INSPIRE

Innovative Solutions for Plastic Free European Rivers

Deliverable 1.2
Monitoring and analysis protocols
Version 4.1

Date of Delivery: 29.11.2023

Dissemination Level: PUBLIC

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Funded by the European Union

Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Climate, Infrastructure and Environment Executive Agency (CINEA). Neither the European Union nor the granting authority can be held responsible for them. This project has received funding under grant agreement No 101112879 (INSPIRE).

Document Information

Grant Agreement	101112879
Project Acronym	INSPIRE
Project Title	Innovative Solutions for Plastic-free European rivers

Deliverable Number	D1.2
Work Package Number	WP1
Deliverable Title	Monitoring and analysis protocols
Lead Beneficiary	UCA, Partner Number 17
Author(s)	Daniel González Fernández (UCA), Ana Isabel Catarino (VLIZ), Mariana Miranda (VLIZ), Kittiphon Boonma (AIT), Tim H.M. van Emmerik (WUR), Liesbeth De Keukelaere (VITO), Stephan Wagner (FRE), Luca Muth (FRE), Marko Petelin (INFOR)
Due Date	29.11.2023
Submission Date	29.11.2023
Dissemination Level	PU[1]
Type of Deliverable	R[2]

Version 1	13.11.2023, Daniel González Fernández (UCA), Ana Isabel Catarino (VLIZ), Mariana Miranda (VLIZ), Kittiphon Boonma (AIT), Tim H.M. van Emmerik (WUR), Liesbeth De Keukelaere (VITO), Stephan Wagner (FRE), Luca Muth (FRE), Marko Petelin (INFOR)
Version 2	22.11.2023, Daniel González Fernández (UCA), Ana Isabel Catarino (VLIZ), Mariana Miranda (VLIZ), Kittiphon Boonma (AIT), Tim H.M. van Emmerik (WUR), Liesbeth De Keukelaere (VITO), Stephan Wagner (FRE), Luca Muth (FRE), Marko Petelin (INFOR)
Version 3	27.11.2023, Daniel González Fernández (UCA), Ana Isabel Catarino (VLIZ), Mariana Miranda (VLIZ), Kittiphon Boonma (AIT), Tim H.M. van Emmerik (WUR), Liesbeth De Keukelaere (VITO), Stephan Wagner (FRE), Luca Muth (FRE), Marko Petelin (INFOR)
Internal review	28.11.2023, Gert Everaert (INSPIRE Coordinator, WP7 Lead) and George Triantaphyllidis (WP2 Lead)
Version 4	29.11.2023, Daniel González Fernández (UCA), Ana Isabel Catarino (VLIZ), Mariana Miranda (VLIZ), Kittiphon Boonma (AIT), Tim H.M. van Emmerik (WUR), Liesbeth De Keukelaere (VITO), Stephan Wagner (FRE), Luca Muth (FRE), Marko Petelin (INFOR)
Version 4.1	14.02.2025, Daniel González Fernández (UCA), Mercedes Vélez-Nicolás (UCA), Miguel Jorge Sánchez-Guerrero-Hernández (UCA), and Ana Isabel Catarino (VLIZ); review based on external reviewer's feedback

Citation: González-Fernández, D.; Catarino, A.I.; Miranda, M.N.; Vélez-Nicolás, M.; Sánchez-Guerrero-Hernández, M.J.; Boonma, K.; van Emmerik, T.H.M.; De Keukelaere, L.; Wagner, S.; Muth, L.; Petelin, M. (2025). Deliverable 1.2: Monitoring and analysis protocols. Version 4.1. University of Cádiz (UCA): Cádiz. 48 pp. https://dx.doi.org/10.48470/101

[1] Dissemination level: **PU:** Public, information as referred to in European Commission Decision 2015/844

[2] Type of deliverable: **R: Document,** Report

Acronyms

Acronym	Description
DMP	Data Management Plan
EMODnet	European Marine Observation and Data network
FRE	University of Fresenius
INSPIRE	Innovative Solutions for Plastic Free European Rivers
JRC	DG Joint Research Centre of the European Commission
RLDB	Riverine Litter Database
UNEP	United Nations Environmental Programme
UCA	Universidad de Cádiz (University of Cádiz), Spain
VITO	Vlaamse Instelling voor Technologisch Onderzoek (Flemish Institute for Technological Research), Belgium
VLIZ	Vlaams Instituut voor de Zee (Flanders Marine Institute), Belgium
WP	Work Package

Executive Summary

This deliverable includes the monitoring protocols that will be used within the implementation of INSPIRE to acquire field data under Work Package 1 (monitoring riverine litter): Tasks 1.2 (microplastic monitoring) and Task 1.3 (meso- and macroplastic monitoring). Field data collection will take place in the use cases described in Work Package 2 for the rivers Scheldt, Po, Douro, Savinja, Rhine and Danube. The monitoring protocols described in this deliverable include sampling and analytical methodologies and are organized according to litter target size (micro-, meso- and macrolitter), and riverine environmental compartment (water surface layer, water column, riverbed sediment and riverbank). The analysis of microlitter is focused on microplastics and tyre wear particles.

Currently, there is a lack of harmonization in the existing monitoring approaches used by the scientific community and the authorities in charge of riverine litter assessment, sometimes lacking well-defined protocols for the sampling of certain litter (and plastics) size ranges and environmental compartments. The selection of monitoring protocols for INSPIRE has considered the state-of-the-art in methodological details for litter monitoring and analysis by considering existing guidance documents, scientific literature, and partners' expertise. The goal of this selection of protocols is to enable assessing baselines levels of litter contamination in each of the INSPIRE use cases, and setting the basis to assess the performance, in terms of efficiency, of the technologies deployed under Work Package 2. The monitoring protocols include the expected outcomes from the acquisition and analysis of field data.

The INSPIRE monitoring approach consists of sampling and analysis protocols, and of a monitoring plan adjusted for the different INSPIRE solutions (technologies and actions) implemented in each use case. A strategy has been defined for the development of the individual monitoring plans, targeting the specificities of the different use cases. A list of overall monitoring goals and general criteria are considered in the elaboration of the monitoring plans. Also, practical considerations are defined for each goal, litter target size, location of the use case and methodologies in place. Finally, a data management proposal is briefly described in relation to the INSPIRE Data Management Plan.

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1. Objective

The objective of this deliverable is to select and describe the monitoring protocols to be used by INSPIRE partners in Tasks 1.2 (microplastic monitoring) and Task 1.3 (meso- and macroplastic monitoring). The monitoring protocols have been selected according to existing guidance documents, scientific literature, and partners' expertise. The monitoring approaches are defined to enable assessing baselines levels of litter contamination in each of the INSPIRE use cases. Furthermore, this deliverable sets the basis for the requirements of the monitoring methodologies that will be used to assess the performance, in terms of efficiency, of technologies deployed under WP2 and further actions implemented within INSPIRE.

2. Background

At European scale, the DG Joint Research Centre (JRC) of the European Commission published a first report on 'Riverine litter monitoring - options and recommendations' back in 2016 (González et al., 2016), when no harmonized methods where in place for plastic monitoring in freshwater environments and field data was very limited in European rivers (mostly focused on microplastics in river and/or lake). Since then, new monitoring approaches have been implemented (e.g., for floating macrolitter assessment) and data collection has increased, spatially and temporally. In 2020, the United Nations Environmental Programme (UNEP) published a report on 'Monitoring plastics in rivers and lakes - guidelines for the harmonization of methodologies'(Wendt-Potthoff et al., 2020), where more recent knowledge gathered at global scale has been put together. However, despite the efforts and recent developments, it is clear that data collection is still not harmonized, and no official monitoring programmes have been defined or implemented in most cases. For example, in Europe, despite the importance of riverine plastic inputs to the marine environment, the Marine Strategy Framework Directive (MSFD, 2008/56/EC) cannot be in charge of monitoring such inputs, and at the same time, the Water Framework Directive (WFD, 2000/60/EC) has not implemented any approaches to ask Member States to monitor litter in freshwater aquatic environments. Therefore, most data collection is performed by scientific research projects and citizen science initiatives, using an array of methodologies that, on many occasions, do not provide comparable data. There are still data and knowledge gaps hindering the calculation of riverine litter baseline levels and assessment at large scale.

In addition, the implementation of solutions, i.e., use of technologies and actions, for the detection, collection, and prevention of plastics in riverine systems has not been assessed quantitatively. The performance of existing commercial solutions, like floating booms, barriers and others, is unknown and no efficiency assessments have been published except in rare occasions. Such assessments require a field data collection before, during and after the implementation of the solutions to allow defining litter baselines and identifying potential changes derived from application of such solutions.

In the framework of INSPIRE, it is necessary to establish monitoring approaches in each use case location that enable both defining the baseline levels in rivers and assessing the performance of the different technologies and actions to be implemented in the set of solutions included by INSPIRE.

3. Methodology

In this deliverable, we have examined existing monitoring approaches, sampling methodologies and analytical techniques to select the most suitable protocols for environmental monitoring of litter and plastics in rivers, inland and transitional waters, which will be used in assessing the baselines levels of litter contamination in European river basins and the performance of Technologies deployed under WP2. The project INSPIRE monitoring approach consists of sampling and analysis protocols, and of a monitoring plan adjusted for the different INSPIRE solutions (technologies and actions) implemented in each use case.

The existing international riverine litter monitoring guidelines (JRC and UNEP) provide the basis to select approaches for monitoring of micro- (<5mm), meso- (5-25 mm) and macrolitter (>25 mm) in aquatic compartments: floating and suspended in water, sediments and riverbanks. In addition, the state-of-the-art in methodological details for plastic monitoring and analysis have been followed in other projects at EU-level (e.g., H2020 EUROqCHARM, JPI Oceans ANDROMEDA, H2020 MONOCLE and GESAMP practices). Finally, the expertise, experience, and laboratory capabilities of the WP1 partners, along with the literature review, were crucial in the selection of fit-for-purpose methodologies to fulfil the INSPIRE objectives. Environmental compartments and selected methods are listed in Tables 1 and 2.

The general strategy to develop the monitoring plans is described: the technologies and actions to be implemented in each use case have been reviewed to define the corresponding monitoring protocols (sampling and analysis protocols) and the monitoring plans to be implemented in further steps of INSPIRE.

Box 1. Definition of microlitter and microplastics

Please note that, throughout this document, the terms 'microlitter' and 'microplastic' will be used with distinct meanings, depending on the procedure and on the sampling and analytical stage. To clarify the distinction between both terms, the following definitions* are considered:

Microlitter: Defined according to the European Commission Decision 2017/848 (2017) and UNEP, 2022: "Marine litter is any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment" (UNEP, 2022). Therefore, microlitter is any litter item with its maximum dimension (length) under 5 mm. Microlitter includes microplastics and human-made particles from different materials such as rubber, natural (cellulose-based or animal-based) fibres, glass, metal, tar, among others.

