INSPIRE

Innovative Solutions for Plastic Free European Rivers

Deliverable 2.1

Development of retention efficiency protocols

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Executive Summary

The 'Innovative Solutions for Plastic Free European Rivers' (INSPIRE) project Deliverable 2.1 "Development of retention efficiency protocols" aims to:

1. Create protocols to assess INSPIRE Work Package 2 solutions for their plastic removal efficiency based on release-catch experiments and other applicable approaches
2. Identify the adequate test materials for the release-catch experiments
3. Establish the plastic removal efficiency concept and terminology, as well as the definition of related concepts such as "release-catch experiment"

The two main concepts used in this deliverable are defined as follows:

Plastic removal efficiency (*%PR*): the proportion (%) of plastic litter removed, both intercepted and collected, from the total plastic litter released during a release-catch experiment, calculated in number *%PR(#)* or mass *%PR(m)* of the plastic items/particles.

Release-catch experiment: an *in-situ* experiment during which a known amount (mass/number) of selected test materials is released at the technology deployment location and is then caught back by the technology, or in its surroundings, i.e., test materials release point, downstream/outlet and other designated 'loss points'. The different test materials fractions, intercepted and collected, intercepted and lost, or not intercepted, are identified, quantified and characterized.

A flexible and modular approach was created which can be used to analyse the plastic removal efficiency of the INSPIRE WP2 solutions, as well as other solutions outside of the INSPIRE project. This approach results from the **combination of the fitting, state-of-art protocols and test materials** established here:

- **Plastic removal efficiency assessment protocols (explored in Chapters 5-7):**
    - Protocols for solutions in riverine environments or ports:
        - Release-catch experiments for floating plastic collection technologies
        - Manual cleanup activities assessment
        - Underwater riverbed macroplastic observations
    - Protocols for technologies installed in urban infrastructure:
        - Release-catch experiments with plastic particles for the assessment of technologies deployed inland
        - Assessment of technologies deployed inland for tyre wear and tyre wear leachables reduction
        - Release-catch experiments for elimination technologies assessment
        - Release-catch experiments for technologies deployed at freshwater abstraction points
    - Laboratory or pilot scale validation
- **Test materials categories for release-catch experiments (explored in Chapter 7):**
    - Plastic items recovered from the environment
    - Laboratory-prepared mixtures of plastic particles, including conventional and commonly observed polymers, tyre wear particles, and biodegradable polymers
    - Non-plastic items that mimic plastic behaviour

- **Additional protocol** for release-catch experiments for floating macroplastic detection using remote sensing techniques

In this approach (Fig. 1), depending on the plastic removal solution (technology or action) characteristics and type of litter targeted at the implementation location, the best fit between i) one subgroup of test materials and ii) one protocol is selected. This makes the protocols and test materials applicable in multiple scenarios, with several combinations possible, and improves the comparability of the results obtained between different release-catch experiments performed, because they share common methodology.

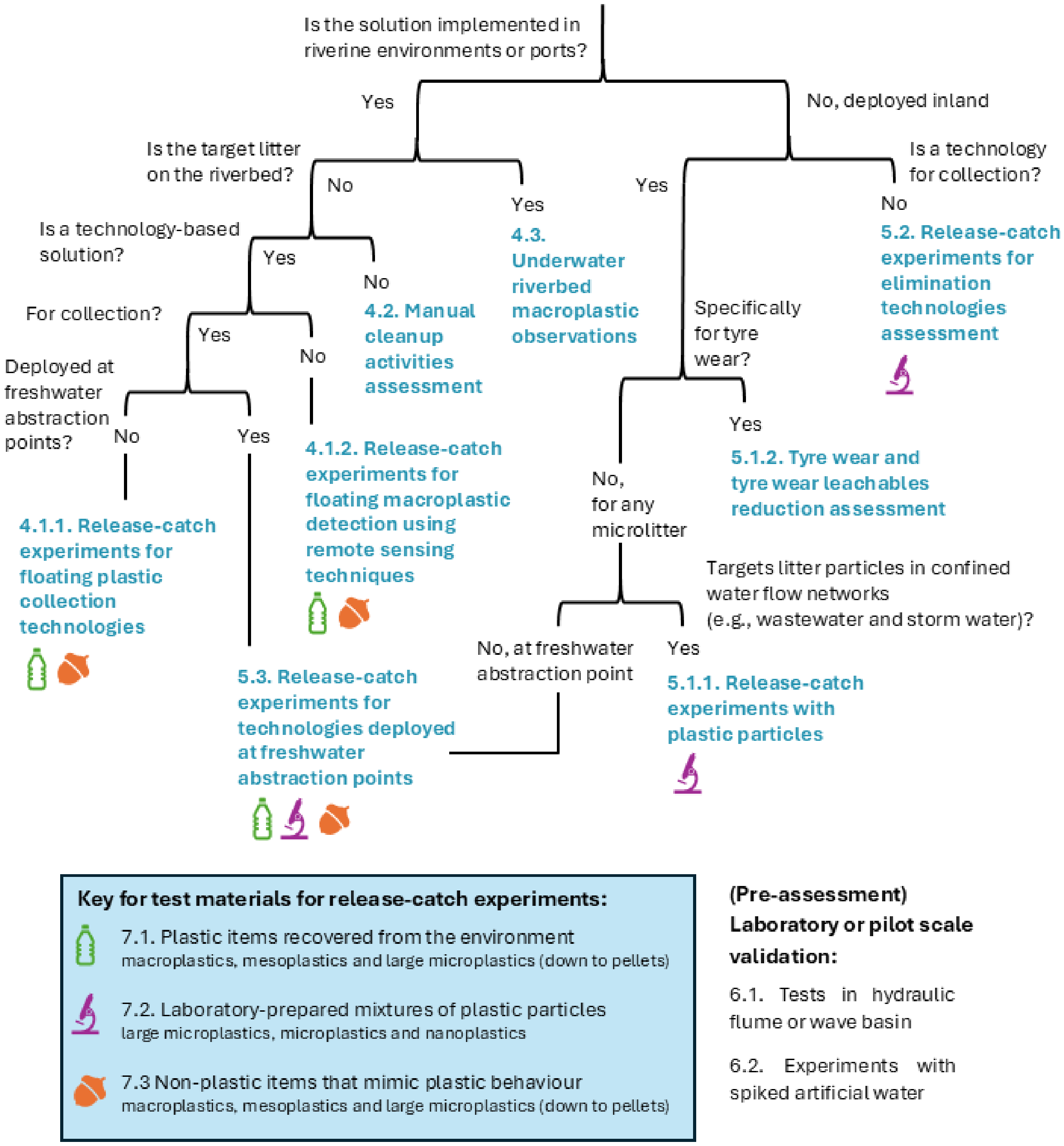

Fig. 1. Map of the INSPIRE flexible and modular approach for plastic removal efficiency assessment, with identification of the protocols and groups of test materials in this deliverable (and respective chapter number).

After the release-catch experiment is performed, the data obtained can be used to calculate the plastic removal efficiency of the solution, following the concepts and terminology that are presented in Chapter 8.

The deliverable includes a section on ethical considerations (Section 7.4), where mitigation actions were identified to comply with EU regulations and reduce/eliminate the environmental impact of the release-catch experiments. Examples include the restriction on the use of some groups of test materials during these experiments in natural environments, as well as the need to inform the local citizens about ongoing scientific studies at the test sites.

The deliverable D2.1 approach is going to be applied to the INSPIRE plastic removal solutions, which comprise 10 technologies used individually or combined, and manual cleanup activities. The refined and validated protocols and test materials, as well as the results obtained from their application to the INSPIRE solutions, will be reported in upcoming project deliverables: D2.2, D2.3, D2.4, D2.5, D2.6 and D2.7.

List of abbreviations and acronyms

Abbreviation or acronym	Description
ABS	Acrylonitrile-butadiene-styrene
AI	Artificial intelligence
ATR	Attenuated total reflectance
CHIRP	Compressed High Intensity Radiated Pulse
CI	Carbonyl index
DLS	Dynamic light scattering
DWTP	Drinking water treatment plant
EU	European Union
E	Test material not intercepted
FSP	Flame Spray Pyrolysis
FTIR	Fourier-transform infrared (spectroscopy)
GC-MS	Gas chromatography - mass spectrometry
GPS	Global Positioning System
HSI	Hyperspectral imaging
INSPIRE	Innovative Solutions for Plastic Free European Rivers
I	Test materials intercepted by the technology
IC	Test materials intercepted and inside the collection net/cage
IBC	Intermediate bulk container
L	Test materials loss
ILO	Outside loss – test materials intercepted but lost outside of the technology (trapped elsewhere)
ILP	Retaining loss – released back into the environment
ILP-O	Retaining loss during operation – test materials intercepted but not retained (found in the treated water at the outlet of the technology)
ILP-E	Retaining loss when emptying collection net/cage – test materials intercepted but not retained during the step to retrieve them from inside the collection cage/net
LC-MS	Liquid chromatography - mass spectrometry
LiDAR	Light Detection and Ranging
LOQ	Limit of Quantification
LR	Litter removal
MP	Microplastic
MRM	multiple reaction monitoring
MSFD	EU Marine Strategy Framework Directive
PA	Polyamide (nylon)
PCS	Photon correlation spectroscopy
PE	Polyethylene
PE-HD HDPE	High-density polyethylene
PE-LD LDPE	Low-density polyethylene
PE-UHMW UHMWPE	Ultra-high-molecular-weight polyethylene
PET	Poly(ethylene terephthalate)
PHA	Polyhydroxyalkanoate
PP	Polypropylene
PR	Plastic removal
PS	Polystyrene
PS-E EPS	Expandable polystyrene
PSD	Particle size distribution

PUR	Polyurethane
PVC	Poly(vinyl chloride)
RGB-D	Red, green, blue and depth
SEM	Scanning electron microscope
SONAR	Sound Navigation Ranging
SPE	Solid phase extraction
STED	Stimulated emission depletion
T	Test materials released at release point
TWP	Tyre wear particles
WP	Work Package
WWTP	Wastewater treatment plant

Polymers terminology and nomenclature according to IUPAC [1].

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1. Objectives

The project 'Innovative Solutions for Plastic Free European Rivers' (INSPIRE) is funded by the European Commission (Grant Agreement no. 101112879), under the call EU Mission 'Restore our Ocean and Waters by 2023' – HORIZON-MISS-2022-OCEAN-01. Under INSPIRE Work Package 2 – Prevention, retention, collection and elimination of plastic litter –, we are developing, testing and optimizing innovative solutions for prevention, retention/collection and/or elimination of macro, meso and microlitter (including microplastic and tyre wear) in riverine environments in Europe. These **plastic removal solutions** comprise 10 technologies used individually or combined, and manual cleanup activities (which can incorporate citizen science events for data collection). The environmental compartments targeted by these solutions are water and sediment/soil, in riverine ecosystems: i) river channel including riverbed, and ii) riverbanks. The performance of the solutions will be measured in terms of their efficiency in retaining, collecting, and eliminating plastic (referred to as **plastic removal efficiency, *%PR*** from now on), cost effectiveness and environmental impact in the deployment locations. Additionally, a life cycle assessment will be conducted (D4.2).

The main objective of this deliverable is to tackle the need to create protocols to be used in the project to assess, as objectively as possible, the removal efficiency of plastic prevention or collection solutions, i.e. amount and characteristics of the plastic litter removed with the INSPIRE technologies/actions. The aim is also to identify test materials for the protocols. From the combination of the protocols and test materials established here, a flexible and modular approach was created that can be used to analyse the efficiency of other technologies outside of the INSPIRE project. With the data collected from the implementation of these protocols, we will estimate the contribution and impact of the INSPIRE project for waste and plastic-free European rivers and seas, by feeding the data to the modelling and sustainability assessment tools selected for the project.

2. Introduction

Different types of responses have been identified over the last decade to mitigate the plastic pollution global environmental problem. These solutions can be classified as upstream when they have a preventive nature that aims at reducing the plastic inputs to the environment, or as downstream when they target the collection of plastic waste that is already polluting the environment [2]. In addition, these responses can be i) based on scientific and technological developments, or ii) build around behaviour change [3] in a voluntary approach by improved awareness, or through the establishment of new regulations and policies that promote the change in the production, consumption, and/or waste management of plastic. A combination of these different types of responses provides a preferred strategy due to the complexity of this environmental issue, the known impacts of plastic during its life cycle [4], the need to remove the plastic already polluting the environment due to its low rate of environmental degradation [5], and the need for a holistic and interdisciplinary approach [3].

Most of the responses that are part of the INSPIRE project Work Package 2 are technology-based solutions (listed and described in Chapter 3). Some of these technologies work in a preventive way by removing, for example, the microplastics that are not eliminated by the conventional wastewater treatment plants or by targeting tyre wear-loaded water runoff, therefore reducing their input when the treated water is discharged into the rivers and ocean. Others can be classified as cleanup technologies [6], which were developed to remove plastics from water or sediments in rivers or in harbours and other urban infrastructure. Actions, such as riverbank manual cleanups (combined with citizen science activities), increase the community and stakeholders' engagement and awareness, closing the INSPIRE holistic approach. Thus, within INSPIRE we will test both upstream and downstream solutions, and technology-based and behaviour-based solutions, with a focus on innovation.

In parallel to the solutions described in Chapter 3, INSPIRE Work Package 3 explores the solutions focused on new materials, circular economy and policies and regulation, for the prevention of plastic waste. These solutions include the development and validation of biodegradable alternative materials to the commonly used polymers, of packaging-free options, and other zero-waste and supply chain innovations. These solutions, which further realize the INSPIRE holistic approach, are going to be evaluated following a different approach and are not explored in this document.

Different prevention or collection technology-based and behaviour-based plastic removal solutions have been developed and implemented over the last decade, yet we identified significant gaps regarding the procedures for the assessment of their plastic removal efficiency. While there have been previous efforts to assess their efficiency (such as those described in [7–11]), these efforts stayed isolated and did not lead, so far, to a standardized approach that enables the solutions efficiency to be quantitatively evaluated and compared. Accomplishing a standard method to assess the different solutions will not only empower the technology providers but it can also support decision-makers in identifying the most appropriate solutions to local or regional plastic pollution problems.

Thus, there is a need to establish a fitting and state-of-art method to quantify and to assess the efficiency of the INSPIRE Work Package 2 solutions to prevent or collect the plastic in the environment: the **plastic removal efficiency (*%PR*)**. Below, we introduce the INSPIRE approach on how to assess and quantify the *%PR* of different plastic pollution solutions. Chapter 8 discusses the concepts of plastic and litter removal efficiency, release-catch experiments and how the results should be presented. Chapter 4 and 5 outline the protocols for evaluating the plastic removal efficiency of the solutions, with Chapter 4 focusing on riverine environments and ports, and Chapter 5 on urban infrastructure (e.g., wastewater treatment plants). In addition, in Chapter 4, we also explore an adapted release-catch experiment for a detection technology, allowing us to assess its detection efficiency. Chapter 6 delves into validation protocols for experiments performed in the laboratory. Chapter 7 explores the test materials used in the release-catch experiments, with particular attention to their origin, selection and preparation, and ethical considerations. In Chapter 3, we introduce how the protocols are being applied in practice at the INSPIRE demo sites.

While this deliverable provides a comprehensive review of potential protocols applicable to various plastic removal solutions, its primary aim is to establish a flexible and modular framework for assessing plastic removal efficiency. The detailed Standard Operational Procedures (SOPs) for each INSPIRE solution will be refined and validated through field testing. These fine-tuned methodologies will be documented in the subsequent deliverables D2.2, D2.3, D2.4, D2.5, D2.6 and D2.7, where the actual performance and efficiency of each device will be reported. This approach ensures that the protocols remain scientifically robust while being adapted to real-world conditions. Additionally, efforts will be made to enhance usability and simplify key aspects to improve practical application.

3. Implementation at INSPIRE demo sites

As identified in the report Chapter 1 (Objectives), the protocols proposed in two following chapters (4 and 5) and the tests materials for release-catch experiments (Chapter 7) will be applied for the INSPIRE solutions, namely for combined technologies/actions and also for their individual assessment when possible. The target litter size for these technologies/actions can be found in Table 1, as well as the locations where they will be deployed in Table 2.

The technologies/actions are the following:

1. **(Collection) Technologies for meso- and macroplastics in the Po River (task 2.1):**
   a. **River Cleaning System (by MOLD, Italy):** modular barrier made of floating buoys which retains and displaces the incoming litter on various types of rivers and waterways. It has its optimal application in flowing waters, where the natural push of the stream spins each module in a manner similar to that of a turbine. The system covers part or the entirety of the waterway and is placed diagonally: this setup, coupled with the natural rotation of the buoys enables the system to displace the incoming trash flow, like a chain of gears, towards the collection point located near the riverbank. As such, it acts like an active collection system, relying on passive and clean forms of energy.
   b. **CLEAN TRASH collection cage (by MINDS, Greece):** collection cage that will be combined with the River Cleaning spinning motion buoying modules in a hybridised technology solution. The CLEAN TRASH hot-dipped galvanized steel collection cage utilizes three separate collection chambers/levels that lower and raise to store the floating litter (down to 5 mm in size). The cage is equipped with lifting points and a sliding door.

2. **(Collection) Technologies for industrial process waters, dams, docks and sluices/locks, in the Rhine, the Scheldt, and/or the Danube rivers (task 2.2 and task 2.6):**
   a. **Archimedean Drum Screen (ADS) (by FishFlow, Netherlands):** variant design of the Archimedes' screw - a slowly rotating screw that transports water. The technology works as a screen allowing 90 % of the filtered water flow to pass through while catching (plastic) debris and floating vegetation. What makes the screen unique is that it is completely fish friendly, so the collection of waste doesn't come at the cost of the local fish species, which exit the screen through a special outlet.
   b. **Patje Plastic (property of the Port of Antwerp-Bruges, Belgium, and developed by AllSeas, Netherlands):** system that collects litter and plastics that are floating or in suspension up to 1.5 m depth. The installation consists of a floating arm (boom) with a length of 100 m and 2 m height (1.5 m underwater and 0.5 floating), which pushes litter to a large bin. A series of filters separates the larger from the smaller waste (> 2 mm). It does not need power supply, since it works on wind, waves, and gravity.
   c. **CirCleaner (by Noria, Netherlands):** an active, sustainable litter removal system running on solar energy that can be operated remotely using smart connectivity. The removal technique consists of a centrally rotating assembly on which a series of blades are mounted. These permeable blades collect pollutants (litter) each time the blade goes underwater. The litter is pushed towards the blades by water flow and wind. The slowly rotating blades collect the trash and pitch it in the container.

3. **(Collection) Technology for litter in the riverbed or bottom of dock channel in Scheldt River (task 2.2):**
    a. **Fish Friendly litter removing trawling net (by FishFlow, Netherlands):** an innovative way of cleaning the bottom of canals and rivers. Fish and marine life pass without damage or harm and plastics and other debris will be removed from the floor of the waterway.

4. **(Prevention) Technology for microplastics in marinas and shipyards in Douro River (task 2.5):**
    a. **EcoPlex Microplastic Remover (by WnW, France):** see 5a (below). During the project preparation stage, it was expected that the Clera.One technology (see 5b) would be used at this demo site. However, after the first demo site visits and field assessments it was agreed within the project consortium to change the approach to better reach the project goal for this location: "innovative water recycling device to clean the greywater coming from vessel washing, which contains substantial amounts of microplastics, metal particles and other toxins" (INSPIRE Grant Agreement). A new deployment plan is, at present, under consideration.

5. **(Prevention) Technologies for microplastics and/or tyre wear from wastewater treatment plants and/or water and sewage runoffs in Savinja or Danube rivers (tasks 2.3 and 2.4):**
    a. **EcoPlex Microplastic Remover (by WnW, France):** consists of two microplastic filtering units that work in series. The system allows to change the connection of the two filtering units from serial connection to parallel. The filtering units are skid mounted and can easily be relocated. Each filtering unit filter contains a bottom layer of fine media and a top layer of coarse media. The devise is self-cleaned and fully automated.
    b. **Special membrane filtration unit (by Clera.One, Slovenia):** chemicals-free water recycling system that enables to eliminate the finest particles, not yet removed in previous steps for water re-use. Its innovative membrane allows a high-water permeation and flow while maintaining low pressure, with membrane pores adjustable down to 0.1 µm. An ozone generator disinfects the membranes and prevents fouling.
    c. **Super-TW-Net (by DELVEC, Greece):** designed to collect nanometric tyre wear particles (TWP). It is based on functional paramagnetic nanoparticles that will be immobilized on an appropriate scaffold and act as high-affinity adsorbents for the TWPs. The filter materials, produced by the industrial-scale Flame Spray Pyrolysis (FSP) technologies of DELVEC, are based on hybrid Fe-based nanoparticles with high affinity for the carbon and sulfur moieties of the weathered TWPs.
    d. **Photocatalytic reactor (KTH, Sweden):** system for photocatalytic degradation of microplastics. It employs nanotechnology-based coatings on filter traps with sunlight activation. It is based on a photo-Fenton process, where hydrogen peroxide (H_2O_2) and ferrous ions (Fe^{2+}) deliver powerful oxidative properties due to the generation of hydroxyl radicals (HO•).

6. **(Collection & Prevention) Manual riverbank cleanup activities with citizen engagement (task 2.2 and task 2.6):**
    a. **Riverbank cleanups (by River Cleanup, Belgium):** activities with a three-part strategy of cleaning, educating, and transforming. The cleanup activities in the riverbanks enable to complement the technology deployment for plastic removal in the river channel, while spreading awareness, educating citizens, and helping businesses and organisations reduce their plastic use.

In addition, some INSPIRE **detection technologies** will also be validated with protocols established in this document, namely the camaras with AI (artificial intelligence) for floating litter detection, which were developed by VITO or AIT.

Table 1. INSPIRE collection technologies/actions and target litter size by use case and location.

Use case	Technologies/ actions	Target litter size	Ferrybox	Nets for macrolitter	Riverbank observations	Filter analysis	Analysis of litter collected
1. Scheldt	1.1. FF trawling net	macro	-	-	-	-	x
	1.1. Riverbank cleanup	macro + meso	-	-	x	-	x
	1.2. FF ADS	> 5 mm	-	x	x	-	x
	1.3. Patje Plastic	2 mm to 1 m	x	x	x	-	x
2. Po	MOLD-MINDS river cleaning system & clean trash collection cage	> 5mm	-	x	x	-	x
3. Douro	WnW EcoPlex Microplastic Remover	30 µm – 5 mm	x	-	-	x	x
4. Kamniška Bistrica	WnW EcoPlex Microplastic Remover	30 µm – 5 mm	x	-	-	x	x
	CLERA water recycling system	> 0.1 µm	x	-	-	x	x
	DELVEC Super-TW-Net filter	10-100 nm	-	-	-	x	-
	KTH Photocatalytic reactor	-	-	-	-	-	*
5. Rhine	5.1. Noria CirCleaner	2 mm to 1 m	x	x	x	-	x
	5.1. Riverbank cleanup	macro + meso	-	-	x	-	x
	5.2. FF ADS	> 5 mm	TBD	x	TBD	-	x
6. Danube	6.1. WnW EcoPlex Microplastic Remover	30 µm – 5 mm	x	-	-	x	x
	6.1 DELVEC Super-TW-Net filter	10-100 nm	-	-	-	x	-
	6.2 FF ADS	> 5 mm or smaller (TBD)	TBD	x	TBD	-	x

TBD: to be defined.
* KTH Photocatalytic reactor will be assessed for plastic/litter elimination and not collection.

Table 2. INSPIRE technologies/actions by plastic removal efficiency protocol.

Environment	Protocol (Chapters 4-6)	Location & technologies/actions
Riverine environments, incl. ports	Release-catch experiments for floating plastic collection technologies	Scheldt (Port of Antwerp - Doeldok) - Patje Plastic Rhine (Port of Rotterdam - Londenhaven) - NORIA CirCleaner “Scheldt” (Port of Oostende – VLIZ science harbour) - FF ADS* Po river (Santa Giulia) - MOLD-MINDS hybridised system
	Release-catch experiments for floating macroplastic detection using remote sensing techniques	Scheldt (Temse bridge) - VITO camaras (> 2.5 cm) Po (Molo bridge) - VITO camaras (> 2.5 cm) Po - AIT camaras (> 5 mm)
	Manual cleanup activities assessment	Scheldt (Temse) - Riverbank cleanups Rhine (Port of Rotterdam - Londenhaven) - Riverbank cleanups Po (Santa Giulia and Molo bridge) - Riverbank cleanups
	Underwater riverbed macroplastic observations	Scheldt (Temse) - FF trawling net
Urban infrastructure	Deployed inland – Release-catch experiments with plastics particles	Douro river (marina) - WnW EcoPlex Microplastic Remover Kamniška Bistrica (WWTP) - WnW EcoPlex Microplastic Remover Kamniška Bistrica (WWTP) - CLERA water recycling system Kamniška Bistrica (WWTP) - DELVEC Super-TW-Net filter
	Deployed inland – Tyre wear and tyre wear leachables reduction assessment	Danube (WWTP & stormwater system) - WnW EcoPlex Microplastic Remover Danube (WWTP & stormwater system) - DELVEC Super-TW-Net filter
	Elimination technologies assessment	Kamniška Bistrica (WWTP) - KTH Photocatalytic reactor
	Release-catch experiments for technologies deployed at freshwater abstraction points	Rhine river (DWTP) - FF ADS Danube (power plant/water abstraction) - FF ADS
Laboratory or pilot scale validation	Tests in hydraulic flume or wave basin Experiments with spiked artificial water	Laboratory tests for validation

* The FF ADS technology was expected to be deployed in Temse, but due to local constrains the technology is being deployed instead in the Port of Oostende. The new location is identical to the one initial planned (a locked dock) and all the activities will be done following the same methodology.

3.1. Data cross-validation and parallel tasks

The approach selected for the assessment of the plastic removal efficiency of the INSPIRE solutions needs to be aligned (Fig. 2) with the methods and corresponding outputs of WP1 - Monitoring Riverine Litter, particularly with:

- The protocols established for plastic litter observations/sampling, sample processing and analysis (D1.2 - Monitoring and analysis protocols).
- The microlitter and microplastic datasets for assessment of technology efficiencies and modelling tasks (D1.3 - Microplastic monitoring datasets at the demo sites).
- The macrolitter and macroplastic datasets for assessment of technology efficiencies and modelling tasks (D1.4 - Macroplastic monitoring datasets at the demo sites).
- The mesolitter and mesoplastic datasets for assessment of technology efficiencies and modelling tasks (which will be incorporated in D1.3 or D1.4).

The results obtained from the release-catch experiments will be cross-validated with the field observations and sampling performed before (baseline pollution levels) and after the implementation of the INSPIRE solutions, and with the plastic litter collected by the INSPIRE solutions after their start operation/activities.

Besides, the plastic removal efficiency assessment is linked (Fig. 2) to the overall measurement of the INSPIRE solutions effectiveness, which also considers other parameters such as power consumption, required maintenance time and operator time (D4.1 - Report on feedback loop including impact pathways generated by measures for prevention). Finally, the environmental impact assessment (D1.5 – Evaluation of the biotic and abiotic alterations) of the solutions needs to be performed in parallel to the quantification and characterization of the plastic and other litter collected by the normal operation/activities of the INSPIRE solutions.

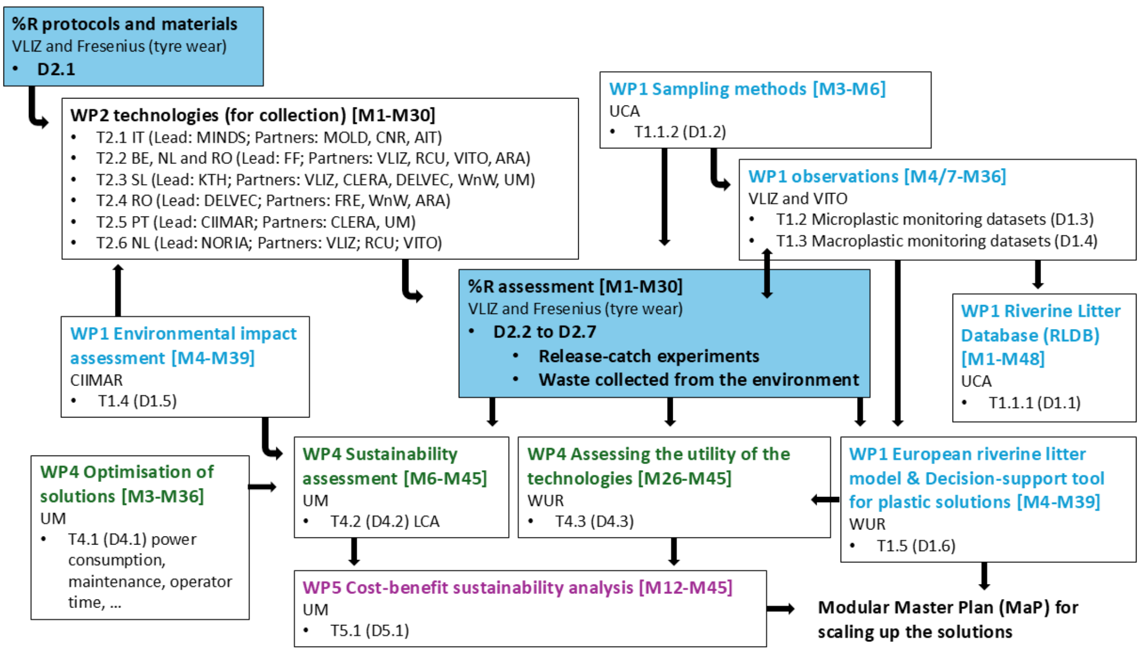

Fig. 2. Schematic of D2.1 within the INSPIRE project, with link to other WPs and tasks.

3.2. Removal efficiency data flow to other INSPIRE WPs and tasks

The data collected by the application of the plastic removal efficiency protocols in WP2 will be reported in D2.2 to D2.7, corresponding to the removal efficiency assessment and performance of the collection technologies of T2.1 to T2.6 (Fig. 2). This data will be also fed to the following tasks:

- Mapping, Assessment and Modelling Framework (T1.5, D1.6, WUR)
    - European scale model of riverine litter & plastics accumulation and transport.
    - Decision-support tool to quantify and visualize plastic mitigation actions.
    - The modelling framework will establish a baseline of river litter & plastic pollution levels in European rivers, will identify the most suitable locations to implement interventions, and will quantify the effect of potential interventions. The resulting tools will provide a multi-scale and dynamic modelling framework for riverine litter allowing the upscale process at basin, country and European scales.
- Life cycle assessment of proposed elimination technologies, biodegradable, circular and zero-waste solutions (T4.2, D4.2, UM)
    - Life cycle assessment for the elimination technologies and > 5 circular products. Impact evaluation for pollution elimination technologies (WP2), considering their design specifications and power consumption, based on a demo site's country location and its telemetric data.
    - 'Unburdening' of the technologies (benefits).
- Assessing the utility of INSPIRE cleaning and circular technologies (T4.3, D4.3, WUR)
    - Compare and contrast the efficiency and utility of INSPIRE solutions for the reduction of pressures from micro-macroplastics on ecosystem services, using modelling tools (EURIPOL – RITA – RIDUC).
    - Assess the potential benefits of scaled up INSPIRE macroplastic and/or microplastic litter cleaning devices on near source local and far field ecosystem services.
    - Quantify the risk reduction by removal of plastic.
    - Number/mass and characteristics of waste collected/prevented that would otherwise be transported by European rivers and potentially be exported to and dispersed in the marine environment.

4. Protocols for solutions in riverine environments or ports

This section describes the tailor-made protocols for the assessment of the *%PR* in riverine environments or port areas. A first aspect in common between these protocols is that the locations targeted are open areas, which are more challenging regarding the recovery of the test materials not caught by the INSPIRE solutions. A second aspect relates to the plastic litter size targeted by the solutions, which goes as lower as large microplastics (> 1 mm) since some of technologies are expected to be able to remove plastic pellets, but are focused mainly on macroplastics and, in some cases, also mesoplastics.

4.1. Controlled release of non-plastic items or plastic items recovered from the environment

In this subsection we describe the protocol for release-catch experiments with floating plastic to assess collection technologies (4.1.1), but also a variation of the same protocol to be applied to remote sensing techniques (4.1.2). Due to the open nature of the riverine environments and ports, it is crucial to use only responsible testing materials in the described experiments, such as those identified in sections 7.1 (Plastic litter items recovered from the environment) and 7.3 (Non-plastic items that mimic plastic behaviour).

4.1.1. Release-catch experiments for floating plastic collection technologies

The goal of this protocol is to assess the plastic removal efficiency for collection technologies that target floating plastic litter in riverine environments or port areas through release-catch experiments (Fig. 3).

The protocol was prepared based on the INSPIRE consortium experience with cleanup technologies, obtained during the first months of this project and from the input of the technology providers, in combination with the results presented by Blettler et al. [7]. In this study from 2023, a floating boom was tested for its effectiveness under realistic conditions.

The INSPIRE protocol includes the following steps:

1. **Selection and preparation of tagged test materials**
   The test materials selected for each technology assessment need to be aligned with baseline pollution levels assessed before the technology deployment, but also with the litter size targeted by the technology (Chapter 7). For example, if the technology has a collection net with a 5 mm mesh size, this should be the lower limit of the plastic items released. There are two main options for the test materials released:
   - Use plastic materials collected from the environment (e.g., from the riverbanks) that are tagged to be able to be distinguished from other plastic litter found at the location (Section 7.1).
   - Use non-plastic items to simulate plastic behaviour (Section 7.3) which should be validated previously at a laboratory scale (Chapter 6).

   A table with a label for each item and its characteristics needs to be created at this point. PP01_TL_T_001 – example of label for technology code PP, release-catch experiment 01, item

001 of the total released. More detailed information on the methodology for the test materials selection, including amount (mass or number of items to release), and for labelling and test materials characterization, in Chapter 7.

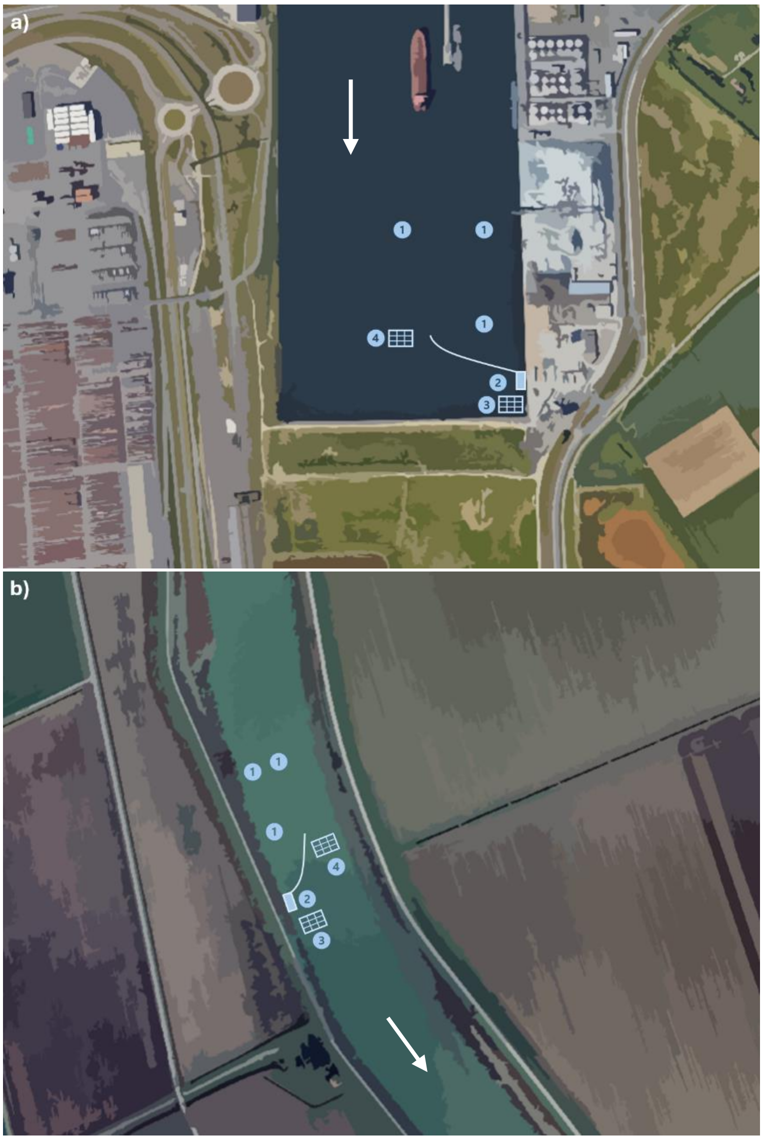

Fig. 3. Schematics of release-catch experiments in one a) dock, and b) river.
Legend: 1 – potential locations for the release point, 2 – collection from the technology and around it, 3 – collection after the technology, 4 – collection of plastic litter not intercepted.

2. **Preparation of the site for the release-catch experiments**
Before the release of the materials in the field, the site needs to be assessed and prepared. This step includes observations and removal of plastics and other litter by manual cleaning to facilitate identifying the tagged plastic. It is recommended that this is conducted shortly before the release-catch experiment due to the potential accumulation in the days between the cleaning activity and the experiment. The cleaning activity should target litter at the riverbanks, inside and around the technology deployed, and any reachable litter in the water. During this preparation phase, the potential use of nets to redirect litter not intercepted or lost by the technology can be judged. These nets will assist in reducing the impact of the

experiment at the location since they will increase the amount of the test materials that are recovered from those not removed (mitigation action described in Section 7.4), and in quantifying all the fractions for the calculation of the *%PR*, namely for the *%I*, *%C*, *%L* and *%E* (Section 8.3), enabling to close the mass/number balance. This has the additional benefit of enabling to use the same test materials on several experiments, to increase the reproducibility of the results. The nets used should target floating plastic but also plastic in the water column up to a few meters of depth, aligned with maximum depth that the technology is targeting and the density of the test materials selected.

3. **Release of tagged materials at release point(s)**

   Release point(s) location: It is recommended that at least 2 release points (Section 8.3) are selected: i) inlet release point, and ii) distant release point. The inlet release point is located just at the entrance of the technology (e.g., 1 to 2 m for the inlet) and allows to determine the technical efficiency of the technology (*%C*), as well as the losses (*%L*). The distant release point should be close enough that most of the plastic litter can be intercepted by the technology, but not at the technology inlet, which would disable the calculation of the interception efficiency (*%I*) and/or rate. Ideally, several distant release points should be tested and need to be realistic of the observed litter transport at the site. Furthermore, the release point location in relation to the river or dock cross section needs to be considered and be mentioned when reporting the results. It can also be different across different release-catch experiments. Since usually the technologies are deployed close to one of the riverbanks, two possible locations are interesting to be explored: at the middle of the cross-section or aligned with the inlet of the system. Likewise, an experiment in which the same type of item is released at multiple points across the cross section of the river will provide relevant information on the plastic transport at the location. This could provide data to support a change of the technology deployment location (cross section wise), to increase the interception of litter. It should be noted that if a bridge or pontoon is located nearby the deployment of the technology, this could be used as a release point, avoiding the requirement of a vessel.

   Time for release: Since riverine systems and port areas can be heavily affected by the tides flow direction, the release-catch experiments need to be aligned with the tides schedule: i) For tidal rivers, since the technologies are usually installed with the inlet oriented upstream, it is recommended that the experiments are performed at the time interval between high and low tide, i.e., when the water flow is directed towards the sea. Since the water flow will also differ with the tide schedule, the water velocity and/flow should be assessed for each experiment conducted. ii) For ports, if it is an open dock (i.e., not behind locks, gates or sluices), it is recommended that the experiments are performed at the time interval between low and high tide, i.e., when the water flow is coming from the sea. The reason for this is that during this time interval the litter flux will be directed inland and the collection technologies deployed in ports are usually installed with the inlet oriented to the sea. iii) For docks behind locks, it is not expected that the tides and river flow will influence the results if the locks are closed at the time of the experiment. In this specific case, the wind speed and direction will play a bigger role in the transport of plastic. Thus, not only the wind parameters should be monitoring but also should be considered when planning the experiments in locked docks. All weather and hydrologic data available or measurable needs to be recorded at the time of each experiment (Section 8.3.2).

Load of materials to be released and timeframe: Different loads of test materials will be tested in different release-catch experiments (Section 7.5). It is recommended that a representative amount (mass/number) is selected considering the baseline pollution levels. However, a second higher load could be selected to test the system in a more challenging scenario of higher pollution levels. In addition, the rate of release of the items needs to be mentioned, i.e., a new item is released every 10 seconds, or all items are released together. Accordingly, the time required for the test materials to reach the technology location needs to be estimated based on the weather and hydrologic conditions. Based on this estimation, the experiment time duration can be defined.

4. **Observations and materials recovery**

   During and after the release of the test materials, it is recommended that key locations have observers positioned to record the test materials transport along the path for both water and riverbanks: at the release point, at the technology and after the technology. Collecting devices, such as the Ferrybox Sampling Device (INSPIRE D1.2 - Monitoring and analysis protocols) and nets, can be used to assist in the recovery of the test materials that are not inside of the technology. These fractions need to be assessed separately and, therefore, should be stored in different containers or bags labelled accordingly.

   Example for technology code PP and release-catch experiment number 01 for that technology:

   - PP01_TL_IC – Test materials intercepted and inside the collection net/cage – used to calculate *%PR*, part of *%I* and *%C*.
   - PP01_TL_ILO – Outside loss: test materials intercepted but lost outside of the technology (trapped elsewhere) – used to calculate part of *%I* and part of *%L*.
   - PP01_TL_ILP – Retaining loss: Test materials intercepted but were not retained during operation (PP01_TL_ILP-O) or during the step to retrieve them from inside the collection cage/net (PP01_TL_ILP-E) – used to calculate part of *%I* and part of *%L*.
   - PP01_TL_E – Test materials not intercepted – used to calculate *%E*.

5. **Analysis of the quantity and characteristics of the materials collected by fraction**

   The test materials from each fraction need to be quantified by number or dry weight and classified by their characteristics. For this, each item will be labelled (e.g., PP01_TL_IC_001) and listed in a table. At this step, the plastic removal efficiency can be calculated according to Chapter 8 for the conditions tested. Based on the characteristics of the fractions, it can be reported for what types of plastic litter the technology is efficient or not.

6. **Repetition of the release-catch experiment for replicate data and in different scenarios**

   It is recommended that each release-catch experiment is repeated to analyse the reproducibility of the data obtained. Furthermore, more release-catch experiments can be conducted with different: release points, test materials (including different loads), and weather and hydrologic conditions (including tide period). It is advisable that at least two release points and two weather and hydrologic conditions are tested, resulting in 4 combinations of experiments. The experiments for the two release points (inlet and distant) can be performed on the same day with two groups of similar test materials. It should be noted that all test materials need to be recovered before performing another experiment or another colour needs to be used to tag the materials.

7. **Analysis and reporting of the technology removal efficiency under different scenarios**
   According to Chapter 8.

4.1.2. Release-catch experiments for floating macroplastic detection using remote sensing techniques

A custom protocol has been developed for calibrating and validating the remote detection of floating macroplastic using bridge-mounted camera systems. This protocol is still based on the general principles outlined in section 4.1.1.

In the operational setup, multiple bridge-mounted cameras will be installed along the river's cross-section, allowing for the collection of data on the plastic flux at that point. Release-catch experiments will enable us to validate the performance of the algorithms used to process the camaras images at two levels: i) validating the detected objects within individual images, and ii) validating the derived plastic flux (i.e., each object appearing in multiple images should only be counted once). If necessary, these experiments can also be used to calibrate the algorithm for improved accuracy.

The deviations for the protocol described in section 4.1.1 are the following:

- **Selection and preparation of tagged test materials**
  The main points described in section 4.1.1 are the same, being recommended that the floating test materials selected are those described in section 7.1 and aligned with the pollution measured through sampling campaigns and field observations conducted before the installation of the camaras. The aim of the bridge-mounted camera systems is to monitor macroplastics, starting from 2.5 cm. However, it is crucial to include a variety of test materials spanning a wide size range to accurately assess the detection limits of the cameras based on this characteristic. Using test materials of varying sizes eliminates the need to monitor a full tidal cycle (i.e., different water levels) with different release-catch experiments.
  Differently from section 4.1.1, it is recommended that both tagged (e.g., in this case painted) and not tagged materials are tested in different release-catch experiments. If the test materials are painted before the release in the field to be able to distinguish them from the litter in the environment, this can impact the detection accuracy of the camaras. For example, camaras have lower accuracy in detecting transparent items, when compared for example to fluorescent orange tagged items. Thus, we recommend a two-step release-catch experiment approach:
    - Step 1: Use painted test materials to validate the protocol and to gather information on the transport of the items at the location.
    - Step 2: Repeat with non-painted test materials.

  Even though all the items selected as test materials can be plastic litter, it is recommended that this type of release-catch experiment uses floating litter found in the environment that covers all the most relevant litter classes (Section 7.1). The use of other litter than plastic litter is important since the bridge-mounted camaras can also be validated to distinguish the plastic from non-plastic items.

- **Preparation of the site for the release-catch experiments**
  The recommendations described in section 4.1.1 apply here. This phase is more critical when performing the step 2 mentioned above, with the non-painted test materials.

- **Release/catch experiments**
  In rivers, the following parameters are relevant to investigate during these release-catch experiments, as they are expected to impact the effectiveness of the detection technology:
  - Tide: In tidal rivers water levels can fluctuate significantly. Conducting release-catch experiments at both high and low water levels will provide valuable information about the limits of object detection and the measurement of plastic flux:
    - High tide: At high tide, data is captured with high resolution. During post-processing, these images can be downscaled to simulate lower water levels. By using test items of varying sizes in the release-catch experiments, this approach allows for an assessment of the system's detection limits.
    - Low tide: At low tide, the overlap between the area covered by two neighboring cameras is high. While some items might be missed at high tide due to incomplete river coverage; the increased overlap at low tide helps in validating the plastic flux product. This overlap facilitates cross-referencing items across different images from various cameras, helping to determine if the plastic flux is being overestimated due to double counting.
  - Sun position: The sun's relative position, in combination with cloud coverage, affects the shadow area in the images, which in turn impacts object detection due to shadowing effects. To assess this, experiments should be conducted both when images are influenced by the shadows of the bridge and when shadows are minimal. Additionally, direct sunlight can cause sun glint, which might be mistakenly identified as plastic. For example, for the Temse bridge case (INSPIRE Scheldt demo site), shadowing effects are minimal when the sun is positioned in the south.
  - Weather: Weather conditions can affect floating debris detection accuracy by increasing the complexity due to higher levels of organic matter, foam or turbulence.
    - Wind: Wind-driven waves, generated by the friction between wind and surface water, can create high turbulence and foam. These conditions may cause plastic debris or foam to be misidentified.
    - Rain: Rainfall increases water turbidity and the amount of organic matter (e.g., reeds and wood). Higher turbidity makes it more difficult to detect transparent plastic items, while floating organic matter may be mistaken for plastic debris.

  Conducting a release-catch experiment one day after heavy rainfall and/or during high wave activity helps to assess the system's efficiency under varying weather conditions.
  While it would be beneficial to conduct release-catch experiments for all these conditions, INSPIRE will prioritize tide and sun position to ensure practical feasibility. Although no specific release-catch experiments will be conducted under various weather conditions, the weather data will be collected and recorded for all experiments performed.

  For tidal rivers, it is recommended that at least two points for release/catch are selected with one being upstream and the other downstream from the bridge (Fig. 4). Then, the experiment can be repeated on the same day when the tide changes the river water flow direction: experiment 1 (water flow to the sea): release point at location A and catch point at location B, and experiment 2: release point at location B and catch point at location A. Two small vessels can be used at the two points to release the test materials but also to try to capture them back, reducing the impact of the experiment and enabling the reuse of the test materials for other experiments. For the Step 1 (of the two-step release-catch experiment approach), it is highly recommended that two different colours are used to tag the similar test materials used in experiment 1 and 2, since some test materials from experiment 1 can be captured during experiment 2 if they were not collected.

Fig. 4. Schematic of release-catch experiments for validation of bridge-mounted camaras. Legend: 1 and 2 – Locations A and B for the release/catch points depending on the tide.

- **Observations with the bridge-mounted camaras**
  In the adapted 4.1.1 protocol, it is only relevant to label and characterize the test materials before the experiment and not after. The list of test materials released and their characteristics for each experiment can be matched with the images captured by the camaras and their outputs after image processing concerning the detection of litter. This will allow to identify the type of items that are not being detected by the bridge-mounted camaras, providing useful information to improve their accuracy through machine learning. A percentage of the accurately detected litter from the total litter released, *%LD(#)*, or the percentage of the accurately detected plastic litter from the total plastic litter released, *%PD(#)*, can be calculated for each experiment to have as reference on the error of the camaras, following Eq. 1.

$$\%D(\#) = \frac{\#(items\ accurately\ detected)}{\#(items\ released)} \times 100 \qquad \textit{Eq. 1}$$

4.2. Manual cleanup activities assessment

The release-catch experiments are not applicable for the assessment of all plastic collection/prevention solutions (limitations explored in section 8.3). This is the case of manual cleanups, which can be conducted by experienced participants or by volunteers. Thus, a different approach was developed and is proposed in this subsection of the document. This protocol was specifically developed for manual cleanup activities in riverbanks; however, it can also be applied to other locations, such as beaches or parks in urban areas. The protocol was prepared based on INSPIRE consortium previous experience with manual cleanup activities or obtained during the first year of the project and based on the discussions carried out during dedicated meetings.

It should be noted that in the scope of INSPIRE, manual cleanups will be combined with drone-based observations. Because of this, this type of litter observation was included in the protocol and is seen as source of added value to cross-validate other data obtained, with the potential of being used as the main assessment tool in the future. Nevertheless, the protocol can be carried out without the drone-based observations, but it is recommended that they are included. During the cleanup activities, the data and metadata should be recorded as established in INSPIRE task 1.3 - Monitoring of macrolitter.

The INSPIRE protocol includes the following steps:

1. **Area and subareas delimitation and first assessment**
   Before any manual cleanup activity is carried out, the area of the activity must be well defined and limited. Since the manual cleanup activity can also have a citizen science component, with the engagement of local citizens, it is crucial to clarify the selected area to all the participants in the cleanup, ensuring that the activity can be repeated under the same conditions.
   The area selected may be divided into subareas, based on different characteristics of the riverbanks, or equal areas along the riverbank. Each subarea should then be allocated to the expected number of required participants to manually clean it under the defined timeframe (e.g., 2 h). Based on this, the required number of total participants can be estimated.
   It is recommended that the selected area and corresponding subareas are visited before the activity for a first visual assessment of the necessary effort (i.e., for the amount and type of litter present) and to identify potential risks for the safety of the participants.

2. **First drone flight**
   Drone flights will be executed on the day of the manual cleanup to record images of the selected area/subarea. This can be done for different flying heights (e.g., 12, 20 or 50 m) to have different resolutions with the equipment used, enabling to detect litter of different size ranges. In INSPIRE, the images collected in this step and in step 5 are later processed with MAPEO Water (VITO), being quality checked, calibrated and georeferenced. After, artificial intelligence (AI) will be used to distinguish the litter on different background types, leading to the detection of litter on the riverbanks.

3. **Manual cleanup activity**
   The manual cleanup activity should be carried out during this step, for the specified time and with a known number of participants allocated to the defined area/subarea. During this type of activity it is expected that the participants will collect all the litter found, not just plastic. Two options are possible: a) all the litter can be collected to the same bag, or b) the plastic items could be already collected into separate bags according to selected categories (e.g. plastics versus non-plastics). If the second option is selected, it is important to inform the participants on the type of items to be collected into the bag for the plastic fraction, since some items (e.g., cigarette butts) might be misplaced during this step and lead to errors in the assessment. The advantage of collecting all the litter into the same bag is that it can be later processed, and the plastic items separated by experts, leading to more robust results in macrolitter enumeration and characterization. One of the main advantages of already

separating litter fractions during collection is that the litter bags not used for further analysis can be promptly and correctly disposed of into the appropriate waste containers according to the local waste management system. Furthermore, it will reduce the required efforts to perform the manual cleanup efficiency assessment, since separating each item will take time and will require the transport of the litter to the location where it will be separated and analysed.

If a subarea of the total area defined for the cleanup is used to assess the plastic removal efficiency, it is critical that the bags of litter/plastic collected for this subarea are correctly labelled (e.g., BE07_ RC_01R1P – plastic fraction bag R1 collected at subarea 01 during a riverbank cleanup, campaign 07 in Belgium) and separated from the rest. The number of items collected in each bag (see step 6) and/or the weight of it can be counted/measured already in the field and registered for the labelled sample.

The effect of the tides must be considered when scheduling the manual cleanup activities, using national/local monitoring information available, and aiming to start when low tide is reached and most of the riverbank area is exposed. Thus, in areas affected by tides, it is recommended that the participants start near the low tide line and progress inland as the water level rises. It is known that the low tide line on the riverbank (due to the water depth) and the time when will occur will change each day because of the Moon cycle. Therefore, the day selected for the cleanup can consider this, specially to select a day when the low tide line can be accessed at an adequate time of the day to conduct the cleanup activity (e.g., 10:00 am).

4. **Inspection of the cleanup area or subareas**

For the selected area or subarea of the cleanup for which the plastic removal efficiency will be determined, a few “verification experts” (minimum two people for a 200 m^2 subarea) are allocated. The verification experts are experienced participants in the cleanup, ideally plastic pollution researchers or trained by one, that go over the sections of the area/subarea already cleaned by the participants to identify missed litter.

When a missed item is found, the verification expert:

- Takes a photograph of the missed item
- Records the GPS location
- Registers the type of item with the J-code [12], as well as main characteristics such as size class and colour
- Does not collect the item

For small subareas, it is recommended that a team of two verifications experts works together, with one taking the picture and the other registering the other data about the missed item. Even though the JRC Floating Litter Monitoring App (in test phase) is designed to detect floating litter, it can be used under the “test” option to record the required data in the field during this step, except for the picture of each item which needs to be currently taken separately. If more than one team of verification experts is allocated, an adequate strategy to delimit the area scanned needs to be in place to avoid that different teams are registering the same item, making the progress less effective and potentially leading to errors in the assessment (i.e., the GPS error may not allow to identify it as the same item).

The verification experts should start assessing the area immediately after the cleanup participants start to move inland to avoid losing riverbank area in case it is a tide affected riverbank.

If only one subarea of the total riverbank cleanup area is selected for this step, the subarea selected should be randomly selected to avoid that more effort is put on the selected subarea compared to the others.

5. **Second drone flight**
   After the cleanup is completed, step 2 is repeated.

6. **Analysis of the characteristics of a subsample of the materials collected**
   If the number of items collected in each bag from the area/subarea selected was not quantified in step 3, it must be assessed in this step. Moreover, if litter fractions were not separated in the field, the number of plastic and non-plastics items can be assessed when counting the total litter. Likewise, the items can be divided into different litter size classes (e.g., macrolitter/macroplastics, mesolitter/mesoplastics) during this step.
   After that, depending on the amount of litter collected and the resources available, all the bags collected (plastic fraction or all litter fractions) or a representative subsample can be transported to the laboratory and more carefully analysed for the material (e.g., polymer type, metal, paper), size class, shape, colour, transparency, dry mass, and J-code [12]. This subsample will provide data on the type of litter present at the location, which can give information on the baseline pollution levels, if it is the first cleanup, or on the type of litter that accumulates at the location between cleanups. Finally, it can provide intel on the type of items that the participants of the cleanup are collecting, in comparison to the items they are missing. This can be used to improve the efficiency of the cleanup activities, by finding gaps in the participants knowledge on plastic pollution. This can be tackled during the briefing usually held before the cleanup starts or on a dedicated workshop.

7. **Analysis of the data recorded and validation of drone-based observations**
   All the data collected during the above steps needs to be complied and jointly analysed. This is particularly relevant for the drones' flights images and subsequent results, which need to be cross validated with the visual observations in the field/laboratory and the litter characterization.
   The drone images can be analysed for individual items sitting on top of the riverbank. However, if too much litter is covering the area, the images can be alternatively analysed for the area covered with litter before and after the cleanup. An example of this would be in areas near polluting industries, where plastic pellets (not usually collected in manual cleanups) are covering the riverbanks, and it is not feasible to count all the pellets before and after the cleanup.
   During this step, the type of items missed by the drones can be identified and used to improve the method of the drone-based data collection in the field, as well as for the machine learning of the litter detection AI. Furthermore, the distribution of the litter missed by the participants, if detected in the drones' images and when crossed with the data collected by the verification experts, can provide insights into potential critical locations and type of litter for future cleanup activities.

8. **Analysis and reporting of the manual cleanup removal efficiency**
   The data collected can be used to report the removal efficiency of litter, *%LR(#)*, and of plastic litter, *%PR(#)*, using Eq. 2.

$$\%R(\#) = \frac{\#(items\ collected)}{\#(items\ collected) + \#(items\ missed)} \times 100 \qquad \textit{Eq. 2}$$

Even though the field manual observations and drone-based observations have shortcomings and still may miss items, we consider that the values reported from following this protocol will provide a fair approximation of the litter and/or plastic removal efficiency of a cleanup activity, particularly when the two observation methods are combined.

While no release-catch experiments were planned for manual cleanup activities, a possible approach would be to collect some plastic/litter items from the cleanup area/subarea, tag them (e.g., adding a small mark) and place them randomly (or in key locations) in the area/subarea selected for the assessment just before the start of the cleanup. Then, their collection or not by the participants in the cleanup could be evaluated at the end.

4.3. Underwater riverbed macroplastic observations

The release-catch experiments were not selected as the method for assessing the plastic removal technologies that target the litter accumulating below water, on the riverbed or in suspension in the lower part of the water column (section 8.3). These technologies are at a less developed stage, compared with the technologies that target the water surface and sub-surface, and so is the study on their efficiency in removing plastic and on the monitoring strategies for underwater litter [13]. Thus, in this section, we only list existing options to assess the efficiency of this type of technology. This will enable us to identify the feasibility of applying any of these options to the single technology in INSPIRE that targets riverbed litter (Chapter 3).

The following options have been identified:

- SONAR (Sound Navigation Ranging) systems [13–15]
- Acoustic Doppler Current Profiler [16]
- LiDAR (Light Detection and Ranging) instruments [17]
- RGB and/or snapshot hyperspectral imaging (HSI) camaras [18,19]

SONAR systems are commonly used for the acoustic detection and identification (size, shape and distance) of underwater objects (e.g., shipwrecks or marine life), through the analysis of the time, strength and frequency of the returning signal, making it useful for military, commercial and research applications. Echo sounding is the technique used to measure the depth of the water body (bathymetry), using a SONAR system for ranging (in this specific case also called echosounder) or another acoustic instrument (such as the Acoustic Doppler Current Profiler). In echo sounding, the depth of the seabed is estimated, and the seafloor is mapped, through the analysis of the time taken for the transmitted sound waves to reflect, making this technique useful for vessel navigation safety.

Valdenegro-Toro [14] proposed the use of Deep Neural Networks to survey and detect marine litter in the bottom of water bodies such as on the seafloor, lake bottom, and riverbeds, based on Forward-Looking SONAR images. The study developed techniques to detect and recognize litter in this type of

SONAR images and proposed the use of Autonomous Underwater Vehicles to survey and detect submerged marine litter with SONAR. Broere et al. [13] explored the use of echo sounding as a low-cost method to quantify and identify suspended macroplastics. For that a single beam SONAR with Compressed High Intensity Radiated Pulse (CHIRP) technology was used. The study concluded that echo sounding can be used for the detection of suspended riverine macroplastics, allowing to count the litter items and exclude the fish. While the technology still needs to be further assessed for this type of application, it can potentially be used to identify different litter types [13]. More recently, Flores et al. [15] performed tests in standing water using two imaging SONAR technologies: Adaptive Resolution Imaging SONAR and a low-cost side-scan SONAR, with the first technology also being tested in flowing water (ca. 0.8 m s^{-1} at a shore channel in the Netherlands). Both technologies performed well in detecting macroplastics in standing water (down to the size of 1 cm^2 with the Adaptive Resolution Imaging SONAR), but the detection decreased in flowing water. This last study recommended that one Adaptive Resolution Imaging SONAR is used in motion, or two SONARs are used simultaneously to provide enough 3D spatial information and images of the suspended macroplastics. The low-cost side-scan SONAR is also promising for this type of application, but it provides lower resolution images.

In parallel, Boon et al. [16] performed tests in a basin, a harbour and a river using a high-end Acoustic Doppler Current Profiler to explore its potential use for monitoring suspended macroplastics. This acoustic device uses the Doppler effect, by transmitting sound waves that are reflected to the instrument when encountering particles in suspension in moving water. These systems work with a series of acoustic transducers that emit and receive from different directions, which can be installed in research or other type of vessels and unmanned underwater or surface vehicles, or can be mounted on stationary structures (e.g., mooring buoy) or on the water body floor/bed. This is an important technique in oceanography due to its use to measure water current velocity and to study the transport of organic and inorganic matter in the Ocean. In the cited study, different items could be detected by this technique, but more developments are needed for this specific application. Since is a highly used device and part of measurement networks in place for current flow and suspended sediment, it could provide a long-term and continuous monitoring for the estimation of suspended plastic transport, and even applied to historical data sets to reconstruct the trends [16].

LiDAR is another remote sensing method that has the potential of being used for monitoring and observations of underwater macrolitter and macroplastics. It uses light, through a pulsed laser, in combination with other data recorded (e.g., with GPS and Inertial Navigation System) to measure distances and obtain three-dimensional data on the shape of the scanned Earth surface. LiDAR has been used for diverse applications, including forestry and land management, for better accuracy in robots' navigation, for surveying and mapping construction sites, for obtaining precise data needed for optimizing renewable energy resources such as wind, among others. It has been applied to plastic pollution detection by PlasticDetect [17], that uses a multispectral LiDAR instrument for real-time detection, classification and tracking of marine plastic pollution (down to 0.5 or 5 mm). Through measurements from a ship or other mobile or fixed platform, it provides quantitative information on plastic litter size and location.

RGB (red, green, blue) and/or snapshot hyperspectral camaras can be used for identifying and locating underwater plastic litter, which can be installed in autonomous underwater vehicles for example. Niu et al. [18] proposed an improved underwater enhancement algorithm for recognizing and localizing underwater litter in images captured, which was tested with the RealSense D415 – an RGB-D (red, green, blue and depth) camera –, used for image and location acquisition. Hanson et al. [19] analyzed the feasibility of the imaging methods, with both RGB and snapshot hyperspectral camaras, for macroplastic detection, especially in river environments. This work showed that imaging-based systems are a viable option for automatic plastic litter detection in rivers, with high accuracy even with relatively low spectral resolution hyperspectral sensors. The snapshot hyperspectral imaging exhibited more promising results than RGB imaging. Nevertheless, the imaging methods still present substantial limitations since minor changes in the environment (e.g., light penetration and turbidity) can lead to differences in the appearance of underwater objects, causing challenges such as noise interference, low contrast, and blurred textures in underwater optical images [18].

The location to be cleaned by the underwater plastic removal technology could be surveyed with one or more of the options listed above, before and after the cleanup. That information, combined with the analysis of the plastic and other litter removed by the technology, can provide some information on the efficiency of the cleanup. However, due to the current challenges and limitations presented, if such an assessment is carried out, its results should be interpreted with caution and as an indicative value.

5. Protocols for technologies installed in urban infrastructure

This section describes the proposed protocols for the assessment of technologies deployed in urban infrastructure or connected to it, namely in boats washing areas at marinas, drinking water treatment plants (DWTPs), wastewater treatment plants (WWTPs), and other industrial facilities (e.g., power plants) and water infrastructure (e.g., storm water systems). The main aspects in common between these protocols are that the deployment locations are inland and/or the technology water outlet is directed inland, with the untreated and/or treated water having a confined flow during part of its path. In addition, the plastic litter size targeted can go lower than in the previous section, to nanoplastics (< 1 μm).

5.1. Assessment of technologies deployed inland

This subsection presents two protocols designed for technologies deployed inland, such as part of the sequence of treatments in a WWTP, in which the untreated water inlet will not come directly from a river. A first protocol, referring to a release-catch experiment, more nonexclusive and applicable for technologies collecting nano-, micro- and/or mesoplastics, is described. Considering the specificities of tyre wear particles, a dedicated protocol was created for these materials.

5.1.1. Release-catch experiments with plastic particles

The goal of this protocol is to assess the plastic removal efficiency for technologies installed in urban infrastructure and deployed inland, being applicable to technologies in WWTPs, in DWTP unless they are located at the abstraction point, in boats washing areas at marinas, in storm water systems, and in other confined water flow networks in industrial facilities or in the urban water cycle. The focus of the protocol is on microplastics, but it can also be used for nanoplastics and mesoplastics. If the release-catch experiments target particles smaller than 1 mm (i.e., if no mesoplastics and large microplastics are used), the results can be reported only in mass if the number of particles removed is not assessed. While this protocol can be adapted to be used for any separation technology, it was specifically devised for filtration technologies.

The protocol was prepared based on the INSPIRE consortium experience with collection and prevention technologies, obtained during the first months of this project and from the input of the technology providers, in combination with the literature available [9,20,21].

The INSPIRE protocol includes the following steps:

1. **Selection and preparation of (tagged) materials**
   The materials selected for each technology assessment need to be aligned with baseline pollution levels measured before the technology deployment, but also with the size targeted by the technology.
   The main test materials option for this type of release-catch experiments are the laboratory-prepared mixtures of plastic particles (Section 7.2), which can be conventional and commonly found polymers, and/or biodegradable polymers. If feasible, these materials should be tagged to more accurately account only for the plastics released. More detailed information on the

methodology for the test materials selection, including amount, in sections 7.2.1 (Conventional and commonly observed polymers) and 7.2.3 (Biodegradable polymers).
Since this protocol focuses on microplastics, the mixtures of microplastics (and/or nanoplastics) should be reported as a table with labels for each group of particles, listing their characteristics, the mass or number, and corresponding percentage of the total amount of material released. PP01_TL_T_001 – example of label for technology code PP, release-catch experiment 01, group 001 of the total released: LDPE particles, mean Feret's diameter 200 µm, 2 g, 20 %, …) (Section 7.2). If mesoplastics and large microplastics (e.g., pellets) are used, each item should have an individual label, similarly to what is recommended in Section 4.1.1.
When evaluating a sequence of technologies, different test materials will be considered to assess the plastic removal efficiency when using a) a specific size range tailored to one technology target, or b) larger size range tailored to all technologies in the series.

2. **Preparation of the technologies and connections**

   It is recommended that each technology and corresponding connections are cleaned before each release-catch experiment. For this, all the different system components, including the feed reservoir, tubing, and other connections, should be rinsed with a flow/volume of ultrapure or distilled water. The type of water used for cleaning and for how long or volume used should be reported. We also suggest that the water used for cleaning is sampled for blanks (to evaluate airborne and other environmental contamination).

3. **Spiked water in feed reservoir**

   In this step, the tagged materials are added to:
   - Option a) Filtered real (waste/storm) water to be tested: obtained by vacuum filtration with successive smaller pore size if the smaller one is easy clogged, e.g., glass fibre membrane of 1 µm followed by cellulose membrane of 0.05 µm.
   - Option b) Ultrapure water, for experimentally determined maximum removal efficiency.
   - Option c) Unfiltered real (waste/storm) water to be tested, in case it is possible to distinguish the added materials from the ones already present.

   It is critical that the reservoir where the feed is stored is stirred throughout the experiment because of potential sedimentation of tagged materials at bottom of reservoir that will not enter the technology. If this is observed, the amount that stays at the feed reservoir corresponds to *%E*, the percentage of plastic litter that is not intercepted by the technology.
   - PP01_TL_E – Test materials not intercepted – used to calculate *%E* and *%I*.

4. **Technologies fed by the spiked water and water sampling**

   In this step, the technology or sequence of technologies are turned on and fed with spiked water. This should be performed for a defined volume or set time, according to the technology's limitations, such as fouling. The feed water flow and pump(s) operation parameters must be recorded and reported as experimental conditions for the release-catch experiment.
   During this step, the permeate (treated water) is collected at the outlet of the technology in a glass bottle (with metal cap or with aluminum foil between the cap and bottle). Alternatively, collection devices can be used, such as the Ferrybox Sampling Device (INSPIRE D1.2 - Monitoring and analysis protocols), with an adequate mesh size for the test materials being

used. It should be noted that the Ferrybox Sampling Device can only recover the test materials down to the lowest mesh size used and available from the supplier (i.e., 20 µm).
The necessary volume to sample will be defined according to the analytical method for micro- and nanoplastics detection and quantification. In case of a long duration release-catch experiment and large volumes of spiked water, it is recommended that more permeate samples are collected, to ensure that the plastic removal efficiency is not reduced over time. For experiments that use low volumes of water, it is advisable that all the permeate volume is stored and analyzed for the presence of plastic particles. The permeate samples should be stored in different containers and labelled accordingly. For example:

- PP01_TL_ILP-O – Retaining loss during operation: Test materials intercepted but not retained (found in the permeate) – used to calculate *%L* and part of *%I*.

It should be noted that for the release-catch experiments in this section, the outside loss as defined in section 8.3 (with example label PP01_TL_ILO) is considered null. Likewise, the "retaining loss when emptying collection net/cage" (with example label PP01_TL_ILP-E) is non-applicable.

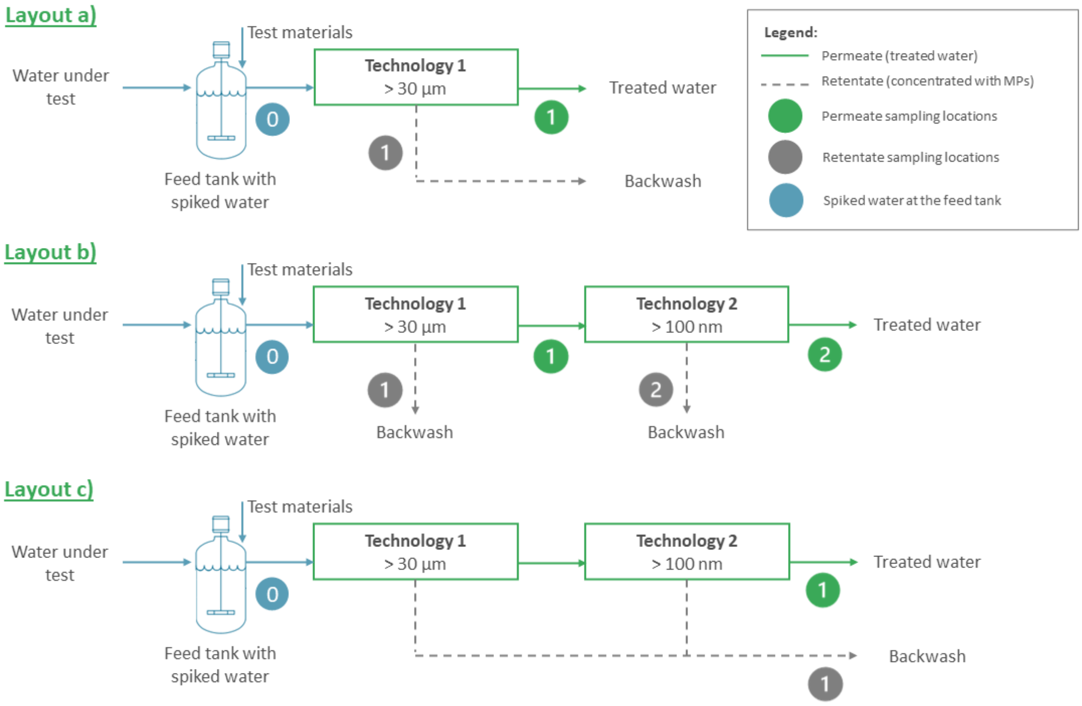

Fig. 5. Schematics of release-catch experiments for technologies deployed inland, under different layouts.

When assessing a treatment sequence with technologies in series, there are three options (Fig. 5) available to assess the retaining loss:

- Layout a) Each technology is first tested individually, with the treated water sample collected at its outlet.
- Layout b) All the technologies are assessed at the same time but individually, with the water samples collected after each technology. This option is only feasible if the experiment is runed over a long period and with a high volume of water. In addition, tap valves need to be installed to redirect for the necessary time the flow into the sample container.

- Layout c) All the technologies are assessed at the same time as one solution, with the treated water sample being only collected at the last technology outlet.

5. **Cleaning of filters to collect the retentate**
   The goal of this step is to collect the retentate (feed that does not pass through the filter). Depending on the technology and system installation, this can also enable us to recover part of the plastic litter in the tubing. The collection of the retentate follows the same considerations as the permeate (see step 4), with similar sampling methods and storage containers.
   If the system produces concentrated water, it can be directly collected and stored. After, the technology filter should be cleaned with ultrapure or distilled water. If applicable, any concentrated water still in the tubing can be collected by operating the technology pumps in reverse rotation (opposite direction from the experiment).
   If the system does not produce concentrated water, the particles retained by it can be collected by backwashing the filters, i.e., reversing the flow and increasing the velocity at which clean water passes back through the filter, obtaining concentrated water at the inlet. Alternatively, and if feasible, the filters can be removed and washed separately from the rest of the technology.
   The amount (i.e., mass) of particles collected during this step will give the amount intercepted and retained by each technology:
   - PP01_TL_IC – Test materials intercepted and retained – used to calculate *%PR*, part of *%I* and *%C*.

6. **(Optional) Recovery of system filter for visual and/or microscope inspection**
   If applicable, the technology filter could be removed for visual and/or microscope inspection. The goal of this step is to detect the materials that are still stuck in the filter and to assess if the filter can be reused and for how many times. For some technologies it might be adequate to clean the filter with specific chemicals to regenerate their permeability for water and impermeability for the plastic litter, increasing their lifetime and, potentially, the cost-benefit of the technology.

7. **Repetition of step 2 to collect remaining plastic litter**
   One extra step of cleaning can be carried out by running the system with ultrapure or distilled water, following the procedure set in step 2. The goal of this step is to ensure that all the plastic litter was removed during step 5, by collecting one control water sample to analyze for the presence of the test materials. This can help closing the mass balance of the test materials recovered at the end of the release-catch experiment. In addition, this will prepare the system for another release-catch experiment.

8. **Repetition of the release-catch experiment for replicate data and in different scenarios**
   It is recommended that each release-catch experiment is repeated to analyse the reproducibility of the data obtained. Furthermore, more release-catch experiments can be conducted with different: test materials (including different loads and mixtures), and water flows. Likewise, if unfiltered real (waste/storm) water is used with tagged materials, different tests can be performed for different water quality levels regarding e.g., organic matter, total

suspended solids, conductivity, and salinity. This will provide the optimal conditions and limitations for the use of the solution(s) to remove plastic litter.

Finally, as explored in step 4, different configurations of the solution can be tested by combining different technologies and testing them individually or together. If several technologies are being tested, it is recommended that they are first test individually (step 4 layout a) and, after, together (step 4 layout b or c).

9. **Analysis and reporting of the technology removal efficiency under different scenarios**
   According to Chapter 8.

5.1.2. Tyre wear and tyre wear leachables reduction assessment

This section provides a protocol for the efficiency assessment of technologies aiming at capturing tyre wear particles (TWP) in aqueous matrices. In INSPIRE, these technologies will be installed on the site of a WWTP for the Danube River case, where the systems´ TWP and tyre wear leachable reduction efficiencies will be determined for two types of aqueous matrices:

a) Effluent from a WWTP for combined wastewater
b) Road runoff stormwater from a nearby highway

The protocol specifies the treatment setup including test materials, the planned sampling points within the treatment setup, the sampling procedure, the sample types, the sample preparation, and sample analysis workflows.

1. **Selection and preparation of test materials**
   For the efficiency assessment no controlled-release experiment will be conducted, but environmental sample materials that typically contain high concentrations of TWP will be used for the assessment [22,23]. This approach prevents new, artificially generated TWP from being released into the environment. In addition, the physical-chemical properties of the TWP generated in the laboratory do not correspond to the properties of TWPs present in the environment [24], which is why the use of real samples is advantageous when examining the efficiency at close to full scale.

   <u>Two types of wastewaters are being used for the reduction tests:</u>

   a) <u>Effluent from a WWTP for combined wastewater:</u> The technologies will be installed on-site of a WWTP with a tubing system that enables them to be switched in and out of the effluent stream. This way it is not necessary to keep the effluent in a separate tank or to agitate it. Before entering the technologies, the effluent will be disinfected in a buffer container to prevent fouling. To assess whether the baseline concentration of TWP in WWTP effluent is above the Limit of Quantification (LOQ) preliminary investigations of WWTP influent and effluent will be performed. Wastewater influent and effluent will be sampled during a rain event because TWP concentration is expected to be much higher during a rain event compared to dry conditions.

   b) <u>Road runoff stormwater:</u> For efficiency assessment of the TWP reduction during stormwater treatment, the local partners will collect highway road runoff at a suitable location once strong rainfalls set in and after an extended dry period. The road runoff water samples can be stored in intermediate bulk container (IBC) storage tanks. To reduce transformation and loss of TWP particles, as well tyre wear leachables, the collected stormwater can only be kept for short period before the reduction assessments with the treatment system are conducted.

2. The sampling setup

The reduction efficiency assessment at the WWTP will take place with the two technologies installed to operate in sequence. The WWTP effluent can be directed into the system (a), but the sample intake can also be adapted to take samples from road runoff storage tanks (b). Samples are taken from the influent and effluent of the treatment technologies. This allows the reduction efficiency of the individual technologies, as well as the overall system performance, to be evaluated.

The efficiencies of the technologies in reducing TWPs and leachables will be evaluated. TWPs are part of the suspended matter, while leachables are dissolved in WWTP effluent and stormwater. Therefore, water and particulate matter samples will be obtained from the treatment system. For the manual collection of samples for both analyte groups taps will be installed at the sampling points. In addition, the WWTP may be able to provide automated samplers to collect samples of the WWTP influent and effluent. From the sampling taps 0.5 L water samples will be collected every hour over 8 hours by the WWTP staff. The one-hour subsamples will then be unified to create a representative master sample for the sampling point and period. The automated samplers may run autonomously over the same period. The sampling schemes of the setups a) and b) will be provided in the next section.

a) <u>Effluent from a WWTP for combined wastewater:</u> According to the sampling scheme in Fig. 6 with sampling point S1 through S6, both deployed Technologies 1 and 2 can be assessed comparing influent and effluent concentrations of the respective Technology. S1 and S2 are automated samplers that may be provided by the WWTP. Sampling points S3 through S5 are water taps and S6, indicated on a backwash line, is the Technology 2 retentate, which can be collected in the form of concentrated water. The WWTP effluent will be disinfected in a disinfection tank prior to reaching the first technology that is expected to reduce TWP down to 30 µm. The second technology will be assessed regarding its capability to eliminate particles down to the nm scale. Both technologies are also assessed regarding their capacity to reduce tyre wear leachables. The effluent of the system is then directed towards the WWTP effluent that discharges into the Danube.

b) <u>Road runoff stormwater:</u> The efficiency assessment for road runoff stormwater will take place the same way as with the WWTP effluent. Here extra care must be taken to ensure homogenization of the stormwater and to prevent settling in the tank storing the untreated road runoff water. The sampling scheme in Fig. 7 provides the possibilities to obtain inflow and outflow samples as well as backwash samples, similar to Fig. 6. S1 and S2 do not exist in this scheme as the system is not connected to the WWTP anymore, S3 will be used to determine the analytes concentrations in the stormwater. The total amount of retained TWP can be determined from the analysis of the backwash.

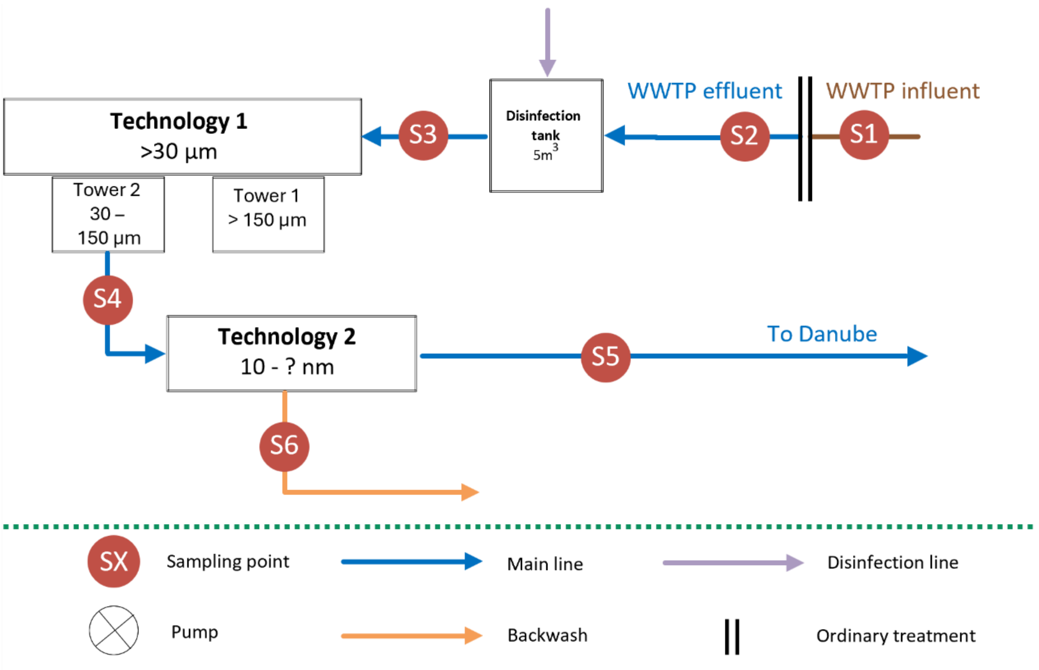

Fig. 6. Schematic of the planned experimental setup for the full-scale efficiency assessment using WWTP effluent.

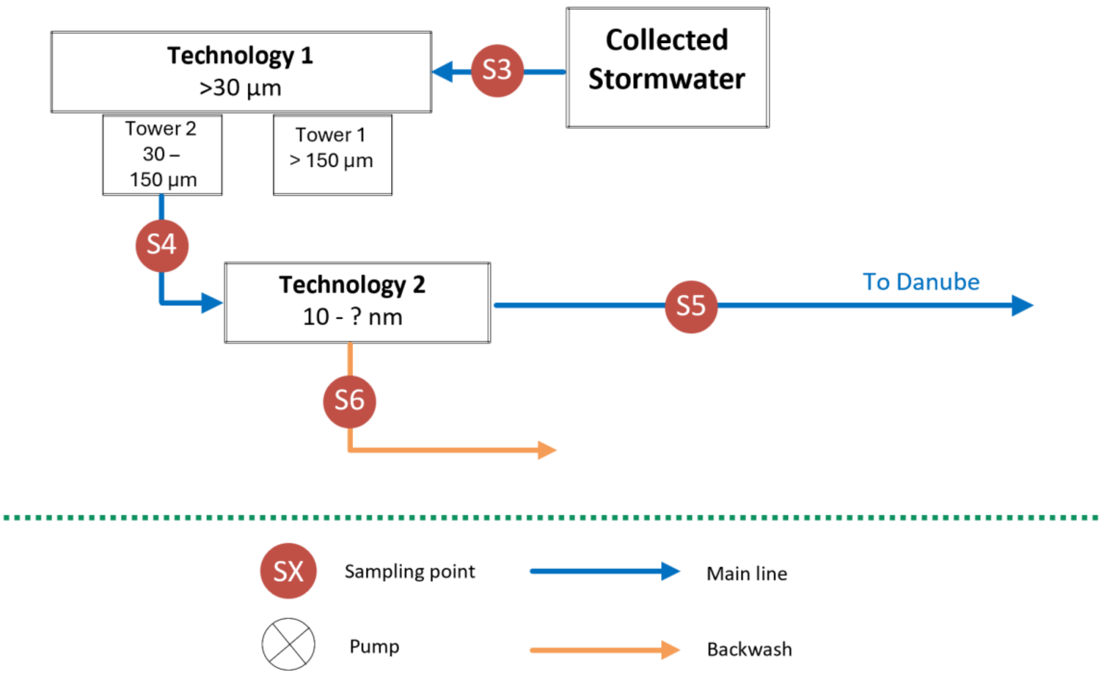

Fig. 7. Schematic of the planned experimental setup for the full-scale efficiency assessment using collected stormwater.

3. Preparation of the technologies

a) Effluent from a WWTP for combined wastewater: After installation of the system on-site, the Technologies and tubing will be rinsed with water. For the on-site efficiency assessment, the technologies will be connected to the effluent stream for most of the time and the efficiency assessment will take place during ongoing operation.

b) Road runoff stormwater: Prior to the efficiency assessment with stormwater the technologies need to be disconnected from the effluent and need to be connected to the stormwater storage container. Here the whole tubing system and the technologies will be thoroughly rinsed with tap or rainwater to minimize carryover from previous tests. As the sample volume in this case is limited, the operation flowrate of all technologies will be adapted to enable time-resolved sampling. Once the system has been cleaned blanks will be collected.

4. Sampling

The sampling is intended to be performed by the WWTP personnel. The person(s) responsible for sampling will do so over the time of approximately one shift (8 h). Ideally, the samples will be prepared right after they have been collected.

The automated samplers installed on-site can be used to collect samples to obtain an overview of analytes entering and leaving the WWTP.

The retentate container of Technology 2 may be accessed to determine how much particulate matter has been retained. This may potentially be used to close an occurring mass balance or to characterize the retentate.

Sampling using the taps:

1. Prepare a large opaque sampling container with 4 L capacity to be kept in the fridge
2. Open the tap and discard the first flush to rinse the tap pipes and tubing
3. Collect approx. 0.5 L of water in glass bottles covered by aluminium foil
4. Place the large sample container on a scale and transfer 500 g of the sample
5. Place the large sample container in the fridge again
6. Repeat this process for all 8 samples to reach a final volume of approx. 4 L in the container
7. If further sample preparation cannot ensue within 24 hours, freeze the samples

Sampling using automated samplers:

1. Rinse the automated sampler using type 2 water
2. Set the sampler to collect time-proportional samples: 0.05 L every 10 minutes, reaching 2.4 L after 8 hours or 7.2 L after 24 hours
3. If the temperature control is operable, set the temperature of the sample container to below 8°C
4. Collect the sample after the run is complete
5. Homogenize the sample and transfer it to opaque containers to store it in the fridge
6. If sample preparation cannot ensue within 24 h, freeