demonstration sites. This is an important finding which must be taken forward into T1.5 (Data platform), D2.4 (Benchmarking of assessed technologies) and more generally in WP3 evaluations at the demo sites. An approach to data restriction to allow for comparison across analytical techniques, laboratories or demo sites will be discussed in further detail in the section "**Restricted datasets based on shared analytical windows**".

Whilst the different particle counting based methods all have varying sensitivity in the lower size regions between 10 – 100 µm, a common assessment and evaluation of the data could be possible through post-analysis processing of the data and restriction of the data range to common size ranges where sensitivity of the two instruments are comparable. For example, taking hypothetical data for a sample generated by µ-FTIR at two different pixel resolutions, 6.25 and 25 µm, it can be seen that whilst the raw datasets could not be compared directly between the two, data restriction to only particles >50 µm could be considered to be a common analytical range in which sensitivity of the two resolutions is similar and so data can be compared (Figure 2).

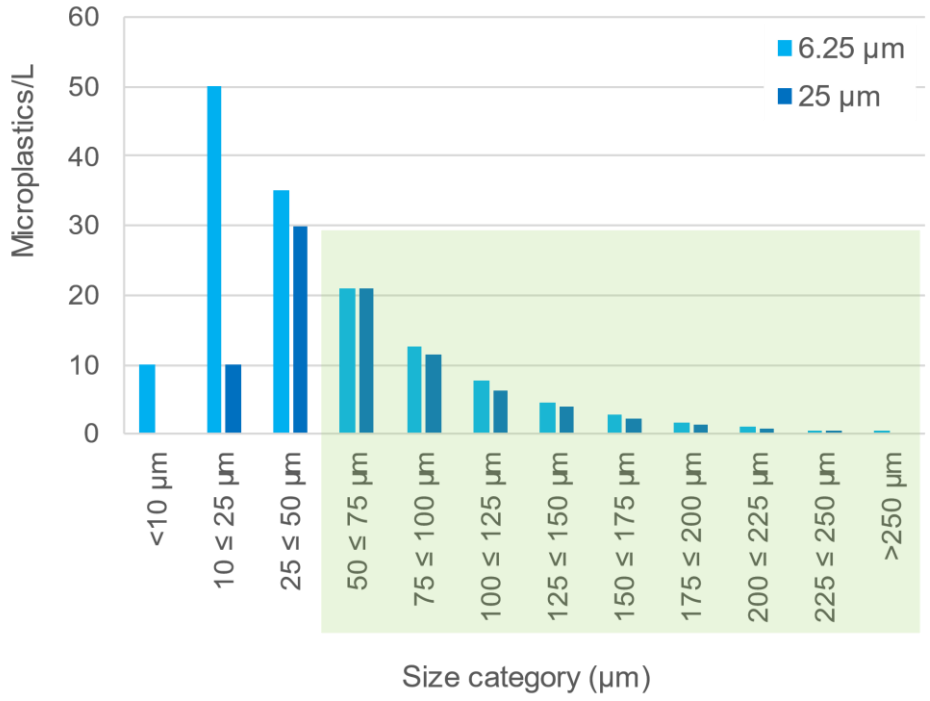

Figure 2: Data restriction approach to harmonise data interpretation.

NOTE: Hypothetical µ-FTIR data at two-pixel resolutions (6.25 and 25 µm). The green shaded area is the restricted data range wherein comparison could be made between the two datasets.

Following this, it may be beneficial to generate both the standard unrestricted datasets generated by the instruments, but also restricted datasets across all techniques, where the lower size of reported particle is fixed across techniques to the lowest common size that all techniques can measure with similar sensitivity (as further explored in the section "

Data interpretation"). In this way, whilst the full data derived from each technique is not directly comparable, the restricted datasets, constrained to a common analytical window and size region across all techniques may allow for comparison across different demonstration sites. There is still the question of whether two techniques may be differently sensitive, even if they are measuring within a common size range. For example, one technique may not be sensitive to a particular polymer or may be less sensitive in identifying fibres than another. In these cases, it must be demonstrated that on similar samples, the total quantification of microplastics is similar, and so it can be confirmed that the two instruments are similarly sensitive within the restricted size range and comparison is valid. This requires corroboration where possible of data generated on similar/ same samples across multiple techniques.

1.2.1. Mapping internal validation and corroboration efforts

A variety of internal validation and corroboration studies are expected in the UPSTREAM project to assist in allowing for a unified assessment of efficacy of different treatments and demo sites across the project. It is therefore important to identify where common analytical ranges may be shared across laboratories and techniques, and where internal comparisons may be performed between partners or across demo sites. There are two types of internal comparisons identified:

- Within technique corroboration, where the same/similar sample is tested across the different labs using the same technique
- Between technique analytical corroboration, where the same/similar sample is tested across different techniques.

The use of imaging µ-FTIR is identified at two demonstration sites (Demo 1, UKCEH; Demo 3 and 5, NIC), whilst (ATR)-FTIR is also used at Demo 1, UKCEH; Demo 2, LEITAT and Demo 5, UNSMF. There may be possibilities of comparison across these techniques at different demonstration sites (within and between technique corroboration). To do so, a harmonised approach to data reporting based on a common lower size limit across these techniques could be co-ordinated for this purpose as described above (data restriction approach).

Similarly, the two staining approaches proposed to be used at Demo 4 (W30) and Demo 2 (LEITAT) also cover a similar analytical window in terms of particle size and so could be included in a cross-demonstration evaluation. Once again, this may require data restriction to ensure that the analytical regions are common in any comparison between the two techniques. In addition, the high throughput fluorescence staining approach represents an opportunity for further validation of this method within the project if corroboration of data from fluorescence staining could be performed with chemically specific techniques such as the µ-FTIR or pyrolysis GC-MS (between technique corroboration). This will be pursued in WP1 between VITO and W30.

Pyrolysis GC-MS is also expected at both Demo 3 (VITO) and Demo 5 (UNSPMF), providing an opportunity for a comparison of baseline mass data for micro and nanoplastics at both a WWTW and the only river demo site. This represents a key opportunity for harmonisation of methods between these two institutes to target, for example, the same polymers and harmonise sample collection so that data is comparable.

To summarise this mapping exercise, Table 1 provides an overview of the internal comparative analyses planned in the project and summarises their aim, the partner(s) involved, the analytical technique(s) that will be used and the demo site(s) at which this assessment will be relevant.

Table 1: Planned internal corroboration and comparative assessments.

Analyte	Aim	Laboratory 1	Laboratory 2	Demo site
Litter	**Between technique** corroboration, validating litter maps with manual collections	UoA (Remote sensing)	UNSMF (On site manual litter survey + FTIR) XRF and DART-MS (VITO)	River Danube (Demo 5)
Microplastics	**Between technique** comparison at SVT comparing data generated by µ-FTIR and fluorescence staining technique (TBC)	UKCEH (µ-FTIR)	W30 (Fluorescence)	SVT Cellulose Recovery site (Demo 1)
Microplastics	**Between technique** comparison	W30 (fluorescence)	VITO (pyr-GC-MS)	Landau (Demo 4)
Microplastics	Between **sampling method comparison** - how to run samples between high and low TSS	W30 (Grab 2.5L sampling)	W30 (PSU 100L sampling)	River Quiech

1.2.2. Gaps in analytical coverage across demonstration sites

Quantitative particle number-based concentrations and size distributions that are chemically specific are not possible with the available methods to measure particles below 10 µm in diameter. All chemically confirmed microplastic counts therefore are restricted to microplastics above a theoretical lower size limit of 10 µm.

Several non-chemically specific techniques including Mastersizer, Non-Invasive Back Scatter (NIBS) dynamic light scattering, nano tracking analysis (NTA) and optical microscopy combined with Rhodamine staining are reported to allow for particles <10 µm to be quantified. These are typically better suited to controlled systems for development of technologies e.g. where a defined population of microplastics are tested and so chemical identification and confirmation of the polymer is less necessary. Documentation of the precision and accuracy of correctly identifying plastic particles down to sub-micrometre scales will be necessary for these techniques.

Mass based techniques extend our capabilities into the small micro and even nano-size range. However, the metrics generated by such techniques are not the same as for the count-based methods. Whilst mass-based data cannot be converted into continuous particle number concentrations and size distributions, there are published methods to estimate the mass of microplastics from two dimensional images based on some simple assumptions (e.g. the approach used in the freeware software siMPle https://simple-plastics.eu/). This will be explored further in the section "**Conversion between metrics and scales**".

It should also be noted that whilst there is overlap in the larger particle sizes that can be analysed and quantified across partners, the sampling approach should also be carefully considered to understand whether there is also an upper size limit quantified in samples. For example, the volume required to collect sufficient particles between 10 and 100 µm in diameter versus that needed to collect particles >5 mm may be orders of magnitude apart. The absence of large particles >1 mm in samples collected with quantification of smaller microplastics in mind e.g. as commonly taken for µ-FTIR analysis are likely to also have an upper size limit that might be expected to be quantified based on the volume of sample captured and analysed. This is discussed in more detail in the chapter concerning "**Sample collection**", in which guidance on representative sampling is provided. This guidance is based on the recent publication by

Cross *et al.*, 2025, in which a statistical tool is provided to allow for prediction of representative sample volumes based on estimated expected concentrations.

Detailed guidance for monitoring litter, microplastics and leachable compounds

The following sections take litter, microplastics and leachable compounds in turn and summarize the critical aspects and decisions for harmonized methods for monitoring each of these analytes.

2. Harmonized methods for monitoring litter

Litter monitoring will take place only at demonstration site 5 on the river Danube. A two-fold approach will be followed:

i. Remote detection and monitoring of litter using UAV and satellite data is performed by the UoA
ii. Visual surveys and litter collection performed by UNSPMF

Since each partner has a distinct role, harmonization within the project is not the focus here, rather ensuring that best practices around monitoring validation, quality control and data reporting are identified, and the final methodology employed adheres to such principles.

i. Remote detection and monitoring is performed using a commercial UAV (DJI Mavic 3E) and its onboard RGB camera.

A specific pre-set flight plan is executed in order to collect images of the surveyed riverbank. The data is then uploaded and processed through the UoA's dedicated CMLO platform. A detailed SOP including the data acquisition protocol, pre-flight checklist and data pre-processing steps can be found in Annex 3. Litter remote detection results are presented in litter density maps, which will form the basis of the D1.2 Litter Density Atlas, along with satellite data for surface litter detection and identification of possible hotspots.

Litter density maps are reported on a 10x10 m grid, in ETRS-LAEA "European Grid" coordinate reference system (CRS). ETRS-LAEA is a multipurpose, pan-European mapping standard, based on the ETRS89 CRS and the Lambert Azimuthal Equal-Area projection. The grid is defined as hierarchical one in metric coordinates in power of 10. The resolution of the grid is 1m, 10m, 100m, 1000m, 10,000m, 100,000m. An example of the litter density map is shown in Figure 3 below. Litter density is reported in units of number of litter items/grid unit, that is number of litter items per 100 m^2.

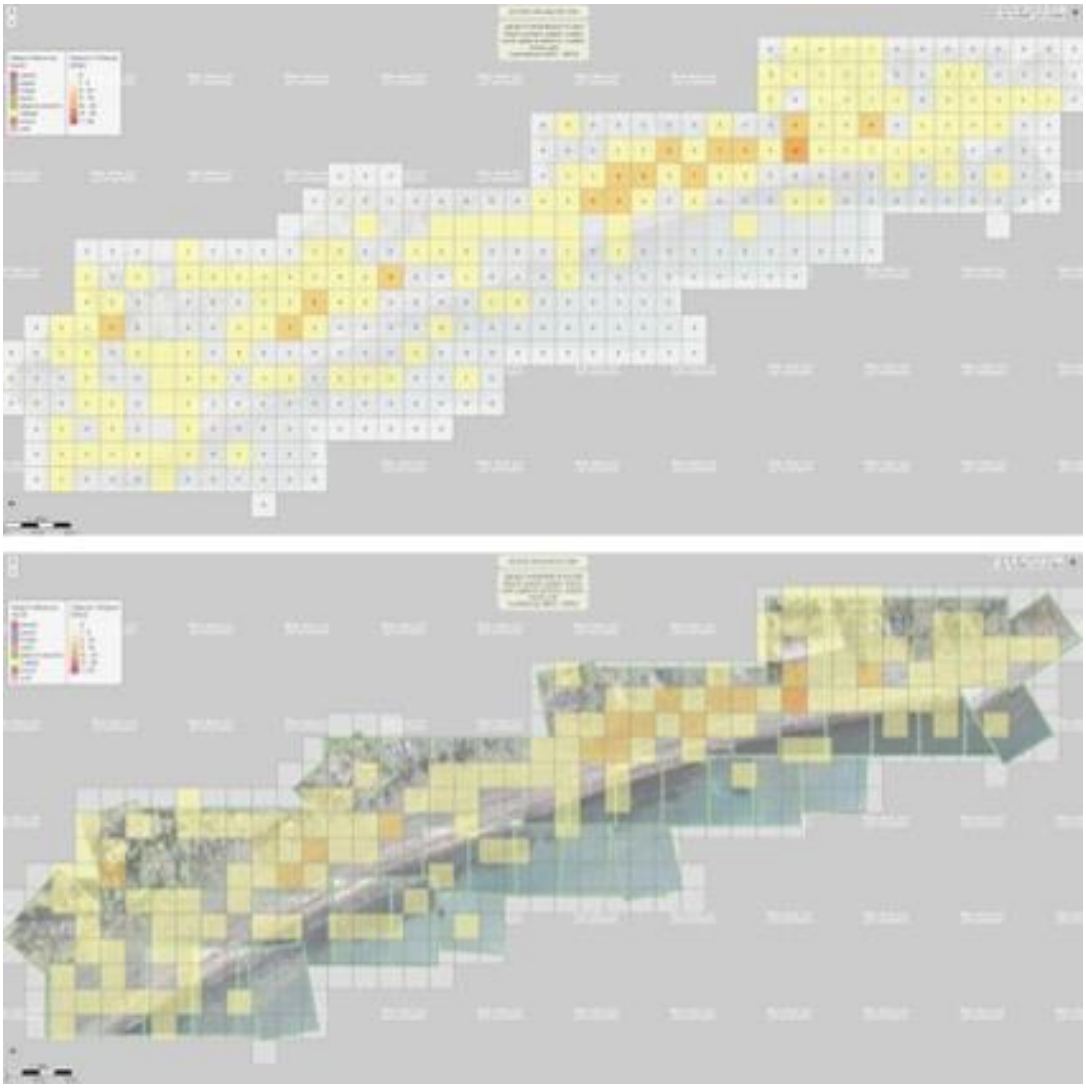

Figure 3: Example litter density map from a beach survey.

NOTE: Litter density is reporting in items/100 sq. meter grid units in ETRS-LAEA. Top view shows the number of items/ grid unit.

UAV monitoring of litter for the Novi Sad demo site is scheduled to take place in the selected survey sites at a monthly rate, except for prolonged harsh weather conditions.

ii. Visual surveys and litter collection is performed as per JRC/MSFD standards (Joint Research Centre (European Commission) and MSFD Technical Group on Marine Litter, 2023)

This standard is modified to a degree to adapt to riverine applications, as per van Emmerik et al, 2020. As per MSFD, monitoring is done in sampling units, which are defined as: “a stretch of coast of 100 m in length covering the area from the water edge to the back of the beach measured at half the actual width as a curved line on curved beaches or a straight line on straight beaches.” In our case the sampling unit is defined as a 100 m long stretch of riverbank, with a width spanning from the high-waterline to the waterline at the time of the survey, at a maximum of 25 m. When the surveyed riverbank is less than 100 m, the survey length is modified accordingly. In case the monitored stretch deviates from the suggested 100 m length, the results are also normalized to 100 m when reported. An example of a sampling unit can be found in Figure 5 below.

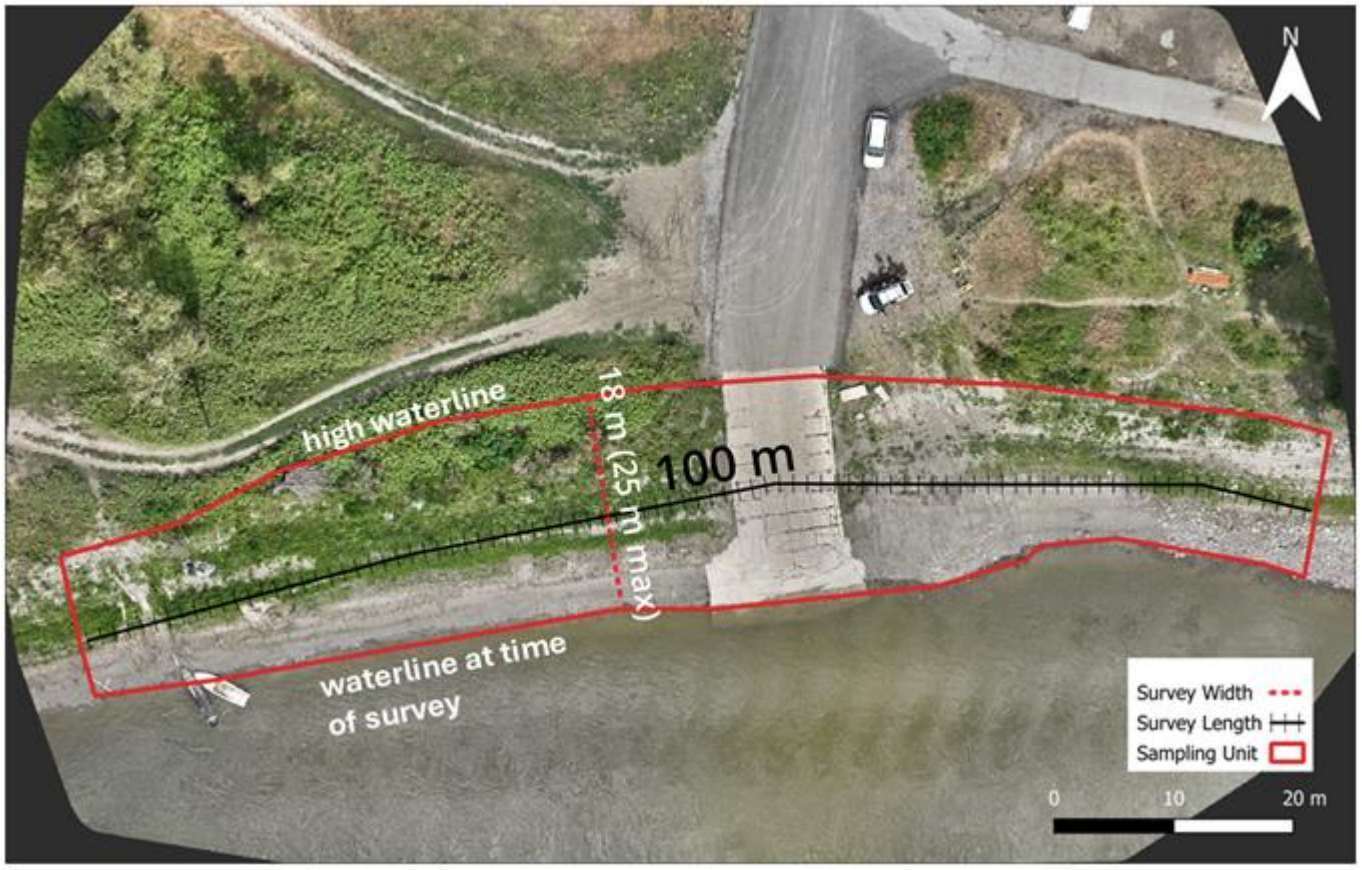

Figure 4: Orthophoto of surveyed riverbank in Novi Sad demo site showing sampling unit FF1 outline for the in situ litter monitoring and collection. Survey Location: Futog Ferry.

Sampling unit selection is done so that it represents the general characteristics of the survey site and the overall state of litter within it. In case the length of the surveyed riverbank allows it, a set of potential sampling units is created, and a number of sampling units are selected at random. In all other cases, a single sampling unit is created for each survey location.

In situ litter monitoring and collection will take place in tandem with UAV monitoring, but at a quarterly rate, every 3rd UAV monitoring.

All items sampled during the survey will be collected and part of them will be delivered to VITO for chemical polymer identification. All items not used in the polymer identification process will be disposed of properly, according to local, regional and national regulations and arrangements for waste disposal.

In addition to survey and survey site metadata that will be reported, Danube river flow and water levels at the time of the surveys will be reported. This data will be used along with river morphology to try and assess any correlation between river flow characteristics and river morphology, with litter beaching and density.

Survey report templates are provided in Annex 4, **Error! Reference source not found.**5, **Error! Reference source not found.**6.

2.1. Quality control and validation of remote sensing method

Quality control and validation of litter monitoring using UAV will be performed in a two-fold manner:

i. Quality control and expert visual validation will be performed for every UAV data acquisition based on the results reporting format, in the 10x10 m litter density reporting grid. A set of random grid units will be selected, the total number of litter items will be sampled visually by an expert, and compared to the litter density map results. The visual identification and counting of the litter items will be performed on a very high resolution orthophoto, allowing for accurate and precise sampling.

ii. In addition to the indirect method of quality control and validation, validation of the litter density maps will also be performed as per RC/EU-MSFD SOP (Joint Research Centre (European Commission) and MSFD Technical Group on Marine Litter, 2023) for litter monitoring. For each sampling unit surveyed, validation will be performed by comparing the litter density map results, to the *in situ* collected litter.

2.2. Harmonization of data reporting and recording with wider community practices

Data reporting for onsite litter monitoring will follow JRC/MSFD standard practices and ontologies as they are outlined above. As per MSFD, the most important elements when surveying macro litter are related to the survey sites' locations and their respective number, the timing of surveys, the positioning of the sampling unit on the survey site, the collection and classification of litter items, the data control and reporting and the metadata documentation. Data reporting will be performed using a standardized survey report template and litter data report (Annex 4, **Error! Reference source not found.**5, **Error! Reference source not found.**6.), as per MSFD requirements. Basic statistical methods will be used to analyse the beach survey data where needed and compare them to the drone litter density maps.

Litter items are categorized into 8 main litter categories (plastic, paper, metal, cloth, glass & ceramic, rubber, wood and unknown). These main litter categories correspond to MSFD directions, with the exception of food waste, which is not reported in the litter density maps. A lower limit of 2.5 cm in the longest dimension is set for macro-litter items monitored during surveys, which corresponds with the lower detection limit reported in the litter density maps.

Survey metadata to be collected:

- sampling unit code/name
- survey date
- surveyor's name and contact information
- length of the surveyed sampling unit, which may differ slightly from the suggested 100 m, measured along the beach curve at the midpoint between the water edge and the back of the beach
- date of the last known cleaning action (e.g. municipality beach cleaning, clean-up days);
- weather conditions during the dates of the surveys
- any deviation from the sampling protocol (e.g. transect length reduction or displacement of the transect, sampling outside the expected period, subsampling) and motivation (e.g. extreme weather events, flooding, new infrastructures in place)
- special circumstances and events that could have caused unusual litter in terms of abundance and/or type (e.g. clean-up actions, mechanical cleaning, beach party or competition, cargo losses nearby,
- extreme weather conditions
- information on any entangled fauna encountered during the survey (details of the organism, nature of entanglement, live or dead)

Survey site metadata to be collected are:

- the sampling unit length, measured along the beach curve at the mid-point between the water edge and the back of the beach
- the sampling unit width (perpendicular to the shoreline line), defined as the distance between the water edge and the back of the beach (base of dunes, cliff, vegetation line or human artefacts) and measured at half its length beach width should be measured at the mean water level in areas with small tidal amplitudes and at the mean high tide level for areas with high tidal amplitude
- start/end GPS coordinates
- direction of the prevailing winds
- direction of the prevailing water currents
- name, distance to and position of the nearest town, and the size its residential population
- distance to and position of the nearest food/drink outlet and the months in which the food/drink outlets are present
- name, distance to and position of the nearest harbour and the type of shipping using the harbour (e.g. passenger, merchant, fishing, military, recreational)
- name, distance to and position of the nearest river mouth
- distance to and position of the nearest wastewater or stormwater discharge point
- distance to and position of the nearest shipping lane and the type and intensity of marine traffic

All data reporting for the density litter maps will be incorporated into the litter data report, for each survey location. The litter data report will follow MSFD standards.

All produced maps will be made publicly available through the dissemination and communication portals of the Project. Additionally, the maps will be presented through the dedicated Coastal Marine Litter Observatory (CMLO) website (cmlo.aegean.gr).

2.3. Harmonization of hotspot definition and reporting

Hotspot definition and identification will follow the framework proposed by Tasseron *et al.*, (2024). The framework incorporates a 5-step hotspot definition, using purpose, units, spatial and temporal scale and threshold values as parameters that define a hotspot. Figure 4 below shows the graphical representation of the methodological framework (adapted for the needs of the project from Tasseron *et al.*, 2024).

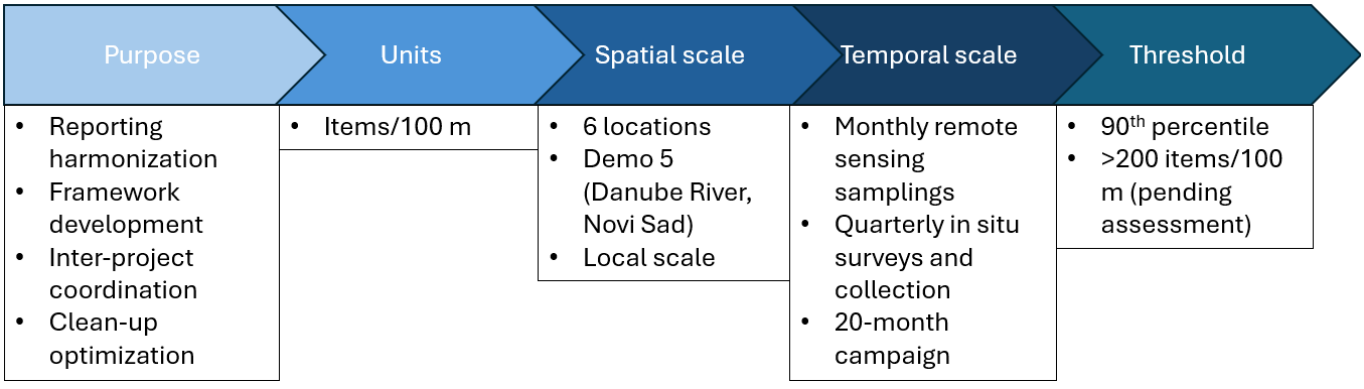

Figure 4: Framework for hotspot definition in UPSTREAM based on Tasseron et al (2024).

In the scope of UPSTREAM, the units used to define a hotspot will be number of items/100 m stretch of surveyed riverbank. Samplings will take place on 6 distinct locations in Demo Site 5 – the riverbanks of Danube in the area of Novi Sad, which will be monitored at a monthly basis through UAV remote sensing, and at a quarterly rate through *in situ* optical surveys and manual collection. Sampling will take place for a 20-month period, resulting in 120 observations in total.

We will be using a two-fold threshold for hotspot definition: one statistical threshold that will be set as the 90th percentile of the most polluted locations throughout the 120 observations, and one absolute empirical threshold of 200 items/ 100 m stretch of surveyed riverbank. This absolute threshold is adjusted van Emmerik *et al.*, (2020), where the median litter density of the surveyed locations in the Dutch Rhine–Meuse delta was 2060 items/km. Additionally, the 200 items/100 m stretch threshold also broadly corresponds to the median pollution rates reported in the OPSAR area from 2018 to 2020 (252 items/100 m). This initial arbitrary absolute threshold is set as above approximately the 50th percentile of observations from van Emmerik *et al.*, (2020) and OSPAR (Lacroix, André and van Loon, 2023), and is also relevant to our initial observations from the Danube Demo Site. This threshold is subject to change after statistical analysis of the litter density data to assess its suitability for our case study. For a survey area to be identified as a hotspot in UPSTREAM, it would need to fulfil both the statistical and absolute thresholds. In this approach we define a hotspot based on our dataset and local conditions, which however aims to remain relative to a more global approach of riverbank litter hotspot definition.

Further to identifying the locality of hotspots in Demo Site 5, we will also be examining the temporal variability of our litter data, to assess hotspot seasonality in each survey location and of the demo site as a whole. Identifying possible seasonal trends in litter density is crucial for clean-up optimization and possible source identification.

In addition to riverbank hotspots, high resolution satellite imagery will be used to survey the surface of the Danube starting from the upstream-most survey location until the downstream-most location. Through this process we will aim to identify any potential accumulation zones at low flow areas or around flow obstructions, which could be considered potential floating litter hotspots. As with the riverbank hotspot, these will result from statistical analysis of our dataset. In the context of UPSTREAM and lacking any concise framework for river surface litter hotspot definition using satellite imagery, we will define river surface litter hotspots as any 100x100 m section of the surveyed area that is above the 80th percentile of sections identified as potential accumulation zones by expert visual analysis of the data and ML classification approaches. In essence, we will assess the identification potential of any locations that consistently accumulate floating debris of any kind, either of natural (i.e. drifting timber or vegetation) or anthropogenic origin. The above approach and specific threshold is subject to change pending data analysis.

Figure 5 below presents the UAV and satellite survey areas for Demo Site 5, Danube River, Novi Sad. The map shows the 6 survey locations along the Danube riverbanks, along with the extent of the river surface that will be monitored using high resolution satellite imagery for the identification of potential accumulation zones

Figure 5: Map of the greater Novi Sad area Demo Site 5, showing the 6 UAV survey locations and the Danube surface that will be monitored with high resolution satellite data.

3. Harmonized methods for monitoring microplastics

Initially, a Sample Collection Record template was drafted to harmonize this metadata collection of sample collection, preparation and analysis data across demo sites. Standardized naming conventions were described to reduce the variability in what kinds of data could be captured, in an effort to increase the precision with which information and metadata about the sample collection could be recorded. This was in the form of an excel template, structured to capture through drop down selection lists, relevant and comprehensive information about:

- Site, location and sample identifying information
- Sample collection
- Shipping and storage
- Contamination controls in the field
- Ancillary field data
- Contamination controls in the laboratory
- Sample preparation steps in the laboratory
- Analytical instrument details
- Data processing

However, it was found during testing of early versions of this template between UKCEH and W30 that the variety of possible endpoints and permutations under each category meant that this structure was not sufficiently flexible for end users, and would require constant bespoke updates if rolled out across the participating laboratories.

Therefore, an alternative, more flexible Sample Collection Record was devised, using the quality scoring approach described in Koelmans et al., 2019. This template adopted the critical reporting standards from

the quality scoring approach and provided a template in which justification against each reporting criteria in the quality score could be demonstrated using open text.

In the following sections concerning sample collection, preparation, analysis and interpretation, select key principles are expanded on in some detail to provide additional guidance to support users to complete the Sample Collection Record Template and to provide specific guidance where necessary on best practices agreed across the participating laboratories.

3.1. Sample collection

Considering sample collection, three key reporting requirements are identified from the Koelmans et al., 2019 quality criteria: clear reporting of the sampling method, representative sample sizes and quality controls for sample preparation and storage during collection.

3.1.1. Sample collection method

A multitude of sample collection methods can be used to capture water samples. These may be specific to the analytical technique to be used. The EUROqCHARM project undertook a systematic review of reported methods for sample collection, preparation and analysis for different environmental matrices. The summary of their findings for the variety of sample collection methods reported in the peer reviewed literature for terrestrial waters and wastewater are reproduced in Figure 6. Typically, these were either nets, grab samples using pumps or bottles, or a combination of pumped filtration. Pumped filtration and grab bottles are expected to be employed in the project. Caution must be taken with grab bottle samples that sufficient particles are captured to be representative of the sampled location. To test whether sufficient sample was collected to be representative we recommend using the RSVP tool (Cross *et al.*, 2025), with guidance provided in the section “

Representative sampling”.

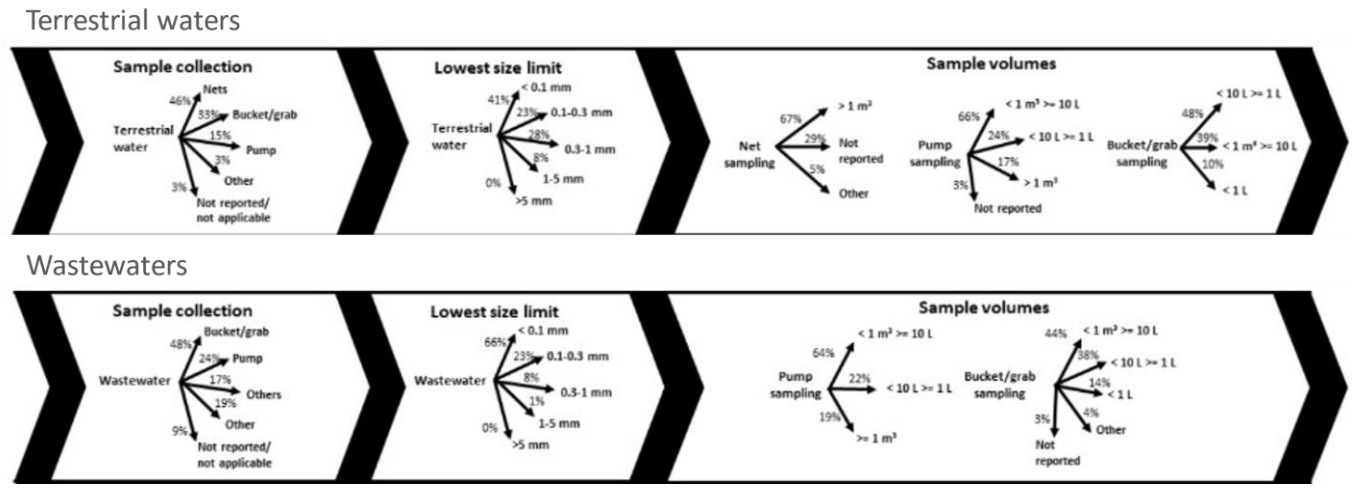

Figure 6: Sample collection methods for terrestrial waters and wastewater.

NOTE: reproduced and adapted from EUROqCHARM short report (Primpke *et al.*, 2023).

Not every demo site will employ the same sample collection protocol due to different site-specific needs. This will be driven by the hypothesis to be addressed and the analytical technique to be used. Therefore, the aim of the harmonization exercise is to provide a template for which the sampling method can be

clearly reported in sufficient detail for the quality of the approach to be judged, and for it to be determined whether the data generated is suitable for comparison against other demonstration sites.

Critical aspects are identified in the original quality scoring approach that must be reported and that are specific to the type of water body sampled. To these, guidance has been added under the "Justification" heading to describe the types of information needed to support scoring either a 0, 1 or 2 in the quality scoring approach for this reporting criteria Table 2.

The term "Campaign ID and description" has also been added to the reporting requirements. This provisional term is intended to link the harmonization and reporting requirements developed in D1.1 and the database task 1.5. To link results back to the original purpose of the monitoring, the hypothesis address, the sites, dates and sampling design undertaken, in essence the meta data required to meaningfully interpret any datapoints generated in UPSTREAM, a unifying identifier could provide a pragmatic solution. Currently, it is implemented in the Sample Collection Record Template as a separate tab in the excel spreadsheet in which a short description of the purpose of the sampling campaign (where, when, why and how samples were collected and the hypothesis/objective of interpretation of the data) is recorded. The application and systematic implementation of this Campaign ID will be further developed in Task 1.5.

Table 2: Sample Collection Record template for "Sample method".

NOTE: Blue text indicates updates to the Koelmans et al., 2019 guidance and additional information to support users in completing the Sample Collection Record.

		Study score			
Reporting criteria	Criteria ID	2	1	0	Justification
Sample method	1	All environments: - Campaign ID and description Surface & Ground water: - Pump - Location - Date - Materials used - Depth of sampling WWTP/DWTP: - Location - Date - Treatment - Sampling method - Materials used	The study reported only a subset of the required characteristics (e.g., date, location, materials used), however is still fairly reproducible.	No/ insufficient reportage of sampling methods.	Here you add all relevant data against the reporting requirements for this criteria. For example, for WWTW you could report: Campaign: details of what the purpose of the sampling campaign is so that data can be linked to hypothesis Location: coordinates Treatment: name technology/process step(s) Date: confirm whether the data sheet contains dates for all samples reported on(yes/no) Sampling method: short description/ can confirm yes/no if details are provided in tab "3. Method statement" Materials used: e.g. pumped filtration over 5um stainless steel filter

3.1.2. Representative sample size

A recent study by the project team at UKCEH has found the sample volume is a critical parameter in explaining some of the variability in quantification of microplastics in aquatic environments (Cross *et al.*, 2025). Publications have generally reported moderately well when assessed against the parameter sample size in the Koelmans quality score approach (e.g. >50% of articles reporting quantification in freshwaters scoring a 1 or 2). However, an interesting observation emerges, where a significant inverse relationship between the volume captured in a sample, and the concentration of microplastics reported is apparent. As the sample volume decreases, when sampling was conducted by grab and pumped samples, the reported concentration was observed to increase (Figure 7A and B).

One explanation could be that the higher reported concentrations correlate with studies quantifying smaller particles. This might be expected, as microplastic particle abundance typically increases dramatically in the smaller size ranges, as larger items disintegrate into ever smaller fragments (Wohlleben *et al.*, 2024). Indeed, a significant, but weak trend was observed in the data, when comparing the reported concentration against the minimum microplastic size quantified in Figure 7C and D. Note that the minimum size is either reported, or inferred through minimum net/filter pore sizes, or the lower limit of detection reported for the analytical technique. This may contribute to the weaker than expected relationship between size and concentration. However, this only further confirms the importance of making sure that justification of what constitutes a "representative sample" takes into account the sample collection method and the minimum quantifiable particle size of the method.

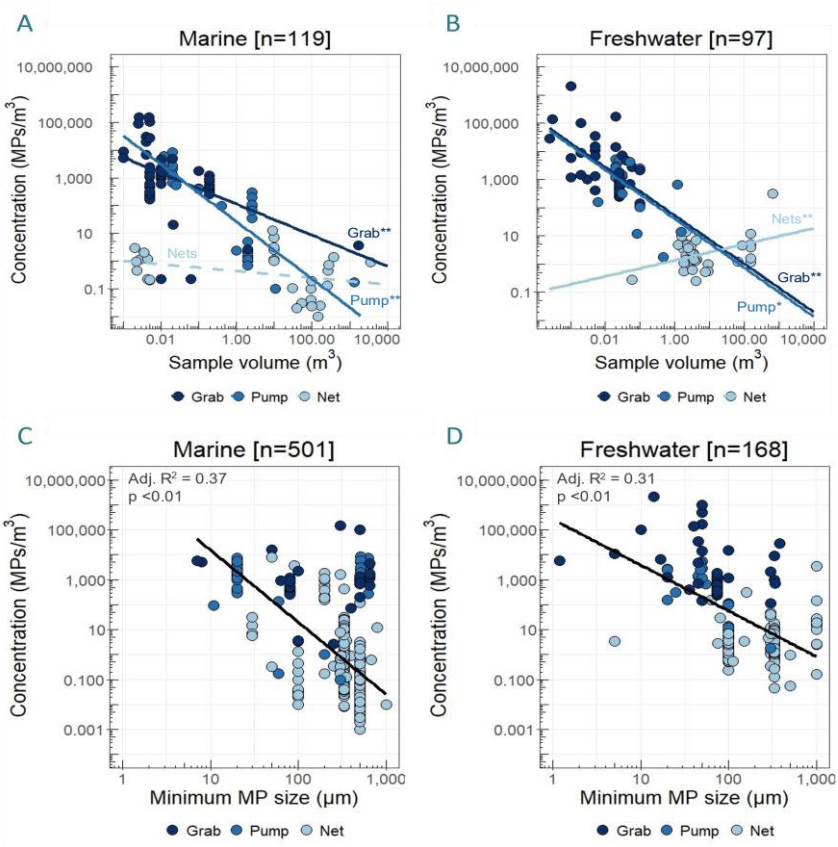

Figure 7: Concentration versus the volume of sample captured or the minimum size of microplastic quantified.

NOTE: Figure adapted and reproduced based on figures first published in (Cross *et al.*, 2025). Regression plots of concentration against the sample volume captured in marine (A) and freshwaters (B), and the concentration plotted against the minimum inferred MP size quantified in marine (C) and freshwaters (D). The collection method is denoted by different coloured points, for grab, net and pumped filtration sampling. Modelled fits for the regressions in (A) and (B) are presented for each of the three collection methods. For (C) and (D), the regressions lines represent the regression for all datapoints, irrespective of the sampling method. Solid lines represent statistical significance (* = $p<0.05$, ** = $p<0.01$) whilst dotted lines were not statistically significant.

Many of the efforts to standardize the quantification of microplastics in environmental samples focus, by necessity, on providing method specific guidance for representative sample volumes. However, given the diversity in analytical techniques across the project partners (Figure 1) an alternative approach focusing on harmonization, rather than standardization is required. For example, it can be seen that a much larger volume is required to be representative of particles 1 mm in diameter, than those 10 µm in diameter from Figure 7C and D (several orders of magnitude higher concentrations expected for 10 µm particles than 1 mm particles). Therefore, rather than providing strict limits on minimum representative sample volumes that apply universally across the project, we introduce a more flexible statistical approach to representative sample volume predictions using the recently published RSVP tool (Representative Sample Volume Prediction Tool - (Cross *et al.*, 2025).

Briefly, the distribution of microplastics within a turbulent mixed body of water can be estimated based on an assumption of a random distribution pattern, following a Poisson point process as demonstrated in Tanaka et al., 2023. This principle is not a unique characteristic of microplastics. Indeed, this statistical method can be employed to predict the number of any discreet objects or “events” that act independently of one another, in a fixed period (e.g. in time or space). This would as equally apply to predicting how long you need to watch the nights sky to see a shooting star, or how many calls are received in any given day on a telephone helpdesk. The chance of counting a specific number in all of these scenarios can be modelled using the Poisson distribution and the related Inverse Gamma Cumulative Distribution Function. This approach in statistics is a discrete probability distribution, that expresses the probability of:

- a given number of discrete events, (here capturing a given number of microplastic particles);
- occurring in a fixed interval, (i.e. in a fixed volume of water);
- if these events occur at a known or expected rate. In this case, this is equivalent to the expected concentration of microplastic particles in the wastewater effluent, or the river you sample from.

In effect, if the user has an estimate of the expected concentration in their select environment and pre-defines the target number of particles they wish to quantify, and the significance level to which they wish to be quantitative, then the RSVP tool can predict the minimum representative sample volume to meet these conditions.

Brief guidance on the different parameters of the RSVP tool and how to use them are provided in Box 1. The text has been kindly reproduced from the original publication (Cross *et al.*, 2025) under a Creative Commons Attribution 4.0 International License http://creativecommons.org/licenses/by/4.0/.

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Box 1: Guidance on using the RSVP tool – text and examples reproduced and adapted from (Cross *et al.*, 2025)

The output to the user is the **volume (*v*= λ/c)** required to capture the **target number of microplastics *k*** at the **given level of confidence *α*** assuming the **numerical microplastic concentration** at the sampling location is *c*.

The **target number of microplastic particles (*k*)** depends on the purpose of the assessment and should be decided *a priori* by the user (Table 3). For example, if you wish to determine presence or absence of microplastics in a location with 99% confidence, the user would set *k* to 1, and *α* as 0.001. The target number of microplastics (*k*) to be measured for different purposes have been proposed elsewhere e.g. *k* required to quantify total microplastics (Tanaka, Kataoka and Nihei, 2023) or multiple characteristics of microplastics in a sample (e.g. Cowger et al., 2024, or Table 3).

The **expected number of particles in a given volume of water λ** should be estimated by identifying the most relevant existing data to inform on expected concentrations at the sampling location. Details of the selection criteria used and justification of the relevance of the data should always be clearly reported. Some key criteria to identify relevant data to inform λ are to select data that:

- represents a similar test system to that under investigation (e.g. similar sized river or catchment)
- represents/ integrates similar environmental fate processes
- collected samples using a similar methodology
- processed samples using a similar methodology
- analysed samples using the same analytical technique and so represents the same "analytical window" i.e. region of the microplastic size continuum, polymer types etc.
- scores highly following quality criteria (e.g. for water samples, Koelmans et al., 2019)
- using the arithmetic mean of suitable data is likely to overestimate the concentration in a given sample because one or more very high values can influence the mean unduly. Ideally one would choose a typical value from a large distribution. In most cases there is not enough data to do this, so either erring on the side of caution or choosing a value less influenced by outliers such as the median or the geometric mean is recommended.

It is acknowledged that in the absence of data representing microplastic particles in the same size range it is challenging to predict the expected number of microplastic particles in a given volume of water. This is why the first recommendation is to use data from analogous analytical methods to inform λ.

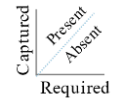
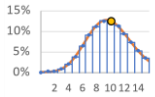
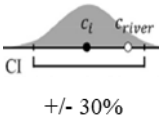
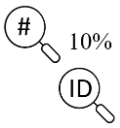
Ultimately, the RSVP tool provides for several useful functions:

1. How much sample must I collect to capture at least a given target number of microplastic particles at a given level of confidence?
2. In the absence of replication, are two values likely to differ at a given level of confidence?
3. Both functions can be applied either to the total number of microplastics when that is of interest, or to subsets of interest, e.g. by polymer, shape, size colour etc.

The desired number of particles to detect is dependent on the purpose of the assessment. There are additional costs with increasing the sample volume, particularly in the clean-up and extraction of microplastics from environmental samples. Therefore, it is not always desirable to capture the maximum possible sample size for a study, rather the representative volume required may be tailored to the purpose of the study. Some general rules of thumb can be found in contemporary studies that are useful as a guide to the number of particles required to be captured and analysed for a given purpose (Table 3).

Box 1 continued:

Table 3: Target number of particles required to evaluate data for specific purposes

	Purpose	Target number of particles	Reference
	Monitoring **presence/absence** at a given level of confidence	1	Cross *et al.*, 2025
	To **calculate the sampling error** using the Poisson point process	10	Tanaka et al., 2023
	To achieve a predicted **95% confidence interval to be within +/- 30%** of the total concentration estimates	50	Tanaka et al., 2023
	To **allow for one additional property** such as polymer identity to be evaluated with an **error of 10%**	96	Cowger et al., 2024
	To **allow for one additional property** such as polymer identity to be evaluated with an **error of 5% or less**	384	Cowger et al., 2024
	To **simultaneously estimate polymer, colour, size, and morphology** distributions with an error of **5% or less**	620	Cowger et al., 2024

As can be seen, the desired or target number of particles to obtain in a sample is a critical parameter in the RSVP tool and is dependent on the purpose of the assessment (Table 1).

It should be cautioned that the RSVP tool is applicable only when the assumptions underpinning the Poisson distribution are adhered too. In particular, assumptions that particles are randomly distributed and acting independently must be met. These conditions have been demonstrated to be met when randomly sampling rivers under turbulent flow (e.g. Tanaka et al., 2022).

For the purpose of UPSTREAM, a link to the full manuscript is included in Annex alongside a downloadable copy of the RSVP tool_v1.0. This will also be stored in the shared project folder for D1.1 for internal project use. A training session with the tool can be organised by UKCEH, opened to all partners in the project involved in sampling microplastics and in data interpretation.

Below is a **worked example** to demonstrate application of the tool:

In this example, the scenario is that the user wishes to capture samples using pumped filtration from the effluent of a WWTW in the UK and will analyse these using µ-FTIR at a pixel resolution of 25 µm.

1. *Identify relevant expected concentration*

First, the relevant existing information to inform on the expected concentration in the sample must be identified. To improve the relevance and thus accuracy of the prediction, the data used to inform on the expected concentration must be as similar as possible in terms of study design, location, sampling method, and analytical window of analysis. To this end, the average concentration of microplastics in wastewater effluent for the UK reported in the Chemicals Investigation Program (CIP3) can be used as the source data, as this report also used pumped filtration, measured microplastics in effluent and used µ-FTIR at a pixel resolution of 25 µm to quantify microplastics. The estimated concentration from this report is 1.4 MP/L (UKWIR, 2022).

2. *Select the target number of particles (k)*

Next the user must select the target number of particles to be quantified in the sample. In this case, the user selects 50 particles, this being the recommended target to achieve a predicted 95% confidence interval to be within +/- 30% of the total concentration estimates Tanaka et al., 2023 (Table 3).

3. *Select the significance level (α)*

Finally, the user must select the significance level and thus the probability of capturing at 50 particles in any given sample. In this case, the user selects a significance level of 0.01, or 99% probability of capturing at least 50 particles.

From this, the user can input into Tab 1 "1. Minimum volume predictor" of the RSVP tool_v1.0:

- *Sample unit used: L*
- *Estimated concentration (MP/L): 1.4*
- *Minimum target number of particles to capture (k): 50*

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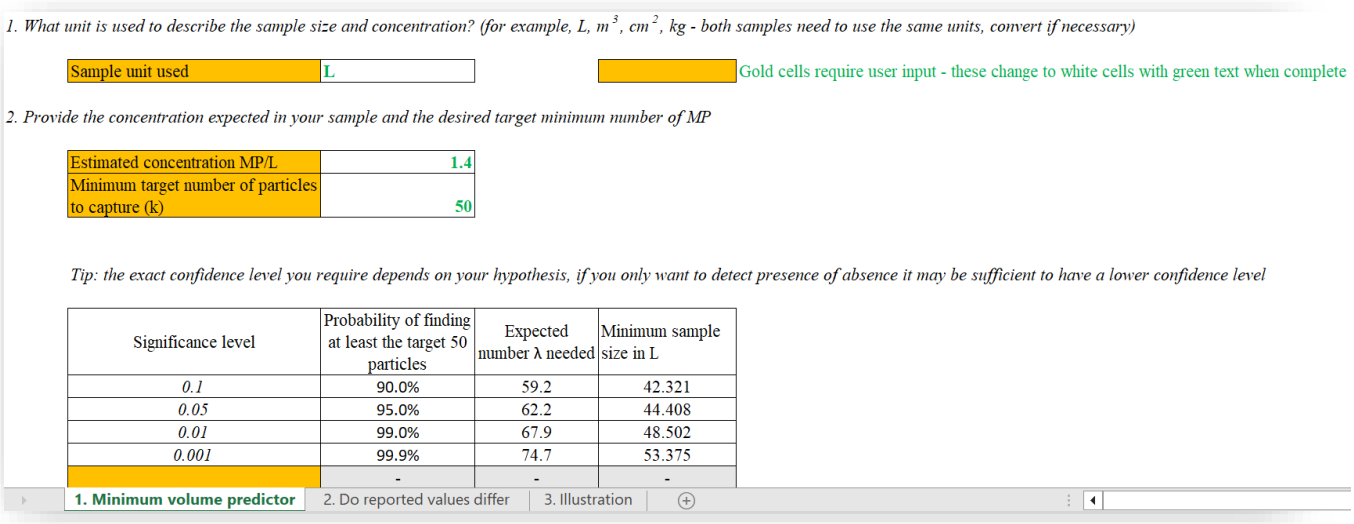
The output from the tool is as follows:



1. What unit is used to describe the sample size and concentration? (for example, L, m^3, cm^2, kg - both samples need to use the same units, convert if necessary)

Sample unit used	L

Gold cells require user input - these change to white cells with green text when complete

2. Provide the concentration expected in your sample and the desired target minimum number of MP

Estimated concentration MP/L	1.4
Minimum target number of particles to capture (k)	50

Tip: the exact confidence level you require depends on your hypothesis, if you only want to detect presence of absence it may be sufficient to have a lower confidence level

Significance level	Probability of finding at least the target 50 particles	Expected number λ needed	Minimum sample size in L
0.1	90.0%	59.2	42.321
0.05	95.0%	62.2	44.408
0.01	99.0%	67.9	48.502
0.001	99.9%	74.7	53.375
	-	-	-

1. Minimum volume predictor | 2. Do reported values differ | 3. Illustration

Figure 8: Example output of the RSVP tool_v1.0

Here the user is guided to collect a minimum of 48.5 L of effluent to achieve a 99% probability of capturing at least 50 particles in the final sample if analysed in its entirety.

The user can then save a copy of this output to provide as supporting information in the Sample Collection Record Template to demonstrate that the conditions of achieving a representative sample are met.

The use of the RSVP tool to validate representative sample volumes within the project represents a significant step towards harmonisation that takes us beyond the guidance of the quality assessment approach for microplastics. This additional justification using the RSVP tool is now integrated into the Sample Collection Record template for “Sample size”, Table 4.

Table 4: Sample Collection Record template for "Sample size".

NOTE: Blue text indicates updates to the Koelmans et al., 2019 guidance and additional information to support users in completing the Sample Collection Record.

		Study score			
Reporting criteria	Criteria ID	2	1	0	Justification
Sample size	2a	Surface & ground water: > 500 L if targeting particles >300 µm. WWTP if targeting particles >300 µm: - Influent: 1L - Effluent: >500 L or until sieve clogging Sample volume may be smaller if statistically justified e.g. if targeting only smaller particles <300 µm. Data should be caveated that sample volumes may be insufficient to capture larger particles e.g. >300 µm.	Surface water: < 500 L "with good cause" e.g. high concentrations expected or only small abundant particles <100 µm targeted, score 1 if RSVP tool is not used to justify statistically Trawls without reporting volume is acceptable. WWTP: If insufficient volume, sampling till clogging would score 1	Surface water: <500 L (if no justification provided) WWTP: Insufficient sampling volume (if no justification provided)	Justification is met either for the standard recommended volumes listed to the left, or if lower volumes are used, these must be justified statistically where appropriate e.g. using the RSVP tool. Sample volume may be smaller if 1. target microplastic sizes are smaller i.e. <100 µm (e.g. Sturm *et al.*, 2024) 2. Concentrations expected to be higher than typical RSVP tool can be used to justify tailored sample size. Copy of the tool output (Excel file) should be supplied alongside the Sample Collection Record. If sample volume collected meets the statistical requirement of the tool, then this can score 2 in the quality score.

3.1.3. Sampling intervals

Due to the high fluctuations of MP concentrations that can be observed in the effluent of WWTPs, a high number of samples is necessary to obtain a representative evaluation of the MP contamination. Single samples are not representative of actual pollution loads and do not capture the temporal variations in MP levels. The minimum recommended MP sampling interval to capture the yearly MP emissions is between 2 to 4 sample collections per month. A higher number of samples is required if seasonal and monthly variations are to be determined.

Based on the results of Sturm *et al.*, (2024), the sampling period outlined in the revised EU Urban Wastewater Treatment Directive will not adequately capture temporal variations in MP contamination levels and may therefore be misrepresentative of actual pollution loads. Some key results of this study that are useful for UPSTREAM quoted here for reference:

- *The presented data show that there are high fluctuations in the microplastic concentrations in the effluent of the WWTP. To capture these fluctuations and obtain a representative evaluation of the microplastic contamination, a high number of samples is necessary. Single samples are not representative. The minimum MP sampling interval to capture the yearly emissions is recommended to be between two to four samplings per month. Based on the results, longer sampling intervals do not provide meaningful results; thus the sampling period outlined in the revised EU Urban Wastewater Treatment Directive will not adequately capture the temporal variations in MP contamination levels and may be misrepresentative of actual pollution loads. To capture both seasonal and monthly variations, higher numbers of samples are needed.*

- *The average microplastic concentration was 27.8 ± 29.8 MP/L, ranging from 0.6 MP/L to 194 MP/L. In 2023, a lower MP contamination of 19.7 ± 17.9 MP/L was detected than in 2022 with 33.0 ± 33.6. This may be caused by increased awareness of the problems associated with MP in the environment resulting in reduced emission by industries and households, regulations on MP in products, or a change in the industries present and contributing to the influent of the WWTP.*
- *Clear seasonal variations could not be statistically proven, but there is a trend towards lower MP concentrations and lower fluctuations of the concentrations in summer, which was visible in the data.*
- *The correlation analysis showed that MPs are not correlated with the investigated wastewater and weather parameters. It should therefore be measured separately as the contamination appears to be driven by other unrelated factors.*
- *Further, the data show that WWTPs are clear point sources for MP into the environment and appropriate measures should be taken to prevent this contamination. Advanced treatment stages targeting MP removal at both upstream sources and at WWTPs should be investigated. (Text quoted from the original manuscript by* Sturm *et al.*, (2024).

The sampling interval needed to understand annual emissions of microplastics from point sources such as WWTWs was not considered as a quality criterion in the original Koelmans framework. However, it is essential to harmonise this parameter in UPSTREAM so that performance at WWTW demonstration sites across the project can be comparable. Therefore, it is recommended in UPSTREAM that monitoring of Demo sites which aims to establish annual performance or variability in a technology should:

- Monitor across an entire year, when possible, to capture seasonal variability.
- Sample at least 2 times per calendar month to capture adequately the annual variation in emissions from WWTW effluent.

One opportunity in the UPSTREAM project would be to further test the findings and recommendations for particular sampling intervals, to see whether the conclusions of Sturm *et al.*, (2024) are consistent across demo sites. As this criterion was not included in the original Koelmans framework, we have added a new line to the Sample Collection Record template with the Criteria ID 2b, “Sampling intervals” as this pertains to representative sampling approaches, similar to Criteria ID 2a “Sample size” (Table 5).

Table 5: Sample Collection Record template for “Sampling intervals”.

NOTE: Blue text indicates updates to the Koelmans et al., 2019 guidance and additional information to support users in completing the Sample Collection Record.

		Study score			
Reporting criteria	Criteria ID	2	1	0	Justification
Sampling intervals	2b	For annual monitoring, 2-4 samples per month across 12 months	Standards partially met, for example: • For annual monitoring, monthly sampling across 12 months • Repeat sampling but not carried out for a full 12-month period to capture complete seasonal cycle	No repeat sampling.	To justify, follow the criteria to the left. If fewer or more intermittent sampling intervals are used, this can be justified if it is demonstrated that the sampling interval was sufficient statistically for the purpose of the campaign.

3.1.4. Sample processing and storage controls - field blanks and contamination controls

The quality scoring approach already provides some guidance to recommended quality controls for the sample processing and storage phase (Table 6). To this, additional detailed guidance is included specifically for contamination controls during sample collection in the field. This guide to best practices agreed across WP1 is summarized in Table 7.

Table 6: Sample Collection Record template for "Sample processing and storage – quality controls".

NOTE: Blue text indicates updates to the Koelmans et al., 2019 guidance and additional information to support users in completing the Sample Collection Record.

		Study score			
Reporting criteria	Criteria ID	2	1	0	Justification
Sample processing and storage – quality controls	3	Sample storing shortly after sampling; any sample handling was avoided before arriving in the laboratory. Sample containers should be rinsed with filtered water. Sample preservation with chemicals should be justified and evaluated for compatibility. Manta trawl nets are allowed to be rinsed with unfiltered water. Sieving in the field is acceptable if sample volume is large. Precautions should be taken to prevent contamination (see detailed best practice in Table 7). Field blanks should be run and documented, and contamination controls should be followed	Standards only partially met or containers are pre-rinsed with samples. Citizen science approach with validation	Samples are handled outside. Storage not mentioned. Citizen science approach without validation	To justify, follow the guidance of reporting requirements listed to the left. Additional field controls for contamination should be followed and reported (see details for best practice in section on field blanks and contamination controls)

Due to the possibility of contamination of samples during sample collection, an assessment of field contamination should be provided by all participants as part of the QA/QC for each monitoring activity. The field blank should be sufficient to conclude that negligible contamination occurs during the collection and transport of samples to the laboratory. As the sample collection method will be specific to each

demonstration site, a set of agreed principles are instead proposed so that the collection of field blanks are harmonized across the different techniques and locations. These principles are as follows:

- Contamination controls (see Table 7) should be employed when working in the field: wear cotton clothing where possible (i.e. not overriding PPE requirements etc.), always stand downwind of any sampling vessels when opening, all equipment used in the field should be washed and cleaned prior to use and stored in a way that limits ambient contamination of the equipment whilst in transit.
- The field blanks should as closely represent all processes and steps followed when capturing a real sample in the field.
- The field blanks should be taken on at least one occasion of real sampling.

Table 7: Harmonised contamination controls used during sample collection

Contamination control method	Description
Clothing and cross contamination controls: Limiting field operator contamination	Cross-contamination from clothing is minimised by standing downwind from the sample and keeping lids on the buckets and bottles whenever possible. Cotton overcoats are also worn where possible. Field blanks for at least one sampling campaign are run as part of the QA/QC to check for contamination
Material substitution: Limiting plastic in equipment	Where possible, non-plastic or uncommon plastic substitutes are used in sample collection equipment and in its preparation for deployment in the field. This includes for example natural fibre brushes, glass Pasteur pipettes, stainless steel buckets, stainless-steel or aluminium filter rigs and stainless steel filters. Where plastic is unavoidable, exotic and hard wearing polymers which would be easily detectable in samples should be used, for example FEP/ETFE wash bottles and PTFE lined lids and ETFE pouring rings for glass bottles. All equipment is washed with filtered (<0.7 µm) RO water prior to use.
Clean air conditions: Limiting airborne contamination	Air filters e.g. HEPA filter removes 99.999% of particles >0.3 µm in size. All preparation of field sampling equipment to be performed under equivalent conditions. When outside of the safety cabinet, all equipment is covered with clean aluminium foil.
Clean washing procedure: Limiting contamination during equipment preparation	All equipment and is washed using only natural fibre scouring brushes to prevent contamination during washing and rinsed repeatedly with filtered water before air drying under foil to prevent airborne contamination. Where possible, any handling of equipment, particularly surfaces which may come into contact with the sample should be performed under clean air conditions, e.g. in a safety cabinet where air is filtered e.g. HEPA filters.
Demonstrate negligible equipment carry over: Limiting sample cross contamination	Field blanks of washed re-used sampling kit is sufficient to demonstrate no carry over between samples. Following clean washing procedure should limit the possibility of cross contamination between samples.

3.2. Sample preparation

A total of five critical areas concerning sample preparation are identified in the quality assessment approach (Table 8). These cover aspects of laboratory preparation, the use of clean air, negative controls, positive controls and sample treatment. Additional specific and more detailed guidance was considered necessary to discuss and harmonize across the consortium for contamination controls, process blanks and process recovery, the results of which are reported in specific sub-sections below.

Table 8: Sample Collection Record template for "Sample preparation".

NOTE: Blue text indicates updates to the Koelmans et al., 2019 guidance and additional information to support users in completing the Sample Collection Record.

		Study score			
Reporting criteria	Criteria ID	2	1	0	Justification
Lab prep	4	Cotton lab coat or non-synthetic clothes Equipment and lab surfaces wiped and rinsed	Solely wiping laboratory surfaces and equipment or not wearing a lab coat IF negative samples were run in parallel and examined for contamination.	No precautions	*To justify, follow the guidance of reporting requirements listed to the left.* *For additional guidance refer to Table 9.*
Clean air	5	Clean room or laminar flow cabinet	Mitigation of airborne contamination by carefully keeping samples closed as much as possible IF negative samples were run in parallel and examined for occurring contamination.	No regard of airborne contamination, or solely use of *fume hood.*	*To justify, follow the guidance of reporting requirements listed to the left.*
Negative controls	6	Controls (minimum in triplicate) treated and analysed in parallel to actual samples. Sample concentrations need to be reported accounting for controls.	Insufficient form of a control, e.g. the filtration of air, or the sole examination of petri dishes/ soaked papers placed next to the samples.	No negative controls	*To justify, follow the guidance of reporting requirements listed to the left.* *For additional guidance refer to "*Process blanks*"*
Positive controls	7	Controls (minimum in triplicate) with an added amount of microplastic particles treated the alongside the samples, and for which the particle recovery rates are determined.	Insufficient form of a positive control (e.g. if only a part of the protocol is tested).	No positive controls	*To justify, follow the guidance of reporting requirements listed to the left.* *For additional guidance refer to "Process recovery"*
Sample treatment	8	Digestion of complete sample using a protocol with KOH, wet peroxidation (WPO) and/or enzymes. If another chemical was used, effects on different polymers should be tested before application. All sample treatments need to be carried out below 50°C to prevent any damage to microplastics.	If proof is missing that polymers are not affected by protocol (e.g. heated KOH) OR in case studies exclusively focus on the bigger microplastics by sieving the samples (mesh size ≥ 300µm). If WPO is carried out without cooling.	No digestion of the sample	*To justify, follow the guidance of reporting requirements listed to the left.*

3.2.1. Laboratory contamination controls

Microplastics are ubiquitous, particularly in the built environment. Contamination controls are critical therefore to ensure that any detected signal from microplastics is attributable to the sample, not ambient sources of contamination during their sampling, preparation and analysis. Sources of contamination include:

- Airborne contamination of any equipment or surfaces that come into direct contact with the sample
- Contamination in reagents used in the processing of samples
- Contamination from operators taking samples in the field (shedding of microplastic fibres from clothing etc.) if the sample is exposed to the air for any period of time
- Contamination from moving parts that may not be in direct contact with the sample, but can shed microplastic fragments to areas of equipment/glassware that are in contact with the sample
- Sampling and storage equipment made (in part) from polymers, in particular this includes lids and pouring rings of glass bottles or jars and seals or valves in filtration equipment and pumps etc.

Even though contamination is well reported and monitoring for contamination should be a minimum requirement when reporting any quantification of microplastics in environmental samples, historically the research community has not been consistent in testing for or reporting results of contamination checks. For example, in a review of the general quality in reporting the detection and quantification of microplastics in freshwater and drinking water, Koelmans et al., (2019) found most publications did not run full procedural blanks - only 18 out of 50 evaluated studies. Whilst some journals are now requiring this as mandatory e.g. (STOTEN, 2024) and so the rate should improve over time, it is critical to harmonise this effort across the project to ensure a consistent approach. General approaches that will be applied across all laboratories to limit contamination are listed in Table 9. This information is also captured in the sample preparation record template, where each Sample Collection Record will be associated with yes/no to each contamination control method listed below, to ensure that all laboratories are working to similar standards, and, where specific contamination control methods are not feasible, this is documented.

Table 9: Harmonised contamination controls during sample preparation

Contamination control method	Description
Material substitution: Limiting plastic in equipment	Where possible, non-plastic or uncommon plastic substitutes are used in during sample preparation, including natural fibre brushes, glass Pasteur pipettes, stainless steel buckets, stainless-steel or aluminium filter rigs, stainless steel or pure silver filters, FEP/ETFE wash bottles and glass bottles with PTFE lined lids and ETFE pouring rings for all sampling and processing vessels. All equipment is washed with filtered (<0.7 µm) RO water prior to use.
Clean air conditions: Limiting airborne contamination	HEPA filter removes 99.999% of particles >0.3 µm in size. All processing steps in the laboratory are performed under these or equivalent conditions when possible. When outside of the safety cabinet, all equipment/samples are covered with clean aluminium foil. If equivalent conditions cannot be met, this must be documented. A systematic use of procedural blanks allows all data to be corrected for any unavoidable background contamination.
Reagent filtration: Limiting contamination from reagents	All reagents used in microplastic processing are filtered through a glass fibre filter with a pore size smaller than the lower limit of detection for the analysis (e.g. 1.2 µm) to remove any particulates prior to use. A systematic use of procedural blanks allows all data to be corrected for any unavoidable background contamination.
Cotton lab coats: Limiting cross contamination from synthetic clothing	All laboratory processing is performed by operators wearing 100% cotton lab-coats.

Demonstrate negligible equipment carry over: Limiting sample cross contamination	Stainless steel filters are commonly used to concentrate water samples. Similarly glass beakers and filtering equipment may be reused between samples. All stainless-steel disc filters are sonicated and washed between samples with detergent, RO or DI water and finally filtered (<0.7 µm) water. Other equipment follows the clean washing procedure below. Absence of carry over between samples should be demonstrated if equipment is to be re-used.
Clean washing procedure: Limiting contamination during equipment preparation	All equipment and glassware is washed using only natural fibre scouring brushes to prevent contamination during washing and rinsed repeatedly with filtered RO or DI water before air drying under foil to prevent airborne contamination

3.2.2. Process blanks

Alongside each batch of samples prepared in the laboratory, at least one process blank should also be included. This blank should represent the entire process that is performed during sample preparation, exactly as it would be a true environmental sample. The duration of steps, reagents used and handling of the sample should be in the same manner as the real samples in the batch and the operator should also be the same. In this way, each batch of samples prepared in the laboratory has a traceable concurrent blank sample which can be used to monitor for unexpected sudden increases in the background contamination within the sample preparation procedure. It is unavoidable that some contamination occurs during sampling handling in the laboratory and so running these blanks is mandatory. Depending on the number of samples and sample batches to be run for each demo, or in each phase of testing at a demo, sufficient blank samples should be run to allow for calculation of average blank concentrations and of limits of detection for the method. In this way, if only a single batch of samples is to be analyzed in an experiment, it is not sufficient that only a single blank is run alongside this batch as neither an average nor the standard deviations required to calculate limits of detection may be calculated. An absolute minimum of 3 blanks is required for any standalone analysis of microplastics, though more than this is encouraged as the variance in the blanks will be constrained with more blanks, and so the limits of detection improved and the final analysis more sensitive in detecting microplastics above this limit.

3.2.3. Process recovery

Process recovery can be performed in a variety of ways. The aim is to understand the recovery of the analyte after all sample preparation steps are complete and thus the efficacy of the method. Similar to process blanks, this is a QA/QC tool that can identify when issues arise in the sample preparation from week to week. The principle of the process recovery is that a known number/concentration of the analyte/ an analogous analyte is spiked into sample(s) and the proportion of this recovered in the analysis represents the recovery from the sample preparation. The peculiar challenge with microplastics analysis is that as discussed, the term represents a huge diversity of polymers, sizes and shapes and so a single optimal material to use as a tracer in recovery assessment is challenging to define. Size, shape and polymer characteristics (e.g. density, hydrophobicity) can all affect the recovery of microplastics during sample preparation. Ideally a mix of particles representing the full diversity of microplastics expected would need to be prepared to perform a truly quantitative assessment of recovery, though it still uncertain that even with such a recovery standard that results could be quantitatively corrected for this recovery. Therefore, it is necessary that pragmatic solutions to process recovery are found across the participants which are harmonized in terms of their core aims, but which provides the flexibility needed for the diverse methods that are represented across the participating laboratories.

Guiding principles for the design of process recovery across the participants should therefore aim to:

- Provide sufficient information to identify batch to batch variability in case of failure of a particular batch of samples during preparation in the laboratory – so at least one recovery sample is required per batch

- Provide sufficient data to give insight into the repeatability of the method across the duration of the project - i.e. requires more than one assessment of one sample, standard deviations must be calculable
- Quantitative correction for recovery is unlikely to be possible, rather the recovery analyte should be easily distinguishable from environmental microplastics in the sample so it can be monitored robustly.

Recovery samples could be prepared as independent samples like for the blank, though in this case they should be prepared in a matrix that is representative of the sample matrix you are investigating.

It is preferable that recovery is performed within routine samples, through spiking each sample with a known amount of microplastic that can be traced through the process but that can also be distinguished from environmental plastics in the sample.

NOTE, for particle count based methods, previous experience has found that it is more consistent to directly count the number of particles added as a recovery standard into each sample, rather than to generate a suspension of particles that is then spiked, as the variability even within a well-mixed suspension when detecting 10's of particles can be high enough to cloud any interpretation of the recovery on a per-sample basis.

Table 10: Recovery assessment planned at each participating laboratory.

			Recovery details			
Demo site	Participant	Analytical method	Polymer	Size	Shape	Reference
1	UKCEH	µ-FTIR	PVC	90-150 µm	Fragments	Defra, (2023)
2	UoB	µ-FTIR		<70 µm	Fragments and fibres	Annex 3
3	W30	Fluorescent Staining	PE, PP, PA, PES, PVC	50-1000 µm	Beads and fragments	https://doi.org/10.3390/microplastics2040026 https://doi.org/10.3390/analytica4010004

3.3. Sample analysis

The critical factor concerning sample analysis reporting requirements according to the quality assessment approach is polymer identification. Reducing uncertainty around correct identification of synthetic microplastics against other naturally occurring particles is paramount for comparison across data.

In addition to the guidance of the quality assessment approach, guidance on target number of particles to quantify for different purposes were established recently using statistical approaches (Cowger *et al.*, 2024). These alongside other guidance from Cross *et al.*, 2025 and Tanaka, Kataoka and Nihei, 2023 are summarized in Table 3. These may also be used to justify the study score for reporting polymer identity.

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Table 11: Sample Collection Record template for "Sample Analysis".

NOTE: Blue text indicates updates to the Koelmans et al., 2019 guidance and additional information to support users in completing the Sample Collection Record.

		Study score			
Reporting criteria	**Criteria ID**	**2**	**1**	**0**	**Justification**
Polymer ID	9	**Per study:** Analytical technique is documented Analysis of all particles when numbers of pre-sorted particles are <100. For particle numbers >100, 50% should be identified, with a minimum of 100 particles. **Per sample:** Analysis of all particles up to a maximum of 50 particles per sample. **Per filter:** ≥25% of the surface area.	Insufficient polymer identification, potentially resulting in an unrepresentative subsample. *See Cowger et al., 2024; Cross et al., 2025 for further guidance*. Identification with SEM/EDX or other measures such as staining/or fluorescence approaches to distinguish polymer vs non-polymeric materials.	No polymer identification	*To justify, follow the guidance of reporting requirements listed to the left.* *Alternatively/in addition, the user may provide supporting justification using the RSVP tool to explain the statistical power in the data on the basis of the target number of particles analysed with confirmed polymer ID, see Table 3.*

3.4. Data interpretation

3.4.1. Blank correction and limits of detection

The issue of inconsistent application of blank correction, or accounting for background contamination through establishing limits of detection have been highlighted as a particular issue in microplastics research, particularly prior to 2019, before the publication of the quality assessment framework by Koelmans et al. Interesting research in 2023 systematically tested 51 different approaches to accounting for background contamination in microplastic quantification that were documented in the literature and found that approaches establishing limits of detection or quantification were the most consistent in reducing background contamination from by between 96.3 and 100% (Dawson *et al.*, 2023).

The limit of detection (LOD) is defined as being 3.3 times the standard deviation of the blank, giving a significance level of 0.05 for the false positive error rate. This means if a sample contains a concentration of microplastics at the same level as the LOD, there is only a 5% chance of a false positive result, where the field sample actually contained no microplastic (Armbruster and Pry, 2008). Microplastics are only quantified if they are detected above this LOD.

The general procedure for data transformation, blank correction and calculation of concentrations is harmonised for both particle count data, and mass data where the data is distinguished on a polymer-by-polymer basis:

- Establish the limits of detection (LOD) on a polymer-by-polymer basis.
- Comparison of the (corrected – if blank correction is used) data against the LOD in each sample.

- Calculation of a concentration of microplastics >LOD on a polymer-by-polymer basis in the sample. The total microplastic concentration is the sum of all values that were >LOD.

For analytical methods that are not chemically specific, a different approach is required, but which can adhere to the same principles and objectives in order to harmonize across the data generated by different participants and across different demos. The two partners using a combination of staining and fluorescence microscopy (W30 and LEITAT) generate particle count data that is not chemically specific to the polymer type.

W30 uses the following approach for blank correction: laboratory blanks are measured during the sample processing and the average of the last 10 blanks is subtracted from the samples.

3.4.2. Recovery assessment/ correction

As discussed in the design of recovery assessment during sample preparation, it is unlikely that quantitative correction by recovery is possible for the particle count based analytical methods. Rather all participants quantifying microplastics using count-based methods (e.g. FTIR, fluorescence microscopy) should quantify the recovery of their selected standard and discuss the repeatability and consistency of this recovery across the period of the project.

For the mass-based methods e.g. pyr-GC-MS, quantitative recovery correction may be possible as here a limited number of polymers are to be quantified and standards for each can be run for assessment of recovery from the full sample preparation and analysis procedure. Recovery and quantification will employ distinct calibration curves, each corresponding to different polymers. Initial steps involve the utilization of a standardized methodology, encompassing parameters such as pyrolysis temperature, split ratio, temperature program and the mode for the mass spectrometer. Subsequently, the matrix effect needs to be assessed to ascertain any potential ion suppression, and to determine the necessity of a recovery correction factor, though its significance is presumed negligible. Finally, real samples will be quantified utilizing specific calibration curves tailored to each polymer type. Additionally, an internal standard, such as polyfluorostyrene, may be employed for necessary corrections.

3.4.3. Restricted datasets based on shared analytical windows

This is specific to the case of count-based data where known relationship between particle size and frequency of detection means that data is only comparable if you are looking at the same size region with your techniques.

We have identified in Figure 1 that across several sites there are quite similar analytical windows that with minor restrictions on the data inclusion (aligning a minimum reported size of particle that is common to all count-based techniques for example), could allow for comparison between demos.

A rough log-log relationship between particle size and number-based concentrations in the environment is generally accepted (Kooi and Koelmans, 2019). In Figure 9, data is reproduced from “Sink to river – river to tap” report UKWIR 2019, Report ref. 19/EQ/01/18, to demonstrate the ubiquity of this relationship between decreasing particle size and increasing particle counts across multiple media from drinking water and wastewater infrastructure (Johnson et al., 2020). As particles fragment from larger items in the environment into ever smaller secondary microplastics, it is apparent why this relationship should be the case (Wohlleben *et al.*, 2024). This has important implications for harmonising data reporting and evaluation of data across different demonstration sites.

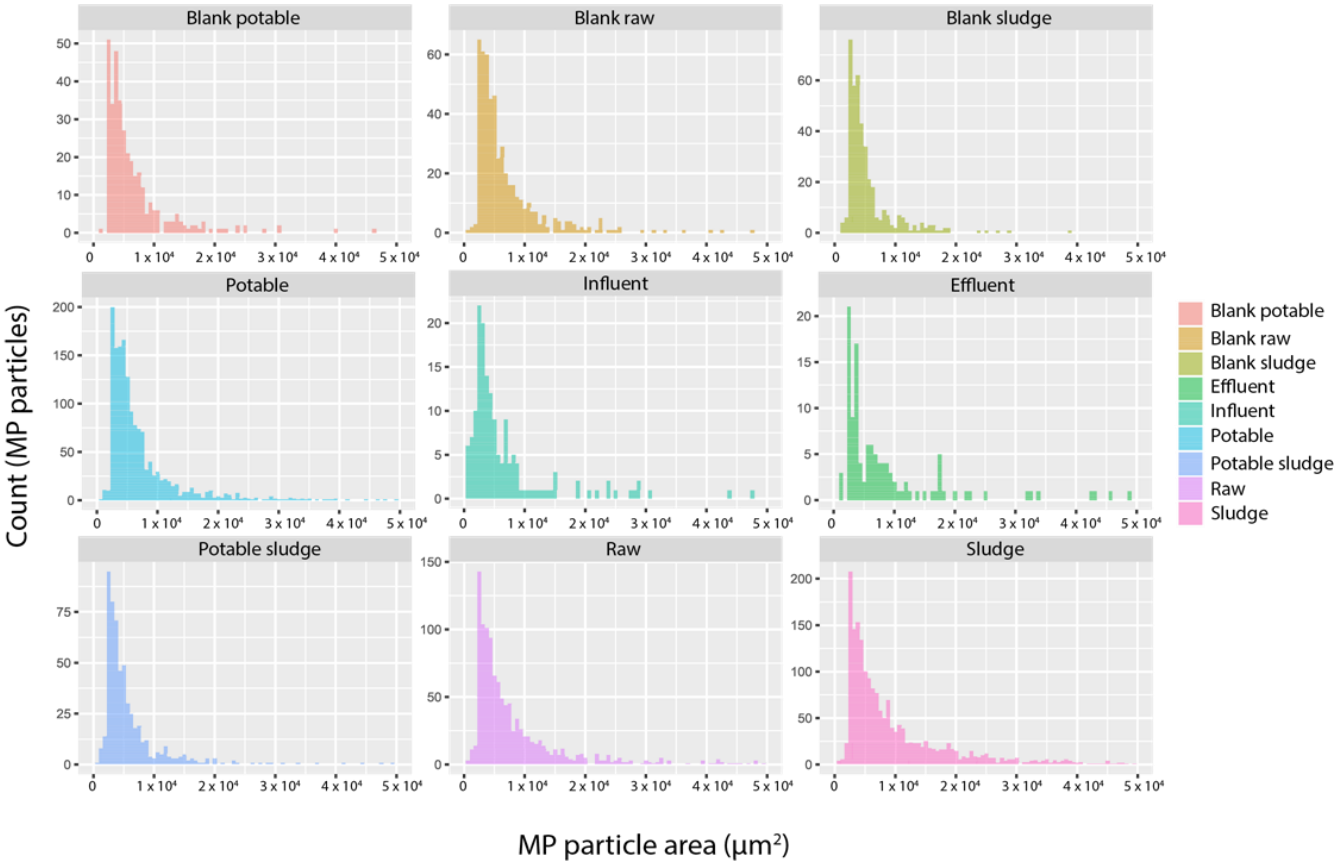

Figure 9: Size distribution of microplastics across 9 different matrices.

Note: Here, data is reproduced from "Sink to river – river to tap" report UKWIR 2019, Report ref. 19/EQ/01/18 (Ball et al., 2019, Johnson et al., 2020). In all media (including in blanks representing different sample preparation workflows) there is a rough log-log relationship between particle size and the frequency of detection. This starts to break down as you reach the particle size limits of detection of the analytical technique. One approach that could be harmonised across the project is defining the lower limits of quantification of each analytical technique (only relevant for particle counting based approaches) based on such frequency plots. In addition, to align measurements across instruments with different analytical windows, minimum size above which microplastics are quantified may be set that are within the analytical window of all techniques so that data can be compared.

For example, µ-FTIR employed for Demo 1 might confidently describe efficacy of the technology for microplastic particles >100 µm in size, whilst the fluorescence staining technique at Demo 4, might be include quantification of smaller particles down to 10 µm. Even this small difference in minimum particle size could result in orders of magnitude differences in reported microplastic particle concentrations between these two demos, simply due to the different lower limits of detection possible at the two locations.

To address this, it is not only important to consider harmonisation of sampling methods and analytical techniques where possible, but also to consider how we harmonise data reporting, particularly when it comes to comparative evaluation of different demo site technologies. One solution that might be considered based on the findings in Figure 9, would be that a common lower size for quantification could be agreed to align data from different methods.

These data restriction approaches will be finalised as part of ongoing discussions in Task 1.5. Below a provisional list of possible restricted datasets that could be generated within the project are indicated Table 12.

Table 12: Provisional restricted datasets to allow comparison across demos

Restricted data set – size range (µm)	Analytical techniques (laboratory)	Relevant demos
>100 µm	µ-FTIR (UKCEH)	Demo 1 (SVT)
	Optical microscope and Rhodamine staining (LEITAT)	Demo 2
	Fluorescence staining (W30)	Demo 4 (Landau)
>10 µm	Optical microscope and Rhodamine-B staining (LEITAT)	Demo 2
	Fluorescence staining with innovative fluorescence dyes abcr eco Wasser 3.0 detect MP-1 and Fluorescence Microscopy (W30)	Demo 4 (Landau)

3.4.4. Conversion between metrics and scales

Spectroscopy based techniques cannot directly measure particle mass. Conversion between each individual detected particle and its estimated mass is therefore required based on particle dimensions and assumptions around volume and density. To allow consistency with the data produced more generally in the research community, it is useful to identify leading approaches which may be typically used. Consistently, µ-FTIR is one of the most commonly employed analytical techniques in international comparison studies by participating laboratories (Belz *et al.*, 2021; van Mourik *et al.*, 2021). The siMPle free software may be one of the most consistently used software by the community for interpreting µ-FTIR due to its applicability to both focal plane array FTIR and linear array FTIR data, and its workflow being designed to allow for analysis across multiple instrument manufacturers (Primpke *et al.*, 2020). In this software, an automated method is implemented to estimate particle mass. Similar methods are also used in recent modelling tools to characterize the multidimensionality of microplastics across environments (Kooi *et al.*, 2021) and databases (Thornton Hampton *et al.*, 2022).

The following method is used to estimate particle mass in the siMPle freeware software (siMPle, 2024). To estimate a mass for each particle detected by the µFTIR, the longest dimension is calculated as the longest distance between pixels of the particle. The minor dimension is calculated by the software assuming that the particle is an ellipse and knowing the two-dimensional area of the particle. The third and final dimension to be calculated, the thickness, is assumed to be 0.67 times the minor dimension. From these dimensions, the volume of the particle is estimated assuming the microplastic particle is ellipsoidal, and the estimated mass is calculated from the volume and the density of the identified plastic polymer. This same approach can be used for any analytical technique where particles are counted, and the polymer identified. This approach will therefore be employed by UKCEH, and UoB (Demo 1), whilst W30 will explore options (Demo 3) based on experiences from UKCEH and UoB.

4. Harmonized methods for monitoring leachable compounds

In the following section, the list of chemicals currently quantified or planned for analysis are reported across partners so as to review where common analytes may be measured across demo sites.

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Three demonstration sites are identified in the mapping exercise as expecting to monitor leachable compounds, Demo 1 Daphnia treatment (UoB), and Demo 3 CAP WWTW (VITO) and Demo 4 Landau WWTW advanced oxidation technology (VRE).

Below are the chemicals quantified in Demo 1 by UoB using high-resolution mass spectrometry (HRMS).

Chemical
Metformin
Acetaminophen
Gabapentin
Codeine
Caffeine
Trimethoprim
Sulfamethoxazole
Tramadol
Metoprolol
Doxycycline
Propranolol
Carbamazepine
Hydrocortisone
Erythromycin-H2O
DEET
Clotrimazole
Mefloquine-HCl
Oxazepam
Diazepam
Ibuprofen
Naproxen
Diclofenac Na
Meclofenamic acid
Glyburide
Gemfibrozil
17-a-ethinyl estradiol
B-estradiol
PFOS
PFOA
MP

To tackle laborious pretreatments, monitoring conducted by VITO will use ambient pressure ionization techniques (e.g., Direct Analysis in Real-Time, DART) coupled to mass spectrometry to rapidly and directly analyse liquid samples at atmospheric pressure without any pretreatment. As a result, the appearance of additives in the plastic life cycle could be monitored.

To date, this method has been tested and is under optimization for the following 10 compounds:

- Dipropyl phthalate
- Dimethyl phthalate
- Dibutyl phthalate
- Diethyl phthalate
- Dipentyl phthalate
- Tripropyl phosphate
- Tributyl phosphate
- Tetraethyl ethylenediphosphate
- Di n-octyl phthalate
- Bisphenol A

Further developments and monitoring of leachable compounds and their transformation products after various remediation technologies will primarily be covered under Task 2.2 “Optimised strategies for L, P and MP release prevention and remediation” and so are not further discussed here.

5. Conclusions

In conclusion, Deliverable 1.1 has summarised recent advances in harmonised methods for monitoring litter, microplastics and nanoplastics which can be leveraged by project partners. This review has refined specific guidance on best practice for monitoring litter and microplastics in the UPSTREAM project. The monitoring capabilities across the project partners have been reviewed, mapping the analytical windows for each partner against different demo sites in order to understand common regions of analysis across demos and technologies as well as where there are gaps in the analytical coverage. This information will be essential in feeding into Task 1.5, development of the data platform.

Litter, microplastics and leachable compounds are taken in turn and specific guidance on agreed harmonised methods for these three major classes of plastic are detailed. For litter this largely pertains to ensuring that the methods used in UPSTREAM are harmonised against current international guidance. Specific definitions of hotspots were established for internal use in the project when monitoring litter based on the latest evidence.

For microplastics, extensive guidance is given on current best practices as there are many partners who will be measuring this class of plastic in the project, each using different analytical approaches to quantify and monitor microplastics (UKCEH, UoB, LEITAT, NVMT, VITO, W30, UNSMF and NIC). No single standardised protocol can be applied for monitoring microplastics across the project as the requirements for sample collection, preparation and analysis are specific to each analytical technique, and there is no single analytical technique that can be commonly employed across all partners in the consortium. It is for this reason that harmonisation rather than standardisation was pursued, to establish a common set of principles for sample collection, preparation and analysis with the aim to ensure consistent quality in the data produced across the consortium. To assist in this and to provide transparency in the data records produced in UPSTREAM, a draft Sample Collection Record Template was designed to allow for a standardised data collection method for all relevant information concerning a microplastic monitoring campaign at a demo site. The quality assessment criteria for freshwaters established by Koelmans *et al.*, (2019) was used as a starting point to establish the Sample Collection Record Template. Each quality assurance criteria was taken in turn and specific updates agreed in UPSTREAM were described. The principle of the Sample Collection Record is that each partner in UPSTREAM will be able to record method

details for a specific sampling campaign (i.e. a study with a defined aim and objective such as a 12M monitoring campaign at a specific demo site) and justify a quality score against each criteria, providing both information required by the criteria, and the justification for the quality score as free text. Data uploaded to the platform will then be associated with this Sample Collection Record so that all meta data around how the sample was collected, prepared and analysed is findable and a harmonised quality score can be transparently reached and reported for all data for microplastic monitoring generated in the project.

The RSVP tool was developed by UKCEH, which allows for a harmonised approach to representative sample volume predictions (the RSVP tool, Cross *et al.*, 2025). This was identified as a gap in the QA/QC criteria as the existing guidance was proscriptive in minimum representative sample volumes based on measurement of large microplastics >300 µm in size. Many partners in UPSTREAM monitor for much smaller particles which are much more abundant and so lower volumes may still be representative, whilst avoiding issues with overloading the samples. The RSVP tool provides a standardised way to justify statistically that the sample volume collected was sufficient to be representative. This is a key criterion in the Sample Collection Record.

Finally, the current status of leachable compound monitoring is also reviewed and the proposed analyte lists measured at each demo site recorded to provide an overview of the chemicals to be monitored during the project.

A series of Annexes are also provided which document the SOPs and data templates for monitoring litter and microplastics by specific partners. In addition to this, the Sample Collection Record Template is provided as an Annex for further development in T1.5, as is the RSVP tool.

References

ASTM (2020) 'D8332-20 Standard Practice for Collection of Water Samples with High, Medium, or Low Suspended Solids for Identification and Quantification of Microplastic Particles and Fibers'. Available at: https://doi.org/10.1520/D8332-20.

ASTM (2023) 'WK87463 New Test Method for Spectroscopic Identification and Quantification of Microplastic Particles in Water Using Infrared (IR) Spectroscopy. Under Development. Work Item.' Available at: https://www.astm.org/workitem-wk87463 (Accessed: 9 December 2024).

Bank, M.S. *et al.* (2021) 'Global Plastic Pollution Observation System to Aid Policy', *Environmental Science & Technology*, 55(12), pp. 7770–7775. Available at: https://doi.org/10.1021/acs.est.1c00818.

Belz, S. *et al.* (2021) *Current status of the quantification of microplastics in water: results of a JRC/BAM interlaboratory comparison study on PET in water*. Publications Office of the European Union. Available at: https://data.europa.eu/doi/10.2760/27641 (Accessed: 9 December 2024).

Bleeker, E.A.J. *et al.* (2023) 'Towards harmonisation of testing of nanomaterials for EU regulatory requirements on chemical safety – A proposal for further actions', *Regulatory Toxicology and Pharmacology*, 139, p. 105360. Available at: https://doi.org/10.1016/j.yrtph.2023.105360.

Cowger, W. *et al.* (2024) 'How many microplastics do you need to (sub)sample?', *Ecotoxicology and Environmental Safety*, 275, p. 116243. Available at: https://doi.org/10.1016/j.ecoenv.2024.116243.

Cross, R.K. *et al.* (2025) 'Ensuring representative sample volume predictions in microplastic monitoring', *Microplastics and Nanoplastics*, 5(1), p. 5. Available at: https://doi.org/10.1186/s43591-024-00109-2.

Dawson, A.L. *et al.* (2023) 'Taking control of microplastics data: A comparison of control and blank data correction methods', *Journal of Hazardous Materials*, 443, p. 130218. Available at: https://doi.org/10.1016/j.jhazmat.2022.130218.

De Frond, H.D. and Wong, D.C. (2021) 'Standard Operating Procedures for Extraction and Measurement by Infrared Spectroscopy of Microplastic Particles in Drinking Water'. Available at: https://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/documents/microplastics/mcrplstcs_ir.pdf.

Defra (2023) *Measurement and Characterisation of Microplastics in English River Catchment Waters and Sediments - WT15135*. WT15135. Defra. Available at: https://sciencesearch.defra.gov.uk/ProjectDetails?ProjectId=20540 (Accessed: 11 December 2024).

van Emmerik, T. *et al.* (2020) 'Riverbank macrolitter in the Dutch Rhine–Meuse delta', *Environmental Research Letters*, 15(10), p. 104087. Available at: https://doi.org/10.1088/1748-9326/abb2c6.

European Commission: Joint Research Centre *et al.* (2021) *Joint list of litter categories for marine macro-litter monitoring: manual for the application of the classification system*. Publications Office of the European Union. Available at: https://data.europa.eu/doi/10.2760/127473 (Accessed: 24 February 2025).

Hartmann, N.B. *et al.* (2019) 'Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris', *Environmental Science & Technology*, 53(3), pp. 1039–1047. Available at: https://doi.org/10.1021/acs.est.8b05297.

Heunisch, E. *et al.* (2022) *Development or revisions of OECD Test Guideline (TG) and Guidance Documents (GD) applicable for nanomaterials*. Available at: https://nanoharmony.eu/wp-content/uploads/2022/10/NanoHarmony-NANOMET-report-on-OECD-TG-and-GD-developments-for-NMs.pdf (Accessed: 22 January 2025).

ISO (2020) 'ISO/TR 21960:2020 Plastics - Environmental aspects - State of knowledge and methodologies'. Available at: https://www.iso.org/standard/72300.html (Accessed: 9 July 2025).

ISO (2023) 'ISO 24187:2023 - Principles for the analysis of microplastics present in the environment'. Available at: https://www.iso.org/standard/78033.html (Accessed: 9 July 2025).

ISO (2024a) 'ISO/DIS 16094-2 Water quality - Analysis of microplastic in water. Part 2: Vibrational spectroscopy methods for waters with low content of suspended solids including drinking water'. Available at: https://www.iso.org/standard/84460.html (Accessed: 7 October 2024).

ISO (2024b) 'ISO/DIS 16094-2 Water quality - Analysis of microplastic in water. Part 2: Vibrational spectroscopy methods for waters with low content of suspended solids including drinking water. Under Development. Stage 40.99.' Available at: https://www.iso.org/standard/84460.html (Accessed: 7 October 2024).

ISO (2024c) 'ISO/DIS 16094-3 Water quality - Analysis of microplastic in water. Part 3: Thermo-analytical methods for waters with low content of suspended solids including drinking water. Under Development. Stage 40.60.' Available at: https://www.iso.org/standard/84463.html (Accessed: 6 December 2024).

ISO (2024d) 'ISO/FDIS 5667-27 Water quality - Sampling. Part 27: Guidance on sampling for microplastics in water. Under Development. Stage 50.20.' Available at: https://www.iso.org/standard/82612.html (Accessed: 6 December 2024).

Joint Research Centre (European Commission) and MSFD Technical Group on Marine Litter (2023) *Guidance on the monitoring of marine litter in European seas: an update to improve the harmonised monitoring of marine litter under the Marine Strategy Framework Directive*. Publications Office of the European Union. Available at: https://data.europa.eu/doi/10.2760/59137 (Accessed: 14 February 2025).

JRC (2013) *Guidance on Monitoring of Marine Litter in European Seas: A guidance document within the Common Implementation Strategy for the Marine Strategy Framework Directive*. JRC83985. Joint Research Centre. Available at: https://circabc.europa.eu/ui/group/326ae5ac-0419-4167-83ca-e3c210534a69/library/ccab7399-89f6-43f8-8eda-25b4747cd911/details (Accessed: 9 July 2025).

JRC (2025) *JRC Floating Litter Monitoring App*, *Inspire Europe*. Available at: https://inspire-europe.org/solutions/jrc-floating-litter-monitoring-app/ (Accessed: 14 February 2025).

Koelmans, A.A. *et al.* (2019a) 'Microplastics in freshwaters and drinking water: Critical review and assessment of data quality', *Water Research*, 155, pp. 410–422. Available at: https://doi.org/10.1016/j.watres.2019.02.054.

Koelmans, A.A. *et al.* (2019b) 'Microplastics in freshwaters and drinking water: Critical review and assessment of data quality', *Water Research*, 155, pp. 410–422. Available at: https://doi.org/10.1016/j.watres.2019.02.054.

Koelmans, A.A. *et al.* (2019c) 'Microplastics in freshwaters and drinking water: Critical review and assessment of data quality', *Water Research*, 155, pp. 410–422. Available at: https://doi.org/10.1016/j.watres.2019.02.054.

Kooi, M. *et al.* (2021) 'Characterizing the multidimensionality of microplastics across environmental compartments', *Water Research*, 202, p. 117429. Available at: https://doi.org/10.1016/j.watres.2021.117429.

Lacroix, C., André, S. and van Loon, W. (2023) *Abundance, Composition and Trends of Beach Litter*. OSPAR Commission. Available at: https://oap.ospar.org/en/ospar-assessments/quality-status-reports/qsr-2023/indicator-assessments/beach-litter/ (Accessed: 17 February 2025).

Lippiatt, S., Opfer, S. and Arthur, C. (2013) *Marine debris monitoring and assessment : recommendations for Monitoring Debris Trends in the Marine Environment*. Technical Memorandum 46. NOAA. Available at: https://repository.library.noaa.gov/view/noaa/2681 (Accessed: 9 July 2025).

Martínez-Francés, E. *et al.* (2023) 'Innovative reference materials for method validation in microplastic analysis including interlaboratory comparison exercises', *Analytical and Bioanalytical Chemistry*, 415(15), pp. 2907–2919. Available at: https://doi.org/10.1007/s00216-023-04636-4.

Morgana, S. *et al.* (2024) 'Fluorescence-based detection: A review of current and emerging techniques to unveil micro/ nanoplastics in environmental samples', *TrAC Trends in Analytical Chemistry*, 172, p. 117559. Available at: https://doi.org/10.1016/j.trac.2024.117559.

van Mourik, L.M. *et al.* (2021) 'Results of WEPAL-QUASIMEME/NORMANs first global interlaboratory study on microplastics reveal urgent need for harmonization', *Science of The Total Environment*, 772, p. 145071. Available at: https://doi.org/10.1016/j.scitotenv.2021.145071.

Primpke, S. *et al.* (2020) 'Toward the Systematic Identification of Microplastics in the Environment: Evaluation of a New Independent Software Tool (siMPle) for Spectroscopic Analysis', *Applied Spectroscopy*, 74(9), pp. 1127–1138. Available at: https://doi.org/10.1177/0003702820917760.

Primpke, S. *et al.* (2023) *Short report on methods and protocols for the analysis of nano-, micro-, and macroplastic in water samples*. Zenodo. Available at: https://doi.org/10.5281/zenodo.8417563.

Schwaferts, C. *et al.* (2019) 'Methods for the analysis of submicrometer- and nanoplastic particles in the environment', *TrAC Trends in Analytical Chemistry*, 112, pp. 52–65. Available at: https://doi.org/10.1016/j.trac.2018.12.014.

Sturm, M.T. *et al.* (2024) 'Long-Term Monitoring of Microplastics in a German Municipal Wastewater Treatment Plant', *Microplastics*, 3(3), pp. 492–502. Available at: https://doi.org/10.3390/microplastics3030031.

Tanaka, M., Kataoka, T. and Nihei, Y. (2022) 'Variance and precision of microplastic sampling in urban rivers', *Environmental Pollution*, 310, p. 119811. Available at: https://doi.org/10.1016/j.envpol.2022.119811.

Tanaka, M., Kataoka, T. and Nihei, Y. (2023) 'An analytical approach to confidence interval estimation of river microplastic sampling', *Environmental Pollution*, 335, p. 122310. Available at: https://doi.org/10.1016/j.envpol.2023.122310.

Tasseron, P.F. *et al.* (2024) 'Defining plastic pollution hotspots', *Science of The Total Environment*, 934, p. 173294. Available at: https://doi.org/10.1016/j.scitotenv.2024.173294.

Thornton Hampton, L.M. *et al.* (2022) 'A living tool for the continued exploration of microplastic toxicity', *Microplastics and Nanoplastics*, 2(1), p. 13. Available at: https://doi.org/10.1186/s43591-022-00032-4.

UKWIR (2022) *The National Chemical Investigations Programme 2020-2022 Volume 2 - Investigations into the fate and behaviour of Microplastics within wastewater treatment works*. Available at: https://UKWIR.org/water-industry-technical-report?object=91d0b63a-a522-4880-9c1e-fa05cdb763a8 (Accessed: 23 May 2024).

United Nations Environment Programme and Intergovernmental Oceanographic Commission (2009) *UNEP/IOC Guidelines on Survey and Monitoring of Marine Litter*. Available at: https://wedocs.unep.org/xmlui/handle/20.500.11822/13604 (Accessed: 14 February 2025).

Wenneker, B. and Oosterbaan, L. (2010) *Guideline for Monitoring Marine Litter on the Beaches in the OSPAR Maritime Area. Edition 1.0.* Report. OSPAR Commission. Available at: https://doi.org/10.25607/OBP-968.

Werner, S. *et al.* (2020) *Threshold Values for Marine Litter.* Report. Publications Office of the European Union. Available at: https://doi.org/10.2760/192427.

Wohlleben, W. *et al.* (2024) 'Everything falls apart: How solids degrade and release nanomaterials, composite fragments, and microplastics', *NanoImpact*, 34, p. 100510. Available at: https://doi.org/10.1016/j.impact.2024.100510.

Wong, D.C. and De Frond, H. (2021) 'Standard Operating Procedures for Extraction and Measurement by Raman Spectroscopy of Microplastic Particles in Drinking Water'. Available at: https://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/documents/microplastics/mcrplstcs_raman.pdf.

Annex 1 – SOP: UAV for beached litter detection (UoA)

Standard Operating Procedure for UAV data acquisition for beached marine litter detection.

1. Introduction

This is a flight and data acquisition protocol compiled to assist with optimal flight parameterisation for data acquisition to perform beached marine litter detection through the Coastal Marine Litter Observatory (CMLO) (cmlo.aegean.gr).

This is not a step-by-step guide as different platforms and capture applications have slightly different settings requirements, but rather a set of parameters that need to be met for the data to be readily useable by the CMLO platform.

All flights must follow the relevant rules and regulations of the local and regional Civil Aviation Authorities (CAA). Pilots are exclusively and solely responsible for their flights and their UAV.

2. Data acquisition protocol

Below is the set of most important parameters that need to be met for full data compliance:

2.1 Primary parameters

i. **GSD at 0.5 cm**. This generally corresponds to a **flight altitude** of about **18 m**. However, different sensors have different characteristics (i.e. sensor width, focal length, image width), and hence the exact flight altitude needed to produce a 0.5 cm GSD must be calculated ad hoc for each sensor. Most UAV flight software will do this calculation automatically. A useful tool to calculate flight altitude for a given GSD based on sensor characteristics can be found here: https://support.pix4d.com/hc/en-us/articles/202560249
Important: In case take off is at a different altitude than the AOI, take off altitude must be adjusted accordingly as most UAV calculate flight altitude from take off point.

ii. **Viewing angle at nadir/vertical/90º.** This is important to ensure that no distortions due to viewing angle characteristics are introduced into the acquired data.

iii. **Side and Front overlap at 20%.** This setting ensures that the acquired data can be merged accurately without distortions due to no-data extrapolation.

iv. **Face parameter to Forward.** This setting ensures the UAV acquires images facing forward relative to its flight path and frontal side.

v. **Speed parameter to SLOW/SLOW+.** This ensures that images are properly lit and no motion blur is present.

2.2 Secondary parameters

i. Triger mode FAST MODE
ii. White balance to AUTO
iii. Look at grids center NO

3. Takeoff checklist

Below is a checklist with conditions that must be met before each flight. Although each UAV is slightly different, the below list is universal and applies to all drone flights performed under controlled conditions.

Drone Pre-takeoff Checklist			
	RC connected to drone		Mission is within range
	Camera ready		Mission uploaded to drone
	Drone flight instruments calibrated		Drone storage adequate
	Homepoint is set		Drone GPS satellites connected

Below is a general pre-flight checklist to ensure that all flights are performed safely and within local CAA regulations. It is assumed that drone registration and insurance are compliant with local regulations. In case flight permits are needed before flight, it is the responsibility of the drone operator to ensure that all permits are acquired and valid.

Pre-flight Checklist			
	You are not in a no-fly zone		Drone meets regulations and is in good condition
	No bystanders or uninvolved persons nearby		No private property/infrastructure nearby, or property owners informed of flight
	Flight altitude within regulations		Flight range within line of sight (VLOS flight)

Important: It is the drone operator's sole responsibility to be aware of the limitations of autonomous flight and capable of taking over manual control if necessary. Manual flight is the only way to avoid previously unseen obstacles or avoid loss of equipment or accidents due to possible GPS interference.

4. Data pre-processing and upload

Data pre-processing is minimal and simply involves collecting all images in a single zipped folder. The folder is subsequently uploaded to the CMLO platform under an authorised account. All data processing to export the litter density maps is performed automatically. An example of the litter density maps that are produced by the platform can be seen below.

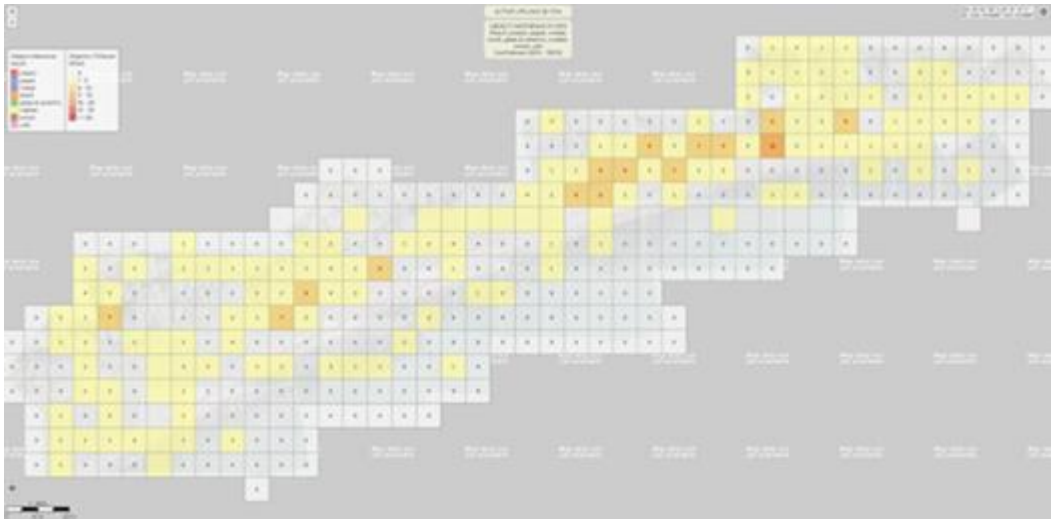

Figure 1: Example of a litter density map from a beach survey reported in items/100 sq. meter grid units showing number of items/grid unit.

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Annex 2 – SOP: Beached litter surveys - site, sampling unit and monitoring identity forms (UNSMF)

UPSTREAM Novi-Sad Survey Site Identity Form (A1)

Name of survey site: …… Date of record: ……
Code of survey site: …… Contact person: ……
Email: ……

Total length of surveyed riverbank: …… (m)
Latitude (central point): …… (polar)
Longitude (central point): …… (polar)
Urbanisation degree: Urban Semi-Urban Remote/Natural

Back of the riverbank: Cliffs Dunes Rocks Forest Bush Crops
Fields Built-up area Road Other: ……
Is there any development behind the riverbank? Yes No
Description of the development behind the riverbank: ……
Looking from the riverbank to the river, what direction is the bank facing (two boxes can be ticked):
N E S W
Riverbank curvature: Linear Concave Convex Sinusoidal
Riverbank substrate (% coverage): ……. % sand……. % pebbles/rocky ……. % other:……..
Objects in river that influence river flow and currents: *(e.g pier, islets etc.)*: ……
Riverbank slope: Level Gentle slope Moderate slope Steep slope

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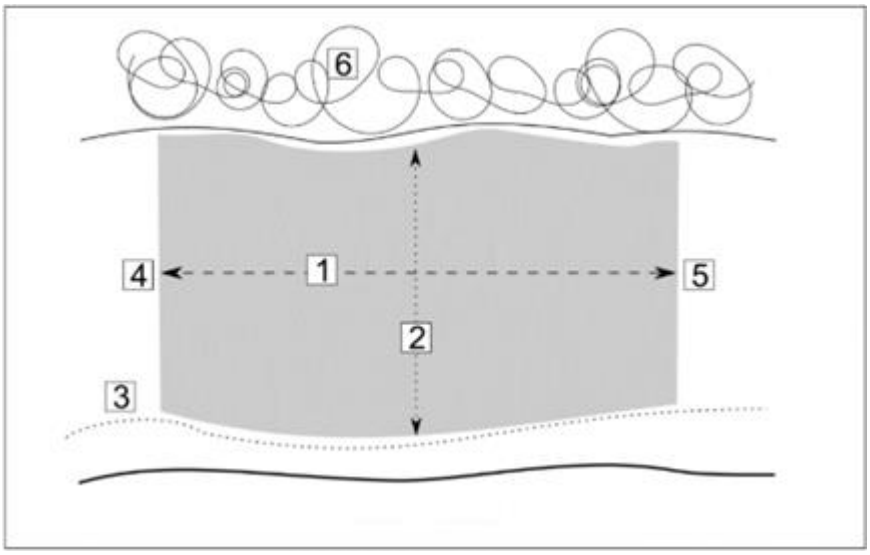
Beach access: Pedestrian Vehicle Boat Primary beach usage *(e.g tourism and recreation, fishing, etc.)*: …… Seasonal All year round Secondary beach usage *(e.g tourism and recreation, fishing, etc.)*: …… Seasonal All year round Estimated average number of people using the beach: winter …… spring …… summer …… autumn ……

Any other noteworthy information: …… …… …… …… …… ……

UPSTREAM Novi-Sad Sampling Unit Identity Form (A2) MSFD format where applicable *(100 m length typical)*

Code of survey site (A1): …… ……	Date of record:
Name of sampling unit (A2): …… ……	Code of sampling unit (A2):
Contact person: ……	Email: ……



1: Sampling unit length

2: Sampling unit width

3: Edge of the water

4 and 5: GPS coordinates of the sampling unit

6: Back of the riverbank

Sampling unit length *(measured along the riverbank curve at the mid-point between the water edge and the back of the riverbank)*: ……. (m)

Sampling unit width *(perpendicular to the shoreline; measured at the mean water level; defined as the distance between the water edge and the back of the riverbank)*: ……. (m)

GPS coordinates 4: N: …… E: …… (polar)

GPS coordinates 5: N: …… E: …… (polar)

Direction of prevailing winds: N E S W

(two boxes may be ticked)

Name of nearest town/suburb:
……

Distance of the town from the sampling unit:
…….. (km)

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Position of the town in relation to the sampling unit: N E S W

Size of residential population of nearest town: ……

Food/drink outlet near the sampling unit: No Yes

Distance of the food/drink outlet near the sampling unit: …… (km)

Position of the food/drink outlet to the sampling unit: N E S W

Present all year round: Yes No, specify months: ……

Name of the nearest harbour: ……

Distance of the harbour from the sampling unit: …… (km)

Position of the harbour in relation to the sampling unit: N E S W

Type of shipping using the harbour: Passenger Merchant Fishing Military

Recreational All kinds Other (specify): ……

Name of the nearest river mouth: ……..

Distance of the nearest river mouth to the sampling unit: …… (km)

Position of the river mouth in relation to the sampling unit: N E S W

Distance of the nearest wastewater or stormwater discharge point from the sampling unit: …… (km)

Position of the discharge point in relation to the sampling unit: N E S W

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Distance of the nearest shipping lane to the sampling unit:
…… (km)

Position of the shipping lane in relation to the sampling unit: N E
S W

Estimated traffic density: …… (n. of ships/year)

Type of shipping using the shipping lane: Passenger Merchant Fishing Military

Recreational All kinds Other (specify):
……

10 x 10 m sampling unit(s) based on CMLO density reporting grid (ETRS-LAEA CRS)

Code of sampling unit 1: ……

Sampling unit 1 GPS central coordinates: N: …… E: …… (polar)

Code of sampling unit 2: ……

Sampling unit 2 GPS central coordinates: N: …… E: …… (polar)

Any other noteworthy information:
……

……
……

……
……

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UPSTREAM Novi-Sad marine litter monitoring survey form (A4)

Code of survey site (A1): …… Date of survey:……

Code of sampling unit (100m) (A2): ……. Name of surveyor 1: ……

Code of sampling unit (grid) (A2): ……. Name of surveyor 2: ……

Code of the survey: ……. Name of surveyor 3: ……

Other information: …… Name of surveyor 4: ……

……

Length of surveyed sampling unit: *(The actual length surveyed, which may differ slightly from the suggested 100 m recorded in the sampling unit identity form (A2). Measured along the riverbank curve at the mid-point between the water edge and the back of the riverbank)*
…….. (m)

Date of the last known cleaning action:
……

Weather conditions during the date of the surveys: Wind Rain Snow Ice

Fog Sandstorm Exceptionally high tide Other: ……

Deviations from sampling protocol: *(e.g. transect length reduction or displacement of the transect, sampling outside the expected period, sub-sampling)*
……

Motivation *(e.g. extreme weather events, flooding, new infrastructures in place)*
……..

Special circumstances that could have caused an unusual occurrence of litter in terms of abundance and/or type: *(e.g. clean-up days, cleaning machine tracks, beach party or competition, cargo losses nearby, extreme weather conditions)*
……

Entangled animals No Yes How many: …….. Alive Dead

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Bird Turtle Fish Mammal Other: …… Sex (if known): ….. Age (if known): …….

Nature of the entanglement and type of litter:

……

……
……

Any other noteworthy information:

……

……
……

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UPSTREAM Novi-Sad litter data form

J-CODE	SUP/FG	NAME	COUNT
ARTIFICIAL POLYMER MATERIALS			
J220		plastic sheeting from greenhouses	
J221		plastic irrigation pipes	
J222		other plastic items from agriculture	
J90		plastic flower pots	
J223		trays for seedlings of foamed plastic	
J46	FG	plastic oyster trays	
J45	FG	plastic mussels/oyster mesh bags, net sack, socks	
J47	FG	plastic sheeting from mussel culture (Tahitians)	
J102		plastic flip-flops	
J136		footwear made of plastic – not flip flops	
J40		plastic gloves (household/dishwashing, gardening)	
J41		plastic gloves (industrial/professional applications)	
J252		single-use plastic gloves	
J69		plastic hard hats/helmets	
J256		foamed plastic insulation including spray foam	
J89		plastic construction waste (not foamed insulation)	
J8	SUP	plastic drink bottles >0.5 l	
J7	SUP	plastic drink bottles ≤ 0.5 l	
J224	SUP	plastic food containers made of foamed polystyrene	
J21*	SUP	plastic caps/lids drinks	
J225	SUP	plastic food containers made of hard non-foamed plastic	
J1	SUP	plastic 4/6-pack yokes & six-pack rings	
J226	SUP	cups and cup lids of foamed polystyrene	
J227	SUP	cups and lids of hard plastic	
J228	SUP	plastic cutlery	
J229	SUP	plastic plates and trays	
J230	SUP	plastic stirrers	
J231	SUP	plastic straws	
J30	SUP	plastic crisps packets/sweets wrappers	
J31	SUP	plastic lolly & ice-cream sticks	
J85	FG	plastic commercial salt packaging	
J58	FG	fish boxes – foamed polystyrene	
J57	FG	fish boxes – hard plastic	
J92	FG	plastic bait containers/packaging	
J60*	FG	plastic fishing light sticks / fishing glow sticks incl. packaging	
J62	FG	plastic floats for fishing nets	
J59	FG	plastic fishing line	
J54	FG	plastic nets and pieces of net > 50cm	
J53	FG	plastic nets and pieces of net 2.5 cm ≥ X ≤ 50 cm	
J232	FG	plastic string and filaments exclusively from dolly ropes	
J233	FG	other plastic string and filaments exclusively from fishery	
J234	FG	plastic tangled nets and rope without dolly rope or mixed with dolly rope	
J235	FG	plastic tangled dolly rope	
J61	FG	other plastic fisheries related items not covered by other categories	
J42	FG	plastic crab/lobster traps (pots) and tops	
J44	FG	plastic octopus pots	
J70		plastic shotgun cartridges	
J11		plastic beach use related body care and cosmetic bottles and containers	
J12		plastic non-beach use related body care and cosmetic bottles and containers	
J95	SUP	plastic cotton bud sticks	
J29		plastic combs/hair brushes/sunglasses	
J98		plastic diapers/nappies	

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J-CODE	SUP/FG	NAME	COUNT
J236		other plastic personal hygiene and care items	
J96	SUP	plastic sanitary towels/panty liners/backing strips	
J144	SUP	plastic tampons and tampon applicators	
J97		plastic toilet fresheners	
J237	SUP	plastic wet wipes	
J253		plastic single-use face-mask	
J211		other plastic medical items (swabs, bandaging, adhesive plasters etc.)	
J100*		plastic medical/ pharmaceuticals containers/tubes/ packaging	
J99		plastic syringes/needles	
J9		plastic bottles and containers of cleaning products	
J15		plastic engine oil bottles & containers >50cm	
J14		plastic engine oil bottles & containers 2.5 cm ≥ ≤ 50 cm	
J17		plastic injection gun containers/cartridges	
J16		plastic jerry cans	
J22*		plastic caps/lids chemicals, detergents (non-food)	
J23*		plastic caps/lids unidentified	
J24*		plastic rings from bottle caps/lids	
J13		other plastic bottles & containers (drums)	
J3	SUP	plastic shopping/carrier/grocery bags	
J101		plastic dog/pet faeces bag	
J5	SUP	the part that remains from tear-off plastic bags	
J36		other plastic heavy-duty sacks	
J238		plastic mesh bags for vegetable, fruit and other products	
J4	SUP	small plastic bags	
J91*		plastic biomass holder from sewage treatment plants and aquaculture	
J18		plastic crates, boxes, baskets	
J65		plastic buckets	
J93		plastic cable ties	

J-CODE	SUP/FG	NAME	COUNT
J84		plastic CDs & DVDs	
J67		plastic sheets, industrial packaging, sheeting	
J64		plastic fenders	
J68		fibreglass items	
J63		plastic floats/buoys other source than fishing or not known	
J239		other foamed plastic items and fragments not made of foamed polystyrene	
J257*		foamed plastic packaging	
J83		fragments of foamed polystyrene > 50cm	
J82		fragments of foamed polystyrene 2.5 cm ≥ ≤ 50 cm	
J80		fragments of non-foamed plastic > 50cm	
J79		fragments of non-foamed plastic 2.5cm ≥ ≤ 50cm	
J240		other identifiable foamed plastic items	
J241		other identifiable non-foamed plastic items	
J166		plastic paint brushes	
J28		plastic pens and pen lids	
J49		plastic rope (diameter more than 1cm)	
J242		plastic string and cord (diameter less than 1cm) not from dolly ropes or unidentified	
J66		plastic strapping bands	
J43		plastic tags (fishing, shipping, farming and industry)	
J87		plastic masking/duct/packing tape	
J88		telephone	
J72		plastic traffic cones	
J86		plastic fin trees (from fins for scuba diving)	
J243		plastic remains of fireworks	
J32*		plastic toys and party poppers	
J27*	SUP	tobacco products with filters (cigarette butts with filters)	
J26		plastic cigarette lighters	
J25		plastic tobacco pouches / plastic cigarette packet packaging	

J-CODE	SUP/FG	NAME	COUNT
J19		plastic vehicle parts	
		RUBBER	
J127		rubber boots	
J133		rubber condoms (incl. packaging)	
J131*		rubber band (small, for kitchen/household/post use)	
J248		rubber sheet	
J134		other rubber pieces	
J249		rubber belts	
J125*	SUP	rubber balloons	
J126		rubber balls	
J250		rubber inner-tubes	
J251		rubber tyres	
		CLOTH/TEXTILE	
J137		clothing	
J138		shoes & sandals made of leather and/or textile	
J141		cloth textile carpet & furnishing	
J140		hessian sacks/packaging	
J143		sails, canvas	
J145		other textiles	
J139		cloth textile backpacks & textile bags	
		PAPER/CARDBOARD	
J150		paper cartons/Tetrapak milk	
J151		paper cartons/Tetrapak (non-milk)	
J244		paper cups	
J245		paper food trays, food wrappers, drink containers	
J246		paper cotton bud sticks	
J247		other paper containers	
J147		paper bags	
J148		cardboard boxes	

J-CODE	SUP/FG	NAME	COUNT
J156		paper fragments	
J154		paper newspapers & magazines	
J158		other paper items	
J155		paper tubes and other pieces of fireworks	
J152		paper cigarette packets	
		PROCESSED/WORKED WOOD	
J159		wooden corks	
J165		wooden ice-cream sticks, chip forks, chopsticks, toothpicks	
J164		wooden fish boxes	
J163		wooden crab/lobster pots	
J162		wooden crates, boxes, baskets for packaging	
J172		other processed wooden items > 50cm	
J171		other processed wooden items 2.5 cm ≥ ≤ 50 cm	
J160		wooden pallets	
J167		wooden fireworks & matches	
		METAL	
J194		metal cables	
J175		metal drinks cans	
J176		metal food cans	
J181		metal tableware (e.g. plates, cups & cutlery)	
J184		metal lobster/crab pots	
J182*		metal fisheries related weights/sinkers, and lures	
J180		metal appliances (refrigerators, washers, etc.)	
J187		metal drums & barrels	
J174		metal aerosol/spray cans	
J188		other metal cans	
J190		metal paint tins	
J178*		metal bottle caps, lids & pull tabs from cans	

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J-CODE	SUP/FG	NAME	COUNT
J195*		metal household batteries	
J177		metal foil wrappers, aluminium foil	
J199		other metal pieces > 50cm	
J198		other metal pieces 2.5cm ≥ ≤ 50cm	
J186		metal industrial scrap	
J191		wire, wire mesh, barbed wire	
J179		metal disposable BBQs	
J193		metal vehicle parts / batteries	
J130		wheels with metal hub	
GLASS/CERAMICS			
J204		glass ceramic construction materials (bricks, tiles, cement)	
J203		glass and ceramic tableware (plates/cups/glasses)	
J207		ceramic or glass octopus pots	
J200		glass bottles	
J201		glass jars	
J208		pieces of glass/ceramic (glass or ceramic fragments ≥ 2.5 cm)	
J205		glass fluorescent light tube	
J202		glass light bulbs	
J219		other ceramic items	
J210		other glass items	
CHEMICALS			
J216		unidentified generally dark-coloured oil-like chemicals	
J217		unidentified generally light-coloured paraffin-like chemicals	
J218		unidentified chemicals	
FOOD WASTE			
J215		organic food waste	

J-CODE	SUP/FG	NAME	COUNT
ADDITIONAL DATA AND NOTES			

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Annex 3 – SOP: Microplastics

Below, Standard Operating Procedures (SOPs) from participating laboratories monitoring microplastics are collated and reproduced where possible, with links to original sources where available.

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UKCEH Standard Operating Procedures: Microplastics

This SOP is reproduced from the publicly available report to Defra WT15135 (2023) “Measurement and Characterisation of Microplastics in English River Catchment Waters and Sediments”.

URL Link to the full report: https://sciencesearch.defra.gov.uk/ProjectDetails?ProjectId=20540

To cite this SOP please refer to the following:

Defra (2023) *Measurement and Characterisation of Microplastics in English River Catchment Waters and Sediments - WT15135*. WT15135. Defra. Available at: https://sciencesearch.defra.gov.uk/ProjectDetails?ProjectId=20540

Sample collection

For the microplastics water sampling a special filtration setup is required (Figure 10A). The sampler must be flushed and conditioned with river water sample before the real sample is taken. The waste pipe is attached to the red bypass tap, the black outlet tap is closed and 2.5 L of river water is pumped to flush out and condition the system, exiting through the bypass.

For sampling, the idea is to pass 50 L spot sample over to collect ~200 µg of solids on the filter for analysis. The flow rate is monitored throughout sampling and recorded at 1-minute intervals for the duration so that the changing flow rate over the duration of each sample can be compared.

Once the sample volume has been confirmed using the inline flow meter, the sampler can be stopped, valves closed and removed from the auto sampler hose. The valve ends of the filter cartridges must be covered with foil to limit dirt ingress. Three samplers will run in parallel and capturing sample from the exact same location within the water column (Figure 10B). Sample hoses will be tied together to allow this to happen.

The opening of the inlet pipe should be approximately 50 cm below the surface of the water where possible. The inlet pipe can be marked at 50 cm as a guide. The inlet hose should be 6 mm internal diameter if sampling from height is required. This has been tested to 7 m vertical lift for bridge sampling.

All water after filtration will be pumped back into the river downstream or away from collection point.

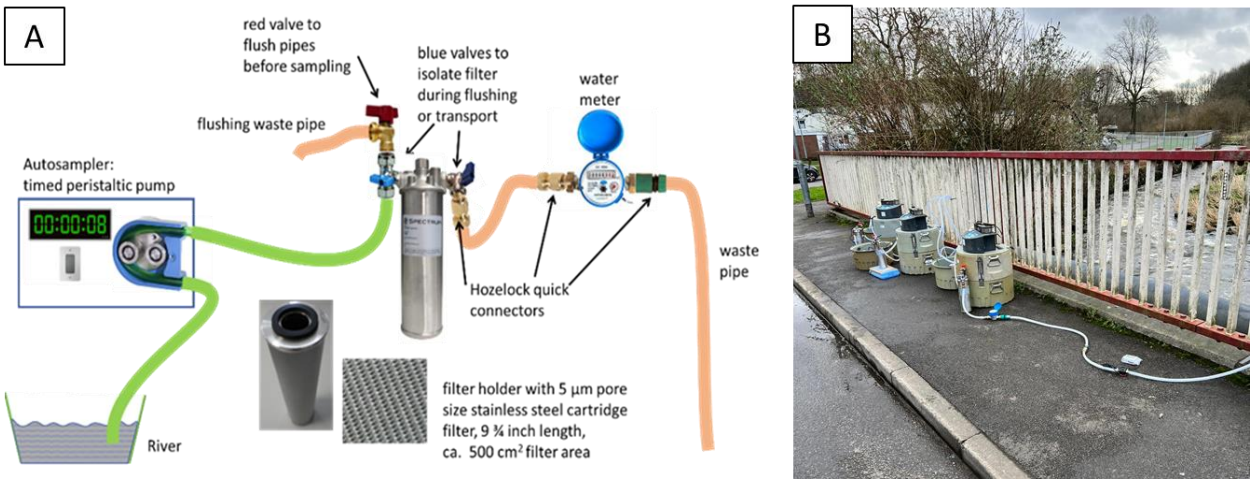

Figure 10: A) Schematic of the pumped filter sampler design used to sample river surface waters. B) an example of triplicate auto-samplers in the field collecting simultaneous replicate samples

Sample preparation

This SOP is reproduced from Defra WT15135 (2023) "Measurement and Characterisation of Microplastics in English River Catchment Waters and Sediments".

URL Link to the full report: https://sciencesearch.defra.gov.uk/ProjectDetails?ProjectId=20540

River water and effluent sample processing

All samples arrive from the field as stainless-steel filter cartridges. Excess water is released from the base of the cartridge and the filter is removed for further processing. Solids were removed from the filter by thorough rinsing with 0.7 µm GF/F filtered DI water and natural hair brush. Approximately 1 L of sample was collected from the filter and stored in a glass beaker. The sample then underwent a Fenton's reaction to break down any organic matter. The Fenton's reaction was left to exhaust for 20 hrs, before being acidified. The samples were then concentrated onto a 5 µm mesh steel filter and submerged in GF/F Filtered 2% HCl for 24 hrs before being 100% deposited on 3 µm silver nitrate filters for µ-FTIR analysis. The use of the Fenton's reaction proved to be effective on the river water samples, however the following issues were observed; 1. A significant fine mineral residue appeared to overload the silver nitrate filter during deposition, 2. The 1 L sample was difficult to work with and reduced the effectiveness of the Fenton's reaction by diluting the reagents. To improve the efficiency of the process and the standard of the final deposited sample, the processing method was refined for trial two. Samples from trial two were removed from the filter using the same method as trial one, however the sample was immediately concentrated on a 5 µm filter and transferred to a 150 mL glass beaker. The samples then underwent the Fenton's reaction, which was much more vigorous than Trial 1 due to the concentration of the reagents. The Fenton's reaction was then acidified and the sample was once again concentrated on the same 5 µm filter before being submerged in 2% HCl for 24hrs. After submersion in 2% HCl, an acid washing stage was added to the process. Samples were concentrated on a 5 µm steel filter to remove the acid and new clean 2 % HCl was flushed through the filter, washing any mineral particles < 5 µm through the steel filter. This washed sample was then washed from the steel filter with 0.7 µm GF/F DI water and 100% deposited.

Sample analysis

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µ-FTIR image analysis

Detection of microplastics and identification the polymer composition is performed by spectroscopic µ-FTIR analysis. The processed sample, suspended in 50% ethanol for storage is deposited onto a 3 µm silver membrane filter. For the cleaner water samples the ambition is for the complete sample to be deposited, however if this results in overloading of the filter, a subsample may be deposited, or the sample may be deposited across several filters. For the sediment samples it is expected that only a sub-sample may be deposited. The proportion of sample represented under the FTIR is calculated from the weighed mass before and after depositing for analysis. The analysis using the Perkin Elmer Spotlight 400µ-FTIR spectrometer will be conducted over a 11 x 11 mm area at a 8 cm^{-1} resolution using 2 accumulations (i.e. four scans per spectra) at 25 µm pixel resolution, and an interferometer speed of 2.2 cm/s. Scanning and this resolution gives a trade-off between mapping time and spectral quality. Under these settings, a single sample takes ~1.5 hours to analyse. Scans from 4000 cm^{-1} to 700 cm^{-1} wavenumbers, cover the main diagnostic areas within the FTIR spectrum. All the generated spectra are analysed using the freely available siMPle software (http://simple-plastics.eu). Spectra are matched against an expanded polymer database of Primpke *et al.* 2018.

University of Birmingham Standard Operating Procedures: Microplastics

University of Birmingham, UK 21st January 2025

Dr Mohamed Abdallah: m.abdallah@bham.ac.uk

Professor Luisa Orsini: l.orsini@bham.ac.uk

University of Birmingham, UK

Microplastics analysis in water and wastewater samples

Sample Collection

The *Daphnia*-based technology was tested in an open flow prototype holding 2.7m³ of secondary treated wastewater at the Spernal Wastewater Treatment Plant owned by Severn Trent Water (UK). The trial lasted 12 months covering both autumn-spring and spring-summer periods. Water samples were collected twice a week in triplicate at the inlet and outlet of the prototype to quantify removal efficiency. This approach guarantees that any variation in flow rate and water quality that may have influenced the input of contaminants is controlled and accounted for when measuring removal. Microplastics removal was quantified between December 2023 and March 2024. Water samples were stored in cold and dark conditions at the wastewater plant and collected once per month to be transferred to the University of Birmingham where they were analysed as follows.

Sample preparation

Non-plastic materials were used in all steps. The outside of the glass sample bottles were wiped (Kimtech™ plastic-free wipes, Fisher Scientific®, UK) with ethanol (HPLC grade, Merck™, Dorset, UK) five times before opening the bottles. The confined workspace was cleaned with ethanol every day and was kept covered with clean aluminium foil changed daily. Filtration equipment were cleaned before and after sample filtration with soap solution in Milli-Q water (18 MΩ.cm – Milli-Q® EQ 7000 Ultrapure Water Purification System, Merck™, Dorset, UK), followed by ethanol then thoroughly rinsed with Milli-Q water, before drying in a plastic-free oven. All sample preparation steps, were performed in a clean room with separate ventilation and under a clean laminar flow fume hood (Air Science® Technologies Ltd. Merseyside, UK), used only for MPs water analysis.

In the current study, water samples were treated using the same protocol according to the guidelines recommended in a recent critical review (Sol et al., 2023) and adopting the method reported by Mukotaka et al. (2021). Briefly, each five samples were analysed together with one blank containing Milli-Q water (18 MΩ.cm) treated as a sample. Sample extraction was performed under the laminar flow hood via vacuum filtration (Rocker® 300 vacuum pump, Thames Restek™, High Wycombe, UK). Water samples were vacuum-filtered through an inorganic silver membrane filter (Sterlitech®, 0.45 µm pore size) housed in a 100% borosilicate glass filter holder kit (Millipore® All-Glass filter holder kit, Merck™, Dorset, UK). Sample bottles were rinsed thrice, with 10 mL Milli-Q water (18 MΩ.cm) each, with the rinse water

passed through the same filter. The filter was then carefully placed on a glass petri dish (Fisher Scientific®, UK), containing 250 µL of hydrogen peroxide (30% w/v, Merck™, Dorset, UK) to digest any natural organic matter on the filter. The digestion step was performed at 60 °C for 24 hours.

Instrumental analysis

Microplastics analysis was conducted using a Perkin Elmer Spotlight™ 400 Fourier-Transform InfraRed microspectroscopy (µ-FTIR) imaging system with remote-controlled stage, coupled to a Spectrum-3™

FT-IR Spectrometer. The system is equipped with SpectrumIMAGE™ and Spectrum MultiSearch™ software. The whole filter was imaged and mapped in reflectance mode. Spectra produced were compared to those from the Perkin Elmer Microplastics library and/or the independent software tool (siMPle®), using a 70% match threshold and visual peak diagnostics to ensure 'best fit'. Spectra were acquired in the wavenumber range: 4000–600 cm−1 with a resolution of 8 cm−1 and accumulation of 16 scans through an aperture size of 20 x 20 µm. If the absorption spectra from 2850 to 3000 cm−1, which derives from C-H vibrational stretching, did not appear, the particle was not an organic compound, i.e., non-plastic, when verified against the library spectrum, and excluded from the MPs count (Harley-Nyang et al., 2022, Mukotaka et al., 2021). The number, shape, and size of the MPs on each filter were determined using microscope images taken by the µ-FTIR microscope in imaging mode, with point mode used for verification of MPs at or near the LOD of 10 µm in size.

Quality assurance and quality control

Conducting studies on microplastics requires avoiding background contamination by MPs in the laboratory. Only pure cotton lab coats and nitrile gloves were worn during the whole experiment process. All sample processing was carried out on the laminar flow bench, which was checked regularly and wiped down with 90 % ethanol. Prior to use, all laboratory equipment/consumables were rinsed thoroughly with Milli-Q water.

Water samples were analysed in baches of 5, to check if sample contamination happened during the sample preparation and filtration, one blank sample (Milli-Q water in glass bottle) was analysed alongside each batch. Moreover, one recovery sample (comprising Milli-Q water spiked with a known number of PE MPs in a glass bottle) was analysed alongside each 20 samples to ensure good recovery of MPs and no interference/loss during sample preparation steps. Results of the blanks and recovery samples are provided in table SI-1. In summary, none of the blank concentrations exceeded 5% of the average MPs concentrations in the respective batch. Therefore, no blank correction was required. The recoveries of MPs in the recovery samples ranged between (80 – 112 %) indicating good performance of the analytical method (Table SI-1).

Table SI-1: Results of QA/QC samples for water analysis Sample batch no.

	Blank (MPs/Sample)	**Recovery sample (MPs/sample)**	**Recovery (%)**
Batch 1	2	24	96
Batch 2		1	
Batch 3		3	
Batch 4		3	
Batch 5	2	27	108
Batch 6		0	
Batch 7		0	
Batch 8		2	

Batch 9	0	20	80
Batch 10		2	
Batch 11		1	
Batch 12		0	
Batch 13	0	28	112
Batch 14		2	

References

MUKOTAKA, A., KATAOKA, T. & NIHEI, Y. 2021. Rapid analytical method for characterization and quantification of microplastics in tap water using a Fourier-transform infrared microscope. *Science of The Total Environment,* 790**,** 148231.

SOL, D., SOLÍS-BALBÍN, C., LACA, A., LACA, A. & DÍAZ, M. 2023. A standard analytical approach and establishing criteria for microplastic concentrations in wastewater, drinking water and tap water. *Science of The Total Environment,* 899**,** 165356.

Wasser 3.0 Standard Operating Procedures: Microplastics

W30's protocols for the collection, preparation, and analysis of MPs can be obtained at the following link:

https://wasserdreinull.de/en/offers-and-services/manuals-for-microplastic-analytics/

- Sample Collection at WWTP using the Particle Sampling Unit (PSU)
- Sample Collection (Surface Waters) using the PSU
- Sample Preparation (WWTP samples)

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Annex 4 – Microplastics Sample Collection Record Template v1.0

Adapted from the quality score framework, Koelmans et al., 2019.

Note that in the finalised template there will also be a column to capture the methodology details that support the justification for the quality scores. In this way there is a harmonised record in which all relevant data to describe the sampling campaign and the sample collection, preparation and analysis methods followed.

			Score				
	Reporting criteria	**Criteria ID**	**2**	**1**	**0**	**Supporting information**	**Justification**
Sample collection	Sample method	1	**Surface & Ground water:** - Pump - Location - Materials used - Date - Depth of sampling **WWTP/DWTP:** - Location - Treatment - Date - Sampling method - Materials used	The study reported only a subset of the required characteristics (e.g., date, location, materials used), however is still fairly reproducible.	No/ insufficient reportage of sampling methods.	*Here you add all relevant data against the reporting requirements for this criteria. For example, for WWTW you could report:* *Campaign: details of what the purpose of the sampling campaign is so that data can be linked to hypothesis* *Location: coordinates* *Treatment: name technology/process step(s)* *Date: confirm whether the data sheet contains dates for all samples reported on(yes/no)* *Sampling method: short description/confirm yes/no if details in "3. Method statement"* *Materials used: e.g. pumped filtration over 5um stainless steel filter*	*Describe why you have scored yourself 2, 1 or 0.* *e.g. 2 - all criteria reported on and values stated in column g* *1 - I don't know one of the criteria e.g. depth of sample taken* *0 - I only have data for <50% of the reporting criteria*

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	Sample size	2a	**Surface & ground water:** > 500 L if targeting particles >300 µm. **WWTP if targeting particles >300 µm:** - Influent: 1L - Effluent: >500 L or until sieve clogging *Sample volume may be smaller if statistically justified e.g. if targeting only smaller particles <300 µm. Data should be caveated that sample volumes may be insufficient to capture larger particles e.g. >300 µm.*	**Surface water:** < 500 L "with good cause" e.g. high concentrations expected or only small abundant particles <100 µm targeted, score 1 if RSVP tool is not used to justify statistically Trawls without reporting volume is acceptable. **WWTP:** If insufficient volume, sampling till clogging	**Surface water:** < 500 L (if no justification provided) **WWTP:** Insufficient sampling volume (if no justification provided)	*Here you add the sample volume you took (if all consistent) or the minimum and maximum if a range of sample volumes taken. If this is below the recommended threshold volumes, then a justification will be required to score 2 or 1.*	*Justification is met either for the standard recommended volumes listed to the left, or if lower volumes are used, these must be justified statistically where appropriate e.g. using the RSVP tool.* *Sample volume may be smaller if:* *1. target microplastic sizes are smaller i.e. <100 µm (e.g. Sturm et al., 2024)* *2. Concentrations expected to be higher than typical* *RSVP tool can be used to justify tailored sample size. Copy of the tool output (Excel file) should be supplied alongside the Sample Collection Record. If sample volume collected meets the statistical requirement of the tool, then this can score 2 in the quality score.*
	Sample intervals	2b	For annual monitoring, 2-4 samples per month across 12 months	Standards partially met, for example: - For annual monitoring, monthly sampling across 12 months - Repeat sampling but not carried out for a full 12-month period to capture complete seasonal cycle	No repeat sampling.	*Here, document the number of repeat sampling events, the interval between them and the total duration of the sampling campaign*	*To justify, follow the criteria to the left.* *If fewer or more intermittent sampling intervals are used, this can be justified if it is demonstrated that the sampling interval was sufficient statistically for the purpose of the campaign.*

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	Sample processing and storage	3	Sample storing shortly after sampling; any sample handling was avoided before arriving in the laboratory. Sample containers should be rinsed with filtered water. Sample preservation with chemicals should be justified and evaluated for compatibility. Manta trawl nets are allowed to be rinsed with unfiltered water. Sieving in the field is acceptable if sample volume is large. Precautions should be taken to prevent contamination (See additional guidance in D1.1) Field blanks should be run and documented, and contamination controls should be followed	Standards only partially met or containers are pre-rinsed with samples. Citizen science approach with validation	Samples are handled outside. Storage not mentioned. Citizen science approach without validation	*Here document how you meet the requirements to score 2, and identify any criteria which are not met*	*To justify, follow the guidance of reporting requirements listed to the left.* *Additional field controls for contamination should be followed and reported (see details for best practice in section on field blanks and contamination controls)*

Sample preparation	Lab prep	4	Cotton lab coat or non-synthetic clothes Equipment and lab surfaces wiped and rinsed	Solely wiping laboratory surfaces and equipment or not wearing a lab coat IF negative samples were run in parallel and examined for contamination.	No precautions	*List which control measures were followed and which were not*	*To justify, follow the guidance of reporting requirements listed to the left.* *For additional guidance refer to guidance on "contamination controls during sample preparation".*
	Clean air	5	Clean room or laminar flow cabinet	Mitigation of airborne contamination by carefully keeping samples closed as much as possible IF negative samples were run in parallel and examined for occurring contamination.	No regard of airborne contamination, or solely use of *fume hood.*	*List which control measures were followed and which were not*	*To justify, follow the guidance of reporting requirements listed to the left.*
	Negative controls	6	Controls (minimum in triplicate) treated and analysed in parallel to actual samples. Sample concentrations need to be reported accounting for controls.	Insufficient form of a control, e.g. the filtration of air, or the sole examination of petri dishes/ soaked papers placed next to the samples.	No negative controls	*List which control measures were followed and which were not*	*To justify, follow the guidance of reporting requirements listed to the left.* *For additional guidance refer to "Process blanks"*

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	Positive controls	7	Controls (minimum in triplicate) with an added amount of microplastic particles treated the alongside the samples, and for which the particle recovery rates are determined.	Insufficient form of a positive control (e.g. if only a part of the protocol is tested).	No positive controls	*List which control measures were followed and which were not*	*To justify, follow the guidance of reporting requirements listed to the left.* *For additional guidance refer to "Process recovery"*
	Sample treatment	8	Digestion of complete sample using a protocol with KOH, WPO and/or enzymes. If another chemical was used, effects on different polymers should be tested before application. All sample treatments need to be carried out below 50°C to prevent any damage to microplastics.	If proof is missing that polymers are not affected by protocol (e.g. heated KOH) OR in case studies exclusively focus on the bigger microplastics by sieving the samples (mesh size ≥ 300µm). If WPO is carried out without cooling.	No digestion of the sample	*List which control measures were followed and which were not*	*To justify, follow the guidance of reporting requirements listed to the left.*

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Sample analysis	Polymer ID	9	**Per study:** Analytical technique is documented Analysis of all particles when numbers of pre-sorted particles are <100. For particle numbers >100, 50% should be identified, with a minimum of 100 particles. **Per sample:** Analysis of all particles up to a maximum of 50 particles per sample. **Per filter:** ≥25% of the surface area.	Insufficient polymer identification, potentially resulting in an unrepresentative subsample. See Cowger *et al.*, 2024; Cross *et al.*, 2025 for further guidance. Identification with SEM/EDX or other measures such as staining/or fluorescence approaches to distinguish polymer vs non-polymeric materials.	No polymer identification	*Document the analytical technique used.* *Record how many particles were detected in the sample or the range of min and max total number of particles measured.* *Record how you meet the requirements per study, per sample and per filter in columns to the left.*	*To justify, follow the guidance of reporting requirements listed to the left.* *Alternatively/in addition, the user may provide supporting justification using the RSVP tool to explain the statistical power in the data on the basis of the target number of particles analysed with confirmed polymer ID, see Table 3 in D1.1*

Annex 5 – Microplastics RSVP Tool v1.0

Full guidance on the RSVP tool is available from Cross *et al.*, 2025.

The link to this DOI is: https://link.springer.com/article/10.1186/s43591-024-00109-2

A downloadable copy of the RSVP Tool_v1.0 is available in the supplementary files for the manuscript and is available to project partners in the Task 1.1 shared file storage.

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