Microplastics: While there is currently no comprehensive European regulation specifically regulating microplastics, microplastics are generally defined as small artificial polymer particles, typically less than 5 mm in size. Microplastics are persistent, highly mobile, and difficult to remove from the environment. They can constitute a portion of microlitter.

Tyre wear: Tyre wear particles are often referred as being part of microplastics because of their mix of natural and synthetically produced rubber compounds. Tyre wear is mostly found as hetero-aggregates of tyre and road wear particles.

**While some of these definitions relied on scientific and regulatory efforts made for the marine environment, the concepts are valid for other environments such as freshwater ecosystems.*

4. Microlitter Monitoring protocols

The target of the microlitter monitoring protocols are particles < 5mm (Arthur et al. 2009), mainly microplastics and tyre and road wear particles. The study of microlitter in rivers, and other inland and transitional waters, considers different monitoring methods depending on the environmental compartment targeted for sampling (Table 1).

Table 1. Riverine and estuarine environmental compartments and corresponding monitoring methods to be applied for microlitter sampling.

	Task	Environmental compartment	Monitoring method
Microlitter	T1.2	Water surface layer	Manta net
		Water column	FerryBox
		Sediment	Grab samplers and corers
		Riverbank	Metal spoons and shovels
		Technology assessment	FerryBox

Sampling at the demo sites will be performed by the local partners (demo site leaders). VLIZ will provide training for harmonization and comparability, prior to the execution of the monitoring plans.

The sampling and analysis of microlitter require some general quality control and quality assurance measures that should be considered to avoid microplastic contamination throughout sampling and processing procedures:

- Wearing of a cotton lab coat is highly recommended. If that is not possible, observers should note down the colours of the clothes of everyone in their vicinity.
- Every piece of equipment, glass- and metalware should be thoroughly washed with tap water and with MilliQ-water (or filtered distilled water) prior to use, in particular any container in direct contact with environmental samples (water or sediments).
- The exposure of samples to air should be minimised to avoid airborne microplastic contamination (e.g., fibres).
- It is highly recommended to include procedural blanks and controls in the throughout the sampling and processing procedures to account for particles' contamination (e.g., dust (plastics) particles fallout). For example, this may include working in laminar flow hoods or clean air cabinets for specific tasks.
- Ensure that the work is done in a clean environment, with designated areas for sample preparation and analysis, and that the observers have undergone adequate training in processing samples for microlitter extraction and quantification.

The MSFD Technical Sub-group on Marine Litter provided a summary table to describe the elements used to manage the microlitter information with specifications for microplastics (Tables 2 and 3) in the Guidance on Monitoring of Marine Litter in European Seas (Galgani et al., 2013). During the

implementation of Task 1.1, INSPIRE will align the MSFD requirements with the vocabularies developed by EMODnet for data acquisition and reporting[3].

Table 2. Physical characteristics and description for microplastics appearance [extracted from Galgani et al. (2013)].

Categorization	
Size	Record size of each item. Minimum resolution is to allocate into bin sizes of 100 µm
Type	Plastic fragments, pellets, filaments, plastic films, foamed plastic, granules and Styrofoam
Shape	For pellets: cylindrical, disks, flat, ovoid, spheroids. For fragments: rounded, subrounded, subangular, angular; For general: irregular, elongated, degraded, rough and broken edges
Colour	Transparent, crystalline, white, clear-white-cream, red, orange, blue, opaque, black, grey, brown, green, pink, tan, yellow

Table 3. Categories for microlitter particles [extracted from Galgani et al. (2013)].

Categories for microlitter < 5mm	
Plastic	Plastic fragments rounded
	Plastic fragments subrounded
	Plastic fragments subangular
	Plastic fragments angular
	Cylindrical pellets
	Disks pellets
	Flat pellets
	Ovoid pellets
	Spheroid pellets
	Filaments
	Plastic films
	Foamed plastic
	Granules
	Styrofoam
Other	(glass, metal, tar)

[3] Vocabularies used by EMODnet: Litter types: https://vocab.nerc.ac.uk/collection/H01/current/; Colour: https://vocab.nerc.ac.uk/collection/H04/current/; Shape: https://vocab.nerc.ac.uk/collection/H02/current/

4.1 Monitoring of microlitter floating in the water surface layer

The manta net device (Fig. 1) has been selected to monitor microlitter (microplastics) floating in the water surface layer. This method enables the collection of volume-reduced samples via in-situ filtering at the water surface.

The Manta net is a device originally designed to sample plankton within in the first few centimetres of the water column, in open but calm seawaters, rivers, or lakes. It has been adapted as a sampling method for microplastics as microlitter also occurs in the same compartments as plankton. Because this methodology enables the filtration of large volumes of water in a standardized way, it is widely used and available, and it is able to collect a large number of microplastics for further analysis in a cost-efficient way.

Fig. 1. Example of manta net model to be used in the sampling activities of INSPIRE (source: Hydro-Bios).

The litter size lower limit for microlitter collection by the device depends on the mesh size of the used net. In INSPIRE, the recommended mesh size for the manta net sampling is 200 µm, and no lower mesh size will be used to avoid fast clogging of nets. Apart from the different mesh sizes and net opening dimensions, the trawling duration, speed, and distance will be established to provide method harmonization and a standard sampling protocol within the various partners of the INSPIRE project. To quantify the water filtered (sample volume), a Mechanical Flow Meter should be mounted in the manta frame.

The floating microlitter sampling protocol selected for INSPIRE is currently used by VLIZ (leader of T1.2 Monitoring of microlitter) and was developed and trialled during the PLUXIN project (Bouwens et al., 2021).

Monitoring output

The sampling of microlitter in the water surface layer will provide samples for the quantification of microplastics (MPs) (and other microlitter) in relation to a volume of filtered water; it is also possible to provide concentration values based on the amount of water surface surveyed:

1. Number of MPs per volume (# particles/m^3).
2. Number of MPs per water surface area (# particles/km^2; # particles/m^2).
3. Mass of MPs per volume of (mg MPs/m^3).
4. Mass of MPs per water surface area (mg MPs/km^2; mg MPs/m^2).

In any case, it is necessary to know the dimension of the sampling device opening (e.g., net mouth) and use a flow meter to calculate surface and volume of water surveyed or measure the river current speed from a fixed location.

Concentration values will be used to estimate daily fluxes of microplastics.

Sampling protocol for manta net from boats/vessels – dynamic sampling

Sampling microlitter using manta nets deployed from small to medium-sized boats or larger vessels enables the performance of dynamic sampling (i.e., the boat moves at a certain speed performing a transect) or stationary sampling (e.g., from a pontoon or when the boat is fixed, and the river/estuary flow facilitates the current to filter the water). The following protocol describes the steps to perform a dynamic sampling activity:

1. Attach the manta net to the boat/vessel with cables and deploy it on the side of the boat (starboard). The net must be towed out of the wake zone to avoid turbulence caused by the boat.
2. The sampling (towing in the water surface) recommended time in turbid waters or waters with sediment in suspension is 5 to 10 minutes, however the time can be tested and re-adjusted for clearer waters. The exact sampling duration depends on the number of particles (including sediment and organic matter) that are accumulating in the net and cod end. Although sampling duration of more than 10 minutes should be avoided, because it can result in heavy loaded samples which are more complex to be processed and in a clogged net, longer times could be used in clear waters.
3. A water current of 2-3 knots (3.7-5.6 km h^{-1}) is recommended for towing manta nets but can be adjusted based on the recommendations of the supplier, as the dimension of the net is optimized to evacuate a certain volume of water per unit of time. Therefore, higher current speeds than those recommended by suppliers may cause overflowing at the net mouth with consequent bias in the quantification of the sampled water volume.
4. Immediately before the deployment and sampling, register the flowmeter starting value (an additional photograph of the flow meter is optional but recommended), as well as starting time and GPS coordinates.
5. At the end of each sampling activity, register the flowmeter final value together with the final sampling time and GPS coordinates.
6. Using clean/filtered water, rinse the net from outside, following the direction from the mouth to the cod end, with the assistance of a water sprayer or hose to direct particles attached to the mesh into the cod end.
7. Detach the cod end from the net and transfer it to a pre-washed metal bucket. Carefully unload the content of the cod end into the bucket. Rinse the cod sample using filtered water from the prepared clean sample recipient (1 L glass bottle with metal cap) and a sprayer.
8. The content of the bucket should then be carefully transferred with a metal funnel into a pre-washed and pre-labelled glass bottle. Any potential remaining microlitter should be collected by rinsing the metal bucket and funnel into the glass bottle. The total amount of rinsing water used cannot exceed 1 L (or below the maximum capacity of the collection bottle)
9. Manually remove large particles (debris > 1 cm) from the bucket before transferring the content to the glass bottle after carefully rinsing three times with filtered water. If macrolitter

items is spotted in a microlitter sample, items should be rinsed threefold with filtered water and stored separately. Record data on meso- and macrolitter separately.

10. Store all samples in the fridge (4 °C) until sample processing (the recommendation is a maximum of 24 h storage time).

The stage of transferring water samples from the cod end to the bucket and subsequently to the collection bottle is susceptible to airborne- and cross-contamination. To minimise the contamination risk throughout the whole sampling and analysis process, the following measures should be adopted:

a. Prewash all equipment and recipients in the laboratory prior to the sampling campaign: threefold rinsing of the glass bottles and metal caps with filtered MilliQ water followed by upside-down drying. Ensure the bottles are prepared to be used for sample collection by adding 500 mL of filtered water, by adding an extra aluminium foil seal between the bottle and cap, and by labelling two-fold with permanent labels (on the cap and the bottle itself). Bottles can be reused after an additional wash with warm tap water, and a metal abrasive sponge.
b. Rinse the manta net (including the net ring) before a second use by towing it for 1 minute through the surface water without the cod end. Other equipment such as the metal bucket, funnel, and cod end should also be rinsed with the surface water and then filtered water between samples.
c. Other measures include avoiding the use of plastic labware whenever possible, keeping track of the researcher´s clothes colours, preferably wearing white cotton laboratory coats and washing hands before handling samples.

For more detailed information, please refer to “Optimized sampling and sample processing protocol (for microplastic)” (Deliverable 4.1a of the PLUXIN project, VLIZ, Belgium) (Bouwens et al., 2021).

Sampling protocol for manta net from fixed locations – stationary sampling

During a stationary sampling, the manta net is deployed from a fixed location and use the river/estuary flow to determine the current speed. Fixed locations may include the riverbanks, pontoons, bridges, and other structures existing in inland waterways that enable deployment of the manta net in similar conditions as described for dynamic sampling. Stationary sampling from a boat/vessel is also considered as a fixed location. The sampling protocol for manta net sampling from fixed locations follows the same steps as described for dynamic sampling, but instead of being towed, the river/estuary current is used to ensure the filtration of the water.

4.2 Monitoring of microlitter suspended in the water column

The FerryBox device is an active water sampler which can be used to sample microlitter (microplastics) suspended in the water column. This method enables collection of volume-reduced samples via in-situ filtering and at predefined depths in the water column. The FerryBox sampling device is a sieving tower system which collects microplastics and other microlitter in the water column within three successive size fractions, currently (at VLIZ) > 50 µm, > 100 µm and > 300 µm (other sieves with different porosity can be used according to sampling needs) (Fig. 2). The device is semi-automated and uses the collection module made of a series of sieves connected to the FerryBox system (see example from installation in a research vessel in 2a-b). The water, which is collected via the pumping system, flows through the collection module and the volume of filtered water and flow rate is recorded using a digital water flow meter (maximum flow rate of around 11-15 L / min for more turbid (particulate matter) water and 15-20 L / min for clearer water). The FerryBox can also be used outside vessels, but it requires a power source for the pumping system (e.g., portable generator).

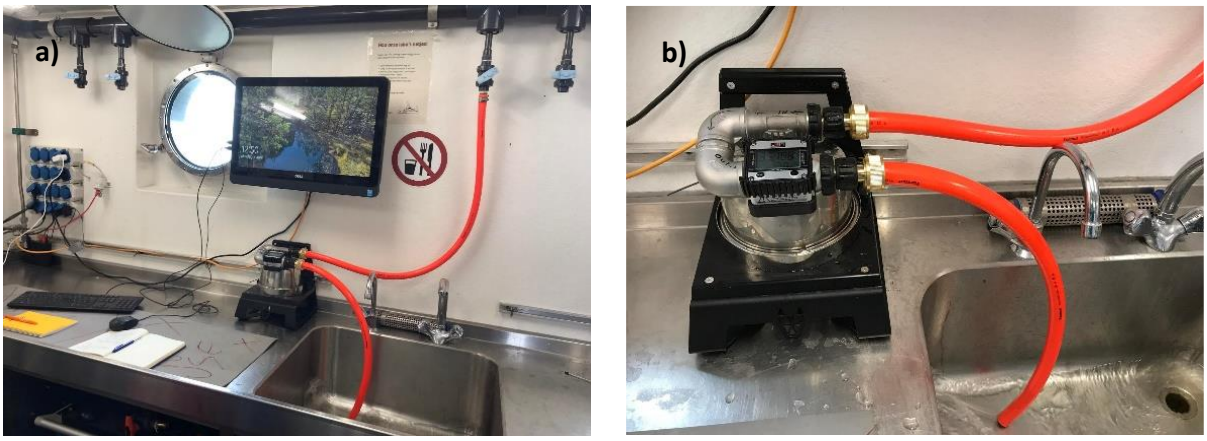

Fig. 2. a) General overview of the FerryBox device, connected to a pumping system available in a research vessel; b) Detailed image of the assembled collection module, with a connection for water input and output. Photo credit: Nelle Meyers, ANDROMEDA JPI Project (www.andromedaproject.net).

The FerryBox device can be coupled to a portable generator and used independently of a vessel, for example from a small boat, riverbank, or a fixed location over the waterways (e.g., floating docks, piers, or pontoons). The rubber tube for water input can be set to reach the selected sampling depth. The generator may have some momentaneous noise nuisance, and so the use of the FerryBox in its portable version should be avoided in protected areas, or during e.g., nesting season of protected bird species.

A Standard Operating Procedure (SOP) document is internally available at VLIZ (Meyers et al 2023, unpublished) for the sampling of microplastics with the FerryBox sampling device and can be provided in full upon request.

Monitoring output

The sampling of microlitter in the water column will provide samples for the quantification of microplastics (MPs) (and other materials) in relation to a volume of filtered water:

1. Number of MPs per volume of water (# particles/m^3).
2. Mass of MP per volume (mg MPs/m^3).

Sampling protocol for FerryBox

1. Stack the three sieves successively (upper sieve: largest mesh size, lower: smallest mesh).
2. Place the stacked sieves above the 'foot' and put the inner housing on top (see Fig. 3). Use the hex key and screws to close the inner housing, but do not force it beyond its limit.
3. Place the inner housing with sieves inside the outer housing with the pipe fitting and close it, then put the device as a whole into the table stand.
4. The upper pipe fitting should be connected to the FerryBox system (or another source of flowing water) using a rubber tube. The lower pipe should ideally be connected to a sink to drain the filtered water using another piece of rubber tubing (Fig. 2a).
5. Let water flow through the sieving system by opening the faucet at a low rate. On the upper part of the screen, you can see the volume of seawater that has been filtered already (Fig. 4). Below it you can see the flow rate. For clear (sea)water, the flow rate should be at most around 15-20 L / min to avoid rupture of the sieve mesh. For more turbid water, e.g., in an estuary, you should reduce the flow rate to around 11-15 L / min.
6. It is recommended to decide the sampling volume beforehand, and to estimate the time needed for sampling, taking into account the chosen flow rate. As an example, to filter 300 L water you will need to operate the device for around 15-20 min.
7. Make sure that throughout the sampling, you frequently verify the flow rate. If it decreases down to 1-2 L / min, it means the sieves are rapidly clogging. In this case, it is strongly recommended to terminate the sampling. If sieves keep getting clogged faster than the estimated time, you could replace to 50 µm-sieve with a 2nd 100 µm-sieve.
8. Once you have reached the desired sampling volume, close the faucet and disconnect the rubber tubing (click system).
9. Carefully open the outer housing as well as the inner housing (hex key) (Fig. 3).
10. Place a large stainless-steel funnel on top of a labelled 1 L-glass bottle and then place one of the sieves upside down on the funnel (so the sample will be facing the inside of the bottle).
11. Use a wash bottle filled with filtered water to rinse off all particles on the sieve and to collect them in the 1 L-glass bottle. Close the bottle with a metal cap and store the sample safely in a cooled, dark environment.
12. While the sieves are being rinsed off, a second stack of sieves and inner housing can be used to start sampling again in the meantime.

Fig. 3. Dissembling the FerryBox sampling device and sample collection.

Fig. 4. FerryBox sampling device with filtered volume and flow rate visible.

4.3 Monitoring of microlitter in riverbed sediments

The Van Veen grab (Fig. 5) has been selected as a suitable sampler to monitor for the presence of microlitter (microplastics) in riverbed sediments (e.g., bottom/subtidal sediment). This methodology enables the collection of bulk samples of surface sediments in inland and transitional waters.

The size of the Van Veen grab will determine the depth of the sediment sample (usually 10-15 cm) with a typical sampling area of 0.1 m^2 (but grab size can vary). For muddy sediments, the sample may maintain the sediment column structure when the grab is retrieved, allowing subsampling the top layer (2-5 cm) of sediment.

The sediment microlitter sampling protocol selected for INSPIRE is currently used by VLIZ (leader of T1.2 Monitoring of microlitter) and was developed and trialled during the PLUXIN project (www.pluxin.be/nl) (Bouwens et al., 2021).

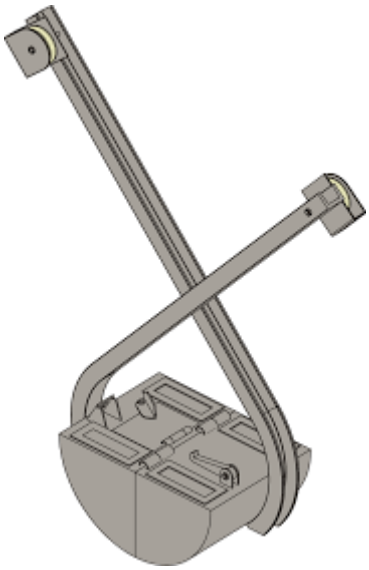

Fig. 5. Van Veen grabs illustration.

Monitoring output

The sampling of microlitter in the riverbed sediments will provide samples for the quantification of microplastics (MPs) (and other materials) in relation to a surface, volume or mass of sediment (Frias et al., 2018):

1. Number of MPs per surface area of sediment (# particles/km^2; # particles/m^2).
2. Number of MPs per volume of sediment (# particles/m^3).
3. Number of MPs per mass of sediment (# particles/kg dry sediment).
4. Mass of MP per surface area of sediment (mg MPs/km^2; mg MPs/m^2).
5. Mass of MP per volume of sediment (mg MPs/l; mg MPs/cm^3).

1. Prior to deployment, clean thoroughly the Van Veen grab by rinsing with water available at the sampling location to remove potential contamination and remains from previous sampling.
2. Deploy large Van Veen grabs from the A-frame (crane) of a vessel. From a small boat or from fixed locations (e.g., pontoon or bridge) deploy the manual Van Veen grabs manually using a (non-synthetic) rope at the sampling location.
3. Carefully register metadata such as time, GPS coordinates, water column depth, sediment type (sandy, muddy, etc.) for each deployment.
4. Repeated deployments may be needed to obtain a grab filled with sediment (limited excess of water). This depends on the nature of the sediment at surface of the bottom (sandy sediments are more difficult to collect than muddy sediments), the water current and the depth at which you aim to collect a sample.
5. To empty the content of the grab, use a pre-cleaned metal tray or bucket (washed to remove potential contamination and remains from previous sampling and rinsed with filtered water) and discard the excess of water.
6. Transfer the sediment sample (within the top cm) (using metal spoons or similar) into the sample recipients (e.g., 800 mL metal containers). Seal the pre-washed metal container with their corresponding lid (all pre-washed in the laboratory with water, rinsed with filtered water and dried). Seal the inside of the lid using a thin aluminium foil to avoid contamination of the sediment from the silicone seals.
7. Rinse the metal containers externally to remove excess of sediment and store at -20 ºC until further processing. Please consider that the containers need a sufficient air gap to enable expansion of the sample during freezing.

For more detailed information, please refer to “Optimized sampling and sample processing protocol (for microplastic)” (Deliverable 4.1a of PLUXIN, VLIZ, Belgium) (Bouwens et al., 2021) and (Frias et al., 2018).

4.4 Monitoring of microlitter in riverbank sediments

Similar to riverbed sediments, the sampling of riverbank sediments consists in the collection of samples of surface sediments. In this case, the samples are collected manually using shovels and spoons to scrap the top layer of the sediment (2-5 cm) and the material transferred into a sample container.

Riverbank sediment will be monitored in parallel with riverbank cleanups at demo sites locations where the monitoring of riverbank macrolitter is planned. The sampling of riverbank sediment shall take place when actual sediment is available for collection, and therefore feasible and suitable for the methodology in place. Some riverbanks are covered with vegetation or boulders, so sediments samples cannot be acquired.

The monitoring protocol is a modification of the methodology developed by Frias et al. (2018) for the stratified random collection of intertidal sediments.

1. In the 100 m transect (along the riverbank) selected for the monitoring of riverbank litter with manual cleanups, identify the two lines along the riverbank corresponding to 1 m above the current river water level and the upper limit (stranding line) from previous high-water level (flood period) in the riverbank.
2. Collect three random samples at each 100 m line: 3 samples at 1 m above the water level, 3 samples at an intermediate distance between the two lines defined in step 1, and 3 samples at the upper limit (stranding line) (9 samples in total).
3. Carefully register metadata such as time, GPS coordinates, sediment type (sandy, muddy, etc.) for each sample.
4. Collect the samples using quadrats 30 x 30 cm as sampling units to collect the top 5 cm of sediment (volume 4.5 l) with a metal spoon or shovel and transfer into labelled sample containers (pre-cleaned glass or metal containers).
5. Store the samples at -20 ºC until further processing.

4.5 Monitoring of tyre and road wear particles

Sampling for tyre and road wear particles (TRWPs) can be performed in a similar fashion compared to microplastic particle sampling. Depending on the applied analytical technique, meaning either microscopic and spectroscopic methods working on a single particle level or mass-based detection techniques analysing the overall content independent of particle size, different sample volumes are required depending on the sample type (Table 4). The required sample volume depends on the particle content in the sample but also the sensitivity of the analytical method and may thus deviate from the values shown in the table.

Table 4. Approximate sample sizes required for TRWPs sampling campaigns for different aqueous sample types and different analytical techniques.

	WWTP influent or storm water road runoff	WWTP effluent	Surface water
Volumes for single particle analysis	0.1 m^3	1 -2 m^3	1 -2 m^3
Volumes for mass-based detection	0.1 m^3	1 -2 m^3	> 1 m^3

Sampling of WWTP influent or storm water and road runoff may be performed using automated sampling systems that can be triggered to collect the sample in case of a rain event. The sampling of WWTP effluent or surface waters may either be performed using fractionated filtration or continuous flow centrifugation. In the latter case fractionation needs to be performed in the laboratory.

Sampling in flowing waterbodies should take place in the turbulent part of the water column. An initial single short-term sample can help to estimate the TRWPs load. Afterwards sampling can be planned accordingly and may also be performed over the span of several days. The sampling protocol considers ISO 24187:2023 (Principles for the analysis of microplastics present in the environment).

4.6 Analysis of microlitter

All microlitter samples will be analysed by VLIZ, except for tyre and road wear particles (TRWPs), which will be analysed by FRE.

Standardized analysis protocols already in used at VLIZ have been selected to fulfil the INSPIRE objectives. Such methodologies are aligned with marine and riverine litter guidance documents published by the European Commission (Galgani et al., 2013; González et al., 2016) and have been partly developed and tested within European and regional projects (ANDROMEDA, JPI; MICROFISH, CEFIC; SeaBioComp, Interreg2Seas; PLUXIN, VLAIO). In addition, the T1.2 partners' expertise (in microplastics, and tyre and road wear particles, monitoring and analysis) has been consulted and integrated.

Once the microlitter samples are received in the laboratory for analysis by VLIZ, the methodology in place for microplastics extraction includes sieving, digestion with a strong base and H_2O_2, density separation, centrifugation, and filtration, after which identification analyses are performed. Two methods for plastic identification will be used: (1) Nile red as fluorescent dye (cost-efficient AI-fluorescent based detection and characterisation), and cross-validated with (2) micro-Fourier transform infrared (µFTIR) spectroscopy (Meyers et al., 2022). The lower particle size limit for µFTIR analysis is set at 50 µm (VLIZ). Randomly selected particles will be analysed by electron microscopy to validate retained particles sizes (VLIZ).

TRWPs detection will be based on mass detection only. Chemical marker compounds for tyre wear will be detected by py-GC-MS, LC-MS and ICP-MS (particulate matter samples) by FRE. Test materials will be used for calibration and to establish a cost-efficient analytical workflow for the detection of tyre and road wear particles (FRE).

4.6.1 Extraction and analysis of microplastics

The PLUXIN project protocol for microplastic identification, characterization and quantification was developed by VLIZ and the University of Ghent (Bouwens et al., 2021). The methodology evolved from previous developments in JPI Andromeda and JPI Baseman projects and was tested and validated with spiked material. The methodology integrates fluorescence microscopy (represented by Nile red staining), image analyses and µFTIR confirmation and characterization. In case no artificial staining with a fluorescent dye is desired, a suitable alternative is offered where relevant in the protocol.

The PLUXIN protocol for microplastic extraction and analysis

The protocol follows a sequence of procedures that enable the extraction and analysis of microplastics in water and sediment samples. The procedures include:

1. Pre-cleaning and pre-washing of laboratory materials to avoid external contamination: pre-washing of glassware and cleaning of all elements and equipment used in the laboratory.
2. Working in a flow cabinet set-up to avoid external contamination.
3. Integrated first sieving and filtration – a filtration system is set up in the flow cabinet with polytetrafluoroethylene (PTFE) filters (pore size according to the microplastic size range of study). A series of stainless-steel sieves are placed in the upper funnel of the filtration system

to avoid clogging in the initial steps. This enables further subdivision into size classes for analysis if desired. If clogging happens in the PTFE filter, then several filters are used and kept in separate carefully labelled Petri dishes. The content of the sieves should be transferred to clean labelled beakers.

4. Digestion of the sample in the beakers and petri dishes can follow two procedures:
    a. Low organic matter content: hydrogen peroxide (30 % H_2O_2) and sonication.
    b. High organic matter content: 48-h digestion with potassium hydroxide (10% KOH at a 1:3 volume sample:solution ratio) on a magnetic hotplate stirrer at 60 °C and 150 rpm (using a magnetic stirring rod). The digested sample is subjected to a second digestion with hydrogen peroxide (33 % H_2O_2) minimum 48 h or until totally digested (additional 33 % H_2O_2 solution may be added).
5. Density separation – saturated salt solutions (e.g., sodium iodine 1.8 g / cm^3, sodium tungstate dehydrate 1.4 g / cm^3, or sodium chloride 1.2 g / cm^3) are used to separate plastics from sediments via flotation according to the methodology described by Frias et al. (2018). Please note that high density plastics will not float when sodium chloride is used and that samples without sediments can skip the density separation step. To perform the density separation, the sample is passed through a sieving system with the saturated salt solution and collected in a pre-washed falcon tube. The sample collected in the falcon tube is centrifuged and the supernatant is filtered to collect the floating particles. This process is repeated threefold by resuspending the pellet in the falcon tube using (the recycled) saturated salt solution.
6. Processing digested samples – the materials resulting from the digestion process in the beakers and petri dishes are filtered again through the filtration system and rinsed thoroughly with filtered MilliQ water.
7. Nile red staining and fluorescent microscope analysis - Nile red staining techniques are applied based on the procedures described in the literature (Meyers et al., 2022). Particles stained with Nile red are observed under UV and Blue fluorescence filters in a fluorescent microscope (Leica). Determination of synthetic versus natural based particles is done after using image analysis techniques (ImageJ and R software) (Meyers et al., 2022). The Feret diameter is assessed using image analysis (ImageJ).
8. The µFTIR analysis is done using a Spotlight 200i FT-IR microscope (Perkin Elmer). Particles are observed directly on the PTFE filter and spectra are captured in a transmittance mode range between 4,000 - 450 cm^{-1}, with a resolution of 4 cm^{-1} (64 scans). Final spectra are matched after running a library search on spectra, excluding the range 1,250 - 1,100 cm^{-1} where the PTFE filter may interfere.

For more detailed information, please refer to “Optimized sampling and sample processing protocol (for microplastic)” (Deliverable 4.1a of PLUXIN) (Bouwens et al., 2021).

4.6.2 Analysis of tyre and road wear particles

The analysis of TRWPs will consider three protocols implemented by FRE (one of them being currently developed), depending on the environmental compartment and analytical technique required (Table 5).

Table 5. Environmental compartments, analytical techniques, and protocols for analysis of tyre and road wear particles (TRWPs).

	Environmental compartment	Analytical Technique	Protocol number
TRWPs	Roadside	ICP-MS	1
	Water column	LC-MS/MS	2
		Py-GC-MS	3
	Sediments	ICP-MS	1
		LC-MS/MS	2
		Py-GC-MS	3

Protocol 1- Zn determination for tyre particle quantification using ICP-MS

Several sample preparation methods are presented here in the context of the Zn-determination, but density separation and size fractionation are also compatible to the other two analysis techniques (Protocols 2 and 3). The analysis consists of the following steps:

1. Density separation of TP and TRWP from particulate samples:

For the density separation procedure for tyre particles (TP), and tyre and road wear particles (TRWP), the following materials are required: analysis 50 mL centrifugation tubes, a vortex mixer, a centrifuge and an ultra-sonication bath. A sodium polytungstate solution in MilliQ-water with a density of 1.9 g / cm^3 serves as separation solution.

To begin, 1 to 2 grams of dry sample are added to the tube and filled up with 50 mL of separation solution, the sample is then shortly vortexed. The tube is then placed in an ultrasonic bath for 15 min, afterwards another 10 min of vortexing at 2000 rpm follow. Centrifugation at 3000 rpm (rcf = 1952 g) for 15 min follows. Then the samples are frozen solid at -73°C. The upper, light, fraction (upper 5 mL) is separated from the lower heavy section by thawing and rinsing it off using ultrapure water. The sample is then filtered using a 1 µm cellulose nitrate membrane filter. The samples are washed with 100 mL of ultrapure water during filtration. Finally, the sample is dried at 70 °C.

2. Sieving for size fractionation

To achieve size fractionation a vibratory sieve shaker equipped with steel sieves of the mesh sizes 20, 50, 100, 250 and 500 µm is used. 50 g of sample are introduced and are subsequently washed through the sieves with tap water. The size fraction < 20 µm is collected in a bucket. After fractionation the

particles are rinsed off the sieves with ultrapure water, the samples are then frozen at – 18°C to be freeze-dried afterwards. The fraction < 20 µm is collected by centrifugation of the bucket content.

3. Sample digestion:

Prior to element analysis, the samples are digested using microwave assisted acid digestion. To this end 6 mL of HNO3 (70 %) and 2 mL of H_2O_2 (30 %) are mixed with the sample obtained from density separation. The microwave power is set to 900 – 1500 W with the maximum temperature reaching 260 °C. The maximum pressure was set to 60 bar. After digestion, the samples are filled up to 50 mL with ultrapure water and centrifuged at 4000 rpm for 10 min.

4. Instrumental analysis:

The instrumental analysis is performed using inductively coupled plasma mass spectrometry. Using the average Zn content of a tyre particle (TP) reference material in mgZN / gTP the TP content of a sample rich in TRWPs can be approximated.

The references for this protocol are Klöckner et al. (2019) and Klöckner et al. (2020).

Protocol 2- Quantitation using organic markers and LC-MS/MS

1. Extraction of organic markers

For the extraction of organic markers 50 mg of tyre material or 100 – 200 mg of environmental samples are transferred into a glass vial. 10 mL of 2-Propanol are added, and the vial is placed inside of an ultrasonic bath for 60 min to achieve solid-liquid extraction. Afterwards the sample is evaporated to dryness under a nitrogen atmosphere at 40 °C in a water bath. 1 mL of 50/50 V/V methanol/ultrapure water is added to the dried extracts and the vial is placed in the sonication bath for 5 min to dissolve the extracts again. Prior to analysis the extracts are filtered using 0.22 µm PTFE syringe filters.

2. Quantification using LC-MS

Three possible organic markers for the quantification of TRWPs have been identified, namely N-formyl-6-PPD, QDI-OH and 6-PPDQ. A standard procedure to determine TRWP content of a sample by determining the concentrations of these markers in an extract yet needs to be developed.

The reference for this protocol is Klöckner et al. (2021).

Protocol 3- Quantification of TP using Pyrolysis-GC-SICRIT-MS

A quantification method using Pyrolysis-GC-SICRIT-MS is currently under development.

5. Meso- and macroplastics monitoring protocols

The target of the monitoring protocols included in this section are mesolitter (5-25 mm) and macrolitter (> 25 mm). Most of the existing monitoring methods for these size ranges have focused on macrolitter only and the study of mesolitter has been mostly neglected, with no harmonized approaches or standardized methods having been developed for riverine (meso)litter monitoring. Currently, most mesolitter is collected occasionally during microlitter sampling, but given the low sample size (volume or area) used for microlitter monitoring and the lower abundances of mesolitter, results can be highly uncertain for the latter, causing potential under- or overestimation. Also, sampling methods designed to monitor macrolitter may exclude the acquisition of mesolitter data by definition of protocols (e.g., visual census of only floating macrolitter > 25 mm) or provide underestimates because the retention (in nets) or counting (visual or image analysis) efficiency is negatively biased, i.e., smaller items are missed.

The study of macrolitter in rivers (and in inland and transitional waters) considers different monitoring methods depending on the environmental compartment selected (Table 6). Some of these methods can be adjusted to simultaneously monitor meso- and macrolitter, minimizing potential biases, as will be described in the following subsections.

Table 6. Riverine environmental compartments and monitoring methods for meso- and macrolitter.

	Task	Environmental compartment	Monitoring method
Meso- & Macrolitter	T1.3.1	Water surface layer	Bridge-mounted cameras
			JRC Floating Litter Monitoring app
			Floating nets
		Water column	Suspended nets
	T1.3.2	Riverbank	Riverbank manual cleanups
			EEA Marine Litter Watch app
			Drone cameras

5.1 Monitoring of meso- and macrolitter floating in the water surface layer

Three different monitoring methods have been selected to obtain complementary and comparable results for data acquisition on floating litter (e.g., for calibration purposes): (i) Bridge-mounted cameras (Section 5.1.1) enables monitoring of floating litter in an operational way for a long(er) time period. Automated litter recognition techniques will be applied on the data to derive the floating macro-plastic flux of a river segment (ii) Visual observation by human observers (Section 5.1.2) is restricted to short periods of time (30-60 mins, to avoid observers' fatigue), and can be used to identify the plastic flux in rivers, after extrapolation, or to observe floating plastic densities in stagnant water (e.g., near dams and sluices). Finally, (iii) nets are used to collect floating litter (Section 5.1.3) and enabling for an actual identification, enumeration, and measuring size and weight of litter. The performance and derived results of these three measuring techniques will be intercompared, and the applied analysing technique (e.g., automated litter recognition or extrapolation technique) can be improved based on conclusion of this intercomparison exercise.

In this section we will focus on the data acquisition part of these techniques.

Considering MSFD monitoring elements recommended for floating litter at sea (Galgani et al 2013), the protocols and reported data should consider the following information when applicable:

Table 6. Monitoring elements to be considered for the observation of floating (marine) litter [extracted from Galgani et al. (2013)].

	Monitoring elements
1	Observation height
2	Observation width
3	Observation distance
4	Observation angle
5	Ship speed
6	Wind speed
7	Sea state
8	Light conditions
9	Sun direction
10	Viewing (quality of vision eventually impaired by fog, etc.)
11	Location (INSPIRE compatible geographical coordinates)
12	Lower size range (detection limit)
13	Upper size range (detection probability)
14	Litter Categories
15	Object properties
16	Windage (protrusion from water surface)
17	Object size
18	Object shape
19	Object description
20	Object depth
21	Source relations
22	Ageing/weathering
23	Biofouling
24	Object colour

Monitoring output

The sampling of meso- and macrolitter floating in the water surface layer will provide samples and observations for the classification of litter items (top ranking lists) and for the quantification of riverine litter (and plastics) concentrations (from net sampling) and estimation of fluxes (from visual and image observations):

1. Number of meso- and/or macrolitter items per surface area (# items/km^2).
2. Number of meso- and/or macrolitter items per volume of water (# items/m^3).
3. Mass of meso- and/or macrolitter items per surface area (g/km^2; g/m^2).
4. Mass of meso- and/or macrolitter items per volume of water (g/m^3).
5. Flux of meso- and/or macrolitter items per unit of time ((# items/h; # items/day).
6. Flux of meso- and/or macrolitter mass per unit of time (g/h; g/day).

The lower litter size limit for data collection will depend on the characteristics of the monitoring site (e.g., height of the bridge for visual observations and fixed cameras), the technical specifications of camera systems (e.g., resolution) and the net mesh size used. All methods will ensure acquisition of macrolitter (>25 mm) data. Certain monitoring setups (monitoring site and technical specifications) will also allow simultaneous acquisition of mesolitter + macrolitter (<5 mm) data.

5.1.1 Monitoring floating litter with bridge-mounted cameras

INSPIRE will implement the monitoring of floating macrolitter from bridges using following camera systems:

- **RGB cameras**
  RGB cameras will be used in the operational set-up for monitoring of floating macro-plastic litter at the different demo sites. They have the advantage of low-cost, making it possible to place multiple RGB cameras at one bridge to cover the full width of the river. Combining all images collected by the different cameras of one bridge makes it possible to derive a plastic flux product (expressed in number of items per time unit). Two different RGB systems will be installed: the RLC-1212a (VITO) and pLitterCCTV (AIT).

- **Multispectral camera**
  Next to the operational RGB cameras, measurements will be taken with a multispectral camera. The spatial resolution of multispectral data is lower than RGB data, but the additional spectral information has the potential to support the discrimination between different floating matter classes. The multispectral camera selected in INSPIRE is the MicaSense RedEdge-MX Dual camera. The protocol for this system differs from the RGB set-up, as the spectral information becomes important, which means that is necessary to correct for changing light conditions.

Monitoring protocol for operational RGB bridge-mounted cameras

To be able to derive the flux of floating macro-litter at a cross section of the river, multiple RGB cameras need to be mounted at the bridge, as depicted in 6. The total number of cameras depends on:

- Field-Of-View (FOV) of the camera.
- Height of the bridge above the water level.
- Width of the river.

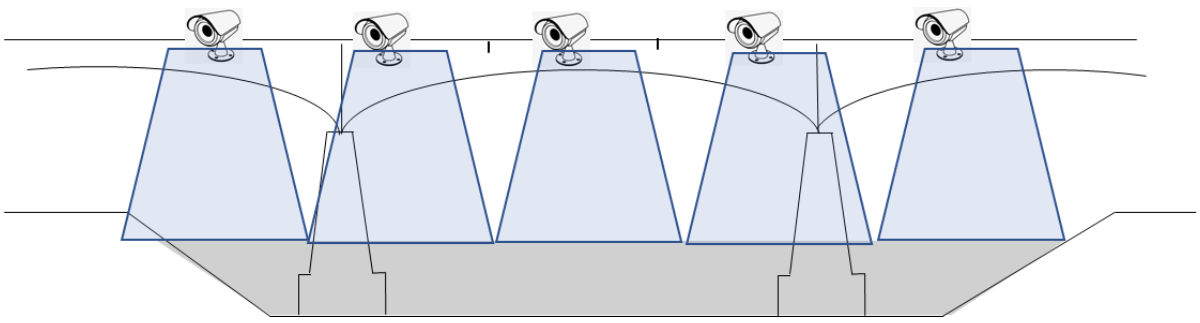

Fig. 6. Schematic representation of multiple cameras at a bridge and their field of views (FOV) depicted in blue. To be able to monitor the full width of the river, multiple cameras are required to be placed next to each other in a well-defined set-up.

It is not necessary to foresee overlapping between the different camera footprints. The number of cameras can be reduced, spacing them at equal distances but leaving gaps in between and interpolate the results to the full river section.

There are two possibilities to install the cameras on the bridge, used in the INSPIRE project:

- A stand-alone set-up. All cameras work as individual stations, relying on batteries and solar panels for power and including 4G/LTE WiFi dongle for each camera unit for internet connection.
- A connected set-up. In this configuration each camera is connected with an ethernet cable to a network PoE (Power over Ethernet) switch that provides both power and network connection towards a network video recorder. The recordings are uploaded to the processing facility through internet.

Both set-ups are discussed in this document.

Installation of the system:

Option 1: RGB camera Stand-Alone set-up (pLitterCCT set-up from AIT):

- The camera should be placed preferably at locations which are not publicly accessible to avoid theft or damage to the camera systems.
- Point the camera towards the water, as nadir as possible, but avoiding features of the bridge being part of the image.
- Avoid placing cameras on the south-side of a bridge. North, East or West sides are accepted. Pointing to the South will introduce a lot of sun glint features in the images, making it more difficult to differentiate between sun glinted areas and plastics in the water.
- The solar panel should face the sun to obtain max sun light.
- Measure the height difference between the water level and the camera.
- The minimum plastic litter size that the camera can detect in the 5m range (water surface to camera) is 35 mm (e.g., plastic-bottle caps).

A schematic of the systems is depicted in Fig. 7.

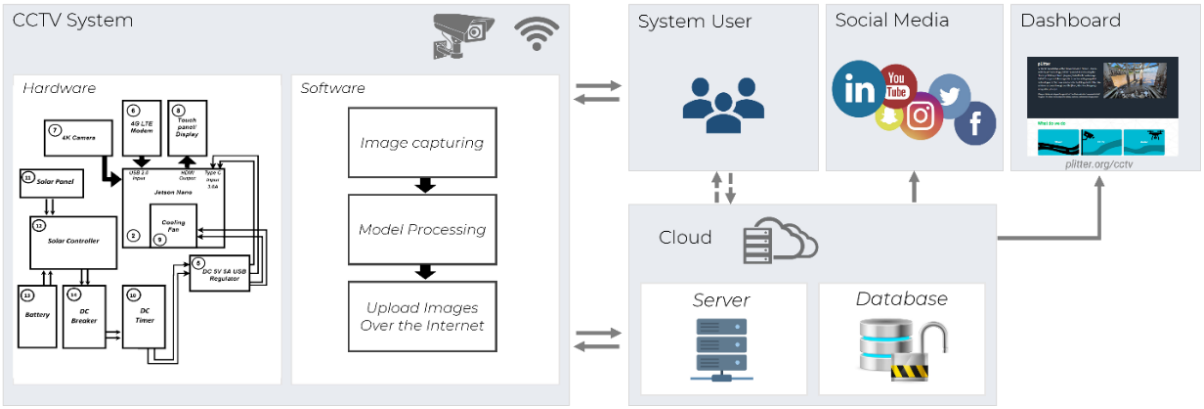

Fig. 7. System schematics of RGB stand-alone set-up.

Option 2: RGB Integrated installation (RLC-1212a set-up from VITO):

- Similar as for the stand-alone set-up, cameras should be place at locations which are not publicly accessible to avoid theft or damage to the camera system.
- Have power and internet supply available
- Look to local requirements for placing cables
- Point the camera towards the water, as nadir as possible, but avoiding features of the bridge being part of the image.
- Avoid placing cameras on the south-side of a bridge. North, East or West sides are accepted. Pointing to the South will introduce a lot of sun glint features in the images, making it more difficult to differentiate between sun glinted areas and plastics in the water.
- Look for a safe place to install the Network Video Recorder (NVR) cabin (see Fig. 8). All cameras are connected to an NVR through an ethernet connection. External hard drives can be exchanged in this cabin.
- Install an (S)FTP server for data upload to the VITO cloud network
- Measure the height difference between the water level and the camera. Optional: include an additional camera/sensor which is pointed towards the horizon, which gives an indication of weather conditions (cloudiness).
- In rivers influenced by tides: include a measuring device, if not yet available, to retrieve the water level.

Fig. 8. Cabin with NVR included. On the left the interior of the cabin is shown. The image on the right represents the exterior of such a cabin.

Operational phase:

Once the system is installed, the operational phase can begin:

- Both set-ups allow remote control of the system.
- No images will be collected during night time.
- Perform regular checks and maintenance of the system.
- If information is available on the flow rate, this information can be used as a first indicator to define the required frame rate.

Monitoring protocol for multispectral bridge-mounted cameras

The protocols for collecting multispectral data differ from collecting RGB images. When working with multispectral data, it is important to be able to convert the images to reflectance data through correction for changing light conditions. The system includes an irradiance sensor. This system will be used in a semi-flexible set-up, collecting only information during the day in presence of an observer. Fig. 9 shows the set-up of semi-flexible approach.

Fig. 9. Multispectral camera at a bridge. Left shows the side view, including a computer and power generator. Right shows the cameras from a bottom-up view.

Installation of the system:

- Find a location at the bridge:
  - Which is representative for data collection
  - Where you have the space available for your instruments
  - Where you don't hinder any passerby
- Place the camera as nadir as possible, avoiding features of the bridge entering your image.
- Avoid placing cameras on the south-side of a bridge. North, East, or West sides are accepted. Pointing to the South will introduce a lot of sun glint features in the images, making it more difficult to differentiate between sun glinted areas and plastics in the water.
- Place the irradiance sensor looking upwards. Avoid features (e.g., high trees, pillars, etc.) blocking the light from the irradiance sensor.
- Take images in .tiff file format.

5.1.2 Monitoring floating litter with JRC Floating Litter Monitoring app

The JRC Floating Litter Monitoring app (https://floating-litter-monitoring.jrc.ec.europa.eu) has been selected as a monitoring tool for floating macrolitter items (Fig. 10). The new mobile app is designed for tablet computers to monitor floating macrolitter (> 2.5 cm) in the sea and rivers. The monitoring method is based on visual observations (counting and classifying) from vantage points over the water surface (e.g., ships, bridges). The app includes a manual for users.

INSPIRE collaborates with the European Joint Research Centre (JRC) in the testing and use of the new mobile app for field macrolitter observations and data collection in European rivers. The use of this methodology enables harmonized data collection and reporting, facilitating comparability of results.

It is an accessible and low-cost methodology that can be applied in any river across Europe and beyond.

Monitoring protocol for visual observations – JRC Floating Litter Monitoring app

The monitoring sessions are performed from vantage points over the water, ideally bridges with recommended maximum height 12 m to enable the visual identification of macrolitter > 25 mm, although higher bridges have been used and reported in the literature. The observation height is determined by the distance from the observer's sight and the water surface.

The observation track width (observation strip width) is defined as the width of the river/waterway surface where the observer scans for identification of floating litter. Depending on the total waterway width and the observation height, the observation track width can cover the whole river or only a portion of a water body width. The observation track width should enable the identification of litter items > 25 mm at both side of the strip (maximum distance to the observer).

When only a portion of the river or waterbody is covered by the observer, it is possible to extrapolate results to the whole river width, but the results may be biased because the litter flux is not homogeneous across the waterways. The recommendation is to perform multiple monitoring strips to cover the whole river width, by using multiple observers simultaneously or an individual observer performing the survey sequentially (strip by strip).

In heavily polluted rivers or fast stream flows, it is recommended to work in teams of two, so one member is dedicated to identifying litter items and the other to register the data.

During the operation of the app, the observers have to fill in metadata fields and acquire GPS position before starting a monitoring session. Once a monitoring session has been started, a full list of litter items filtered by specific plastic types and general litter categories is available to classify and count the observed floating items (harmonized with the EU Joint List of Litter Categories for Marine Macrolitter Monitoring (Fleet et al., 2021)). Once an item is selected, a secondary menu pops up allow to insert additional information such as quantity, size and colour of the object and comments. The selected litter items are recorded along with time and GPS data. When the monitoring session is finished the operator has to use Stop button to access to the review page with the spotted litter list. To complete the monitoring the data has to be confirmed and uploaded to the portal with the specific button. The monitoring activities then can be consulted on the dedicated portal: https://floating-litter-monitoring.jrc.ec.europa.eu.

To use the app, the observer needs an Android device and login to it with an EU Login Account, if missing can be create from dedicated link (https://ecas.ec.europa.eu/cas/login). Monitoring sessions are stored locally in the android device (e.g., tablet or smart phone) but not accessible, it is necessary to upload monitoring sessions to the Floating Litter Monitoring data portal (check link above). Users can then manage their data files (export, import, etc.) at the data portal.

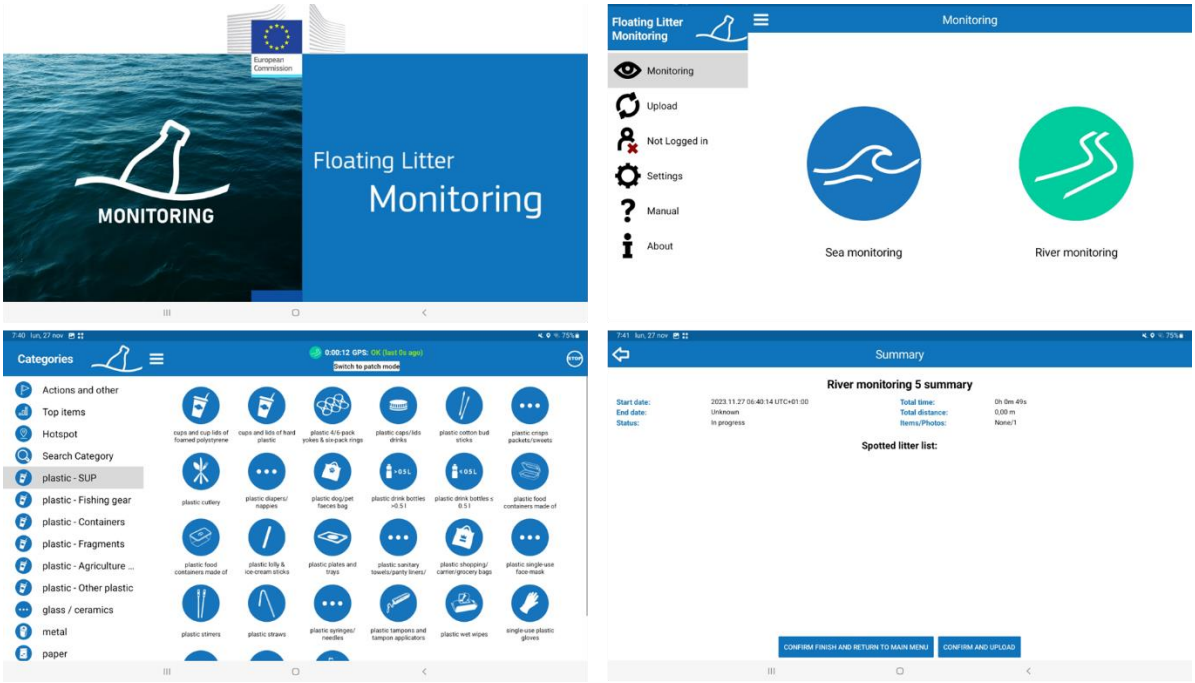

Fig. 10. The JRC Floating Litter Monitoring app and monitoring setup for visual observation of floating litter in rivers.

5.1.3 Monitoring floating litter with floating nets

Collection of litter items floating in the river surface water layer is performed by using nets with mesh sizes that target meso- (lower limit 5 mm) and macrolitter (lower limit 25 mm). Therefore, the mesh sizes are selected at a smaller size than the litter size target. Nets for meso- and macrolitter collection have rarely been used for riverine litter monitoring. However, some protocols exist and have been considered for the needs for INSPIRE (Barnardo and Ribbink, 2020; van Emmerik et al., 2018; Wendt-Potthoff et al., 2020).

Small nets 2 m x 1m are recommended for manual deployment from boats and fixed locations (bridges). Ideal mesh size for simultaneous sampling of meso- and macroplastics is 2-4 mm, and for macroplastics is 10-20 mm. the bottom of the frame of the net is

These nets may bycatch fish and other biota, so it is important to communicate with local authorities to obtain corresponding permits for research purposes.

The monitoring with nets should be use as a complementary methodology to the visual observations and fixed camera monitoring plans. Therefore, the net samples should only be acquired for comparison and calibration purposes at certain times and locations where the other methodologies are applied.

1. Consult weather and river flow conditions, communicate with authorities, avoid maritime traffic sections, check for presence of large items, tree branches, etc.).
2. Estimate water current speed using a flow meter or by measuring distance/time of floating items seen in the river (or using targets, like a piece of orange peel). Ideally, a flow meter should be installed in the net to calculate area and/or volume of water filtered.
3. In case a flow meter is used, register the flowmeter starting value just before deployment (an additional photograph of the flow meter is optional but recommended), as well as starting time and GPS coordinates.
4. Deploy the net from a boat or fixed location (bridge) using adequate ropes under safety conditions. The verticality of the net frame is adjusting by the ropes length.
5. The net deployment duration should match that of the visual observations, or be matched with a certain time frame, to obtain samples simultaneously for comparison and calibration with the other methodologies. In any case, the net deployment duration will be determined by local conditions and litter abundance to enable safety manoeuvring.
6. Retrieve the net by pulling the ropes and register the flowmeter final value together with the final sampling time and GPS coordinates.
7. In multi-strip visual observations and multi-camera installations, the net is deployed for each matching section across the river.
8. Collect all the litter items and classified them according to the EU Joint List of Litter Categories for Marine Macrolitter Monitoring (Fleet et al., 2021).
9. All litter categories are photographed (zenithally) on a flat surface with homogeneous background (e.g., concrete, tarp) using a size scale reference for image analysis.
10. All litter items are cleaned and dried up for specific size and weight measurement.
11. The numbers of litter items per categories are reported.
12. River current speed or flow meter data is used to calculate litter concentration and flux.

5.2 Monitoring of meso- and macrolitter in the water column

Similar to what has been described in section 5.1.3, collection of litter items in the water column is performed by using nets with mesh sizes that target meso- (lower limit 5 mm) and macrolitter (lower limit 25 mm).

Nets can vary in size and cover partially or totally the water column. Deployment is preferably done from boats, but small nets (2 m x 1 m) can be deployed manually from fixed locations (bridges) by adding weight at the bottom of the net framework to facilitate sinking.

Monitoring output

The sampling of meso- and macrolitter floating in the water column will provide samples for the classification of litter items (top ranking lists) and for the quantification of riverine litter (and plastics) concentrations and estimation of fluxes:

1. Number of meso- and/or macrolitter items per volume of water (# items/m^3).
2. Mass of meso- and/or macrolitter items per volume of water (g/m^3).

3. Flux of meso- and/or macrolitter items per unit of time ((# items/h; # items/day).
4. Flux of meso- and/or macrolitter mass per unit of time (g/h; g/day).

Monitoring protocol for suspended nets

1. Consult weather and river flow conditions, communicate with authorities, avoid maritime traffic sections, check for presence of large items, tree branches, etc.).
2. Estimate water current speed using a flow meter or by measuring distance/time of floating items seen in the river (or using targets, like a piece of orange peel). Ideally, a flow meter should be installed in the net to calculate area and/or volume of water filtered.
3. In case a flow meter is used, register the flowmeter starting value just before deployment (an additional photograph of the flow meter is optional but recommended), as well as starting time and GPS coordinates.
4. Deploy the net from a boat or fixed location (bridge) using adequate ropes under safety conditions. Weights are added to the bottom of the net frame to facilitate sinking. The verticality of the net frame is adjusting by the ropes length.
5. The net deployment duration should match that of the visual observations, or be matched with a certain time frame, to obtain samples simultaneously for comparison and calibration with the other methodologies. In any case, the net deployment duration will be determined by local conditions and litter abundance to enable safety manoeuvring.
6. Retrieve the net by pulling the ropes and register the flowmeter final value together with the final sampling time and GPS coordinates.
7. In multi-strip visual observations and multi-camera installations, the net is deployed for each matching section across the river.
8. Collect all the litter items and classified them according to the EU Joint List of Litter Categories for Marine Macrolitter Monitoring (Fleet et al., 2021).
9. All litter categories are photographed (zenithally) on a flat surface with homogeneous background (e.g., concrete, tarp) using a size scale reference for image analysis.
10. All litter items are cleaned and dried up for specific size and weight measurement.
11. The numbers of litter items per categories are reported.
12. River current speed or flow meter data is used to calculate litter concentration and flux.

5.3 Monitoring of meso- and macroplastics at riverbanks

Three different monitoring methods have been selected to obtain complementary and comparable results for data acquisition of meso- and macroplastics at the riverbanks r (e.g., for calibration purposes). The manual cleanup of predefined surface areas in riverbanks enables the collection of litter and registration of scientific data simultaneously. This method can be combined with the use of the EEA Marine Litterwatch app to report data in a harmonized way following the litter classification lists implemented by the European Commission for the Marine Strategy Framework Directive (2008/56/EC). This way, data collected in along rivers, inland and transitional waters can follow a common methodology, linking sources of litter from freshwater to marine environments. Furthermore, the use of drone imaging for mapping litter accumulation in riverbanks can extend the monitoring area size beyond the area covered by the manual cleanup methodology, reducing human effort in the field.

The simultaneous use of manual cleanups and the use of the EEA app will help in the calibration and training of AI models for litter recognition from drone imaging according to harmonized litter classification lists. Sampling campaigns will be designed to, first, perform image drone mapping, and second, register the manual cleanup to provide classification and quantification of litter items per sections within the riverbank monitoring section.

Monitoring output

The sampling of meso- and macrolitter in riverbanks will provide samples and observations for the classification of litter items (top ranking lists), the creation of waste maps, and for the quantification of riverine litter (and plastics) concentrations:

1. Number of meso- and/or macrolitter items per surface area (# items/km^2; # items/m^2).
2. Mass of meso- and/or macrolitter items per surface area (g/km^2; g/m^2).

The lower litter size limit for data collection will depend on the methodology applied (e.g., manual cleanup or drone imaging) and the technical specifications of drone imaging (e.g., flight height and camera resolution). All methods will ensure acquisition of macrolitter (>25 mm) data. Certain monitoring setups (technical specifications) will also allow simultaneous acquisition of mesolitter+macrolitter (<5 mm) data.

5.3.1 Monitoring riverbank litter with manual cleanups

Manual cleanup activities will be conducted by citizens. The manual cleanup protocol is defined to be aligned with the existing protocols for beach litter and riverbank litter monitoring (Galgani et al., 2013; Van Emmerik et al., 2020).

Monitoring protocol for riverbanks - Manual cleanups

1. Sampling area is selected by considering a 100 m stretch along the riverbank shore and 25 m width from the water line.
2. During the manual cleanup, all litter found in the riverbank inside the sampling area is picked up and taken to a predefine common spot for classification.
3. The litter is classified according to the EU Joint List of Litter Categories for Marine Macrolitter Monitoring (Fleet et al., 2021).
4. All litter categories are photographed (zenithally) on a flat surface with homogeneous background (e.g., concrete, tarp) using a size scale reference for image analysis.
5. A selection of representative litter items is cleaned and dried up for specific size and weight measurement.
6. The numbers of litter items per categories are reported.

5.3.2 Monitoring riverbank litter with EEA Marine Litterwatch app

The EEA Marine LitterWatch app enables the monitoring of beach and riverbank macro litter by organizing community clean-up events (as citizen science actions). The app has been converted to a web app available at https://marinelitterwatch.discomap.eea.europa.eu. The current version (2023) of the app uses the EU Master List of Litter Categories (G codes) from 2013 (Fig. 11). The list will be updated to the new EU Joint List of Litter Categories during 2024. To access all features in the app and the data portal, users must be registered and logged in their profiles.

Monitoring protocol for riverbank – EEA Marine Litterwatch app

Cleanup events are set up by the community coordinator (general public) and an event code is provided to users. Communities refer to specific locations for identification of beaches and riverbanks. Users are recommended to join a local community in their area and can start a monitoring survey by login in the app and using an event code. Performing independent surveys is also possible. However, to submit your data to the EEA data portal, you need an event code.

During the survey, each user follows indications given by the community coordinator, following the app guidelines: 'Register the litter items by typing the number of items you find during your survey, or pressing the "+" and "-". The list can be scrolled up and down. The items are organized by type of material. The survey list shows the top 20 items found in Europe´s beaches or in your community. You can add items from the full MLW list (green circle). You can also search for an item by typing its name in the search field.

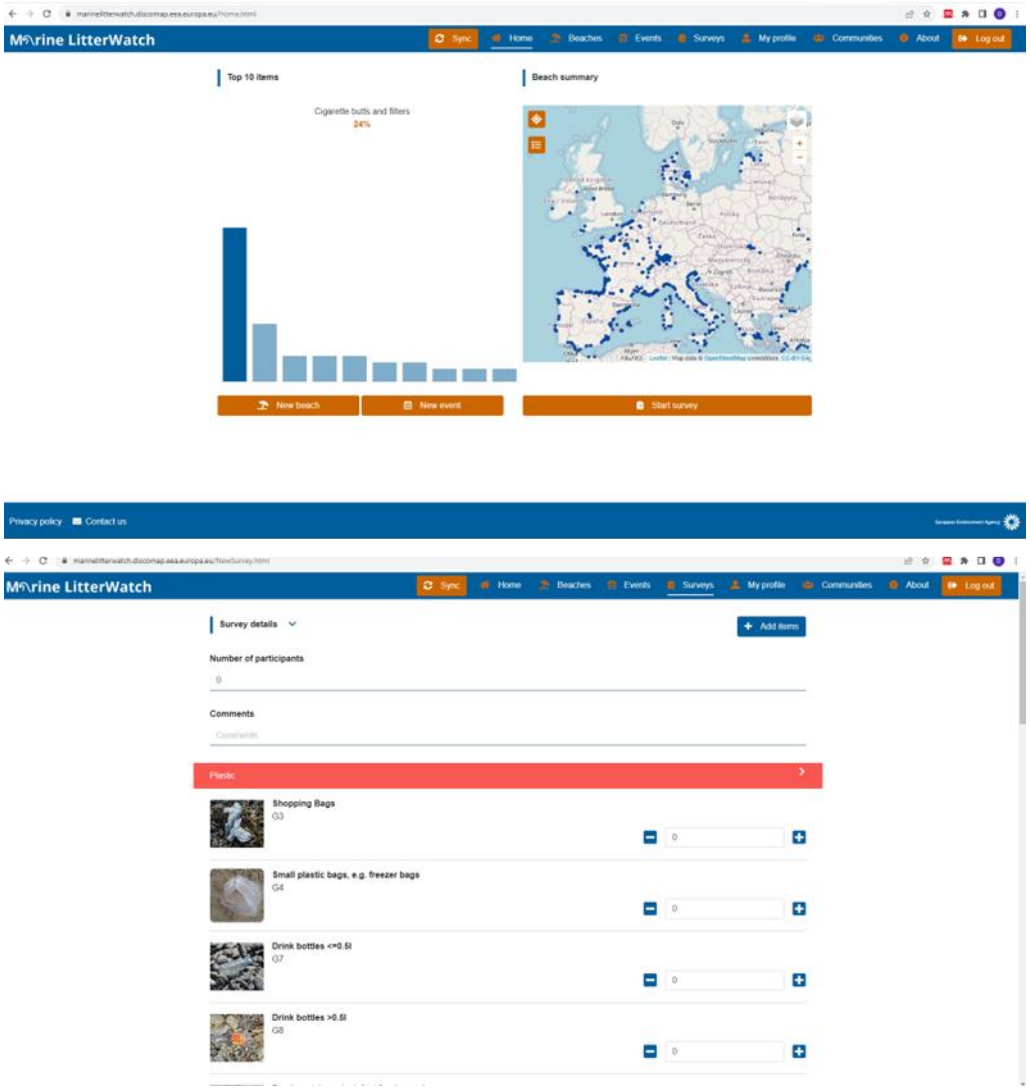

Fig. 11. The EEA Marine LitterWatch wep app.

5.3.3 Monitoring macro-litter at riverbanks with drone imaging

Drone data will be used to monitor riverbanks and observe macro-plastic items. Most flights will be conducted with RGB cameras to obtain high spatial resolution data. The output will be a waste map, indicating hotspots of litter at the riverbanks (Fig. 12).

Fig. 12. Left: Patches of litter identified from drone data. Blue patches indicate presents of plastic items > 2.5cm, red coloured patches are areas not polluted by litter. Right: shows a derived waste map, showing hotspots of litter along the riverbank.

Monitoring protocol for drone data collection riverbank – Drone imaging

The protocol for drone image data collection for monitoring of macrolitter on riverbanks has been defined in the WasteWatchers project (www.river-cleanup.org/en/waste-watchers).

Legally compliancy

Make sure you are legally compliant before performing drone flights for data collection.
Requirements may vary for each use case based on local or regional regulations.

- For most UAS drones, you need the EU Drone Licence. This consists of two parts: the Basic Certificate (A1-A3) and the Supplementary Certificate (A2).
- Mandatory for drones from 250 grams
- The drone may fly a maximum height of 120 metres
- The drone must be flown within direct sight of the pilots
- You must register yourself as a drone pilot
- You have to attach an operator number to your drone
- When planning a flight check if the activity is allowed in defined zone and if you need to inform about your flight the local authorities.
- To fly a drone weighing less than 250 g, it is basically enough to read the manual. Training is not mandatory, but getting the theoretical Open A1 certificate is easy, free and online. If you fly with heavier drones, training is mandatory.

Flights Instructions

- Set up somewhere central and check your surroundings for obstacles such as tall trees or lampposts.

- Always leave with a full battery.
- Steer your drone in parallel flight lines along the towpath and bank.
- Maintain a constant altitude of 15 m above the ground.
- Fly at as constant a speed as possible of no more than 3 m/s. To limit speed, it may help to switch your remote control to C (Cine) or T (Tripod) mode, and/or change the maximum speed in the settings (depending on the type of drone). Of course, it's not bad if you deviate from this occasionally.
- Before you take off, don't forget to check your drone's safety settings. Set the maximum flight distance at 300 meters, the maximum flight altitude at 120 meters and the return-to-home altitude above obstacles. That way, you will always fly within the legal limits.

The flight path

- How far you fly the drone depends on how far you can still keep the drone in sight and how comfortable you feel. We recommend flying no further than 250 m from you.
- First let your drone fly a certain distance to the left, move up a bit and let the drone return. Then you can continue flying the same distance to the right, eventually returning to the original flight line.
- Aim your drone so that the front of the drone (the side where the camera hangs) points in the direction of flight. When you have the drone move up, make sure that you still recognize in the edge of the image the landscape elements that you were also just in view on the previous flight line. That way, you can keep the flight lines tight, and a little overlap ensures that you don't skip spots.

How to take pictures

- Take photos, not videos.
- Set the camera to "timelapse" at 5 second intervals.
- Leave the file format set to JPEG at maximum resolution, the exposure setting and focus to AUTO, but set the exposure compensation (EV) to -1.
- Before you start, check that you have enough space left on the SD card: with 500 photos left on the counter, you are definitely safe.
- Once the camera is set, ascend to 15 meters, turn the camera down and press the photo button to start the photo series. (Image 2)
- When you get back to the take-off site at the end of the flight, press the photo button again to stop the sequence, and set up for landing. It is a good idea to point your camera back forward just before landing to avoid damage to the lens from any stones or windblown dust.

6. Monitoring plans

To establish a monitoring plan targeted to each use case, the sampling plans will be aligned with the activities, i.e., technologies deployment and actions, taking place in each of the locations, in a joint effort between WP1 and WP2. The major goal of the monitoring plans is to establish the baseline levels of litter contamination and to assess how each target activity will tackle local accumulation. Each plan will includes a dedicated power analysis to estimate the sampling effort (minimum number of samples). The identification of the statistical power to detect significant differences (at a specified significance level) is essential to estimate, for example, the number of samples per sampling occasion, sampling frequency etc., required for each use case. This power will decrease as sources of variance (analytical variance, natural environmental variance) increase. Our analysis will use three components to estimate the sample size (Cohen, 1987): (1) significance level (a = 0.05), i.e., the probability of finding in case this effect is existing (type I error); (2) power (0.8), which is the probability of finding an effect when there is one; and (3) effect size, i.e., the quantification of the difference between two (or more) levels of a factor (De Witte et al., 2022; Goldstein et al., 2013). The analysis will be based ANOVA, with a power of 0.8 and an effect size calculated for each use case specifically (Cohen, 1987; Montgomery and Runger, 2010). All analyses will be performed in R (R Core Team, 2013) (version 3.6.1) using the packages “pwr” (Champely et al., 2020), “car” (Fox and Weisberg, 2018), and “power” (Sassi Pereira, 2021).

For each use case we will follow the statistical sampling requirements as close as possible within the budget available. It is important to consider that permits are sometimes needed to perform sampling, and that we will do all necessary and possible to obtain the permissions for monitoring. The monitoring plan will also consider weather conditions and other environmental variables that may hinder implementing the activities foreseen in the project. The monitoring plans may need to be adapted to unexpected conditions and events in order to fulfil the purpose of the different monitoring tasks.

Monitoring strategy design

We will use a harmonized approach to design the monitoring strategy for each case study (Fig. 13; van Emmerik et al.(2023)). For each river basin we will determine a specific goal or set of goals (step 1). Then we identify the river characteristics, including river dimensions, flow characteristics, available infrastructure (step 2). Together with project partners and the case study team we inventory the available resources, funding, and staff to execute the monitoring (step 3). All input from step 1-3 is used to design the monitoring strategy (step 4), including method selection and implementation (e.g., frequency, duration, duplicates). We will propose an initial “best case” strategy, which will be further tuned in collaboration with the project partners based on limitations and additional local experience. Finally, the selected monitoring strategy is evaluated to check whether it aligns with the goal(s) (step 5). If that is not the case, we initiate a new design iteration.

Fig. 13. Workflow for developing a targeted river plastic monitoring strategy following a harmonized approach [adapted from van Emmerik et al. (2023)].

Overall monitoring goals

In addition to the case specific monitoring goals, we have identified several overall goals that the monitoring strategies should be based on:

- Quantify the current state of plastic pollution (baseline).
- Quantify the standing stock and fluxes in the monitoring area.
- Produce input data for the model(s) to be developed in Task 1.5.
- Quantify changes in plastic pollution over time.
- Optional: attribution of change in plastic pollution to specific interventions.

General criteria

We have summarized the compartments and size ranges to be included in the monitoring strategies in the Table 7.

Table 7. Riverine environmental compartments and size ranges to be included in the INSPIRE monitoring strategies.

		Surface			Suspended			Riverbank			Sediment		
		Macro (>25 mm)	Meso (5-25 mm)	Micro (<5 mm)	Macro (>25 mm)	Meso (5-25 mm)	Micro (<5 mm)	Macro (>25 mm)	Meso (5-25 mm)	Micro (<5 mm)	Macro (>25 mm)	Meso (5-25 mm)	Micro (<5 mm)
Scheldt	Temse	x	x	o	x	x	o	x	x		x		
	Doeldok	x	x	x	x	x	x	o					
Po		x	x		x	x		o					
Douro		o		x			x	o		x			x
Savinja		o						o					
Rhine	Rotterdam	x	x					x	x				
	Evides	x	x	x	x	x	x	o					
Danube	Festesti	o		x			x	o		x			x
	ELCEN	x	x	x	x	x	x	o					
x = required, o = nice to have													

The size ranges and environmental compartment are based on the technologies and interventions that are planned in each of the case studies. Besides the required size/compartment combinations, we also included several combinations that are “nice to have”. These additional measurements are relatively cost effective to implement and will allow to directly compare the state of plastic pollution between all rivers in INSPIRE.

Monitoring plan: Practical considerations

The table below shows the practical considerations that should be considered when designing the final monitoring strategy. For a (non-exhaustive) list of monitoring goals we show what steps are required to work towards the implementation. The most important decisions that need to be made relate to the number of observation points, measurement frequency, duration, sampling area or volume, duplicates, level of detail of the classification. Furthermore, for tidal systems we need to determine how to account for the daily (ebb/flood) and/or monthly (neap/spring) tidal dynamics.

Table 8. Overview of practical considerations for the monitoring strategies.

Goal	Size range	Location	Method	Implementation	Limitations, risks, uncertainties
Estimate daily total and net surface plastic flux	Macro/meso	Bridge	Visual counting	o observation points across river width o frequency of measurement round o duration measurements o number of repetitions o ebb and flood (daily/monthly) o total items with(out) categories	Tidal dynamics Unknown variations
		Bridge	Camera (multisp	o observation points o frequency of photos o band selection o installation height above water surface o image resolution o manual or AI processing	o Image processing o Item categorization o only daytime o legal and practical constraints o loss of equipment (theft) o power supply
	Micro	Open water	Mantra trawl	o observation points o duplicates o ebb + flood o deployment time o collect macro for mass statistics	o Tidal dynamics
Estimate daily mean suspended plastic concentration + flux	Micro/meso	Open water	Ferrybox	o observation point o sampling depths o duplicates o ebb + flood o deployment time	o Variation across river width o Variations along vertical
Estimate total plastic mass on riverbanks	Macro/meso	Riverbank	Visual counting	o locations on each side o sampling area (width and length) o frequency measurements o counting or classifying only o use mass data from cleanups to convert to mass	
	Macro	Riverbank	Drone	o numbe rof locations on each side o observation area o frequency measurements o flying altitude o image resolution o percentage of overlap between images o manual or AI processing	o legal and practical constraints
Estimate total plastic mass in sediment	Macro/meso	Sediment	Trawling	o frequency measurements o same or different locations o length of trawling	o unknown density o monitoring through collection o impacts data interpretation

7. Data management proposal

All data and metadata records will be standardized throughout the project and delivered in open file formats to allow for re-use, according to the Data Management Plan (DMP) of the INSPIRE project (D7.3) and its update (D7.8). The DMP describes how the collected data will be handled during and after the project with regards to standardization, documentation, storage, accessibility, re-useability, security, etc., and according to the FAIR principles. The monitoring and analysis protocols in this deliverable help ensure the quality of the generated data.

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Funded by the European Union

Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Climate, Infrastructure and Environment Executive Agency (CINEA). Neither the European Union nor the granting authority can be held responsible for them. This project has received funding under grant agreement No 101112879 (INSPIRE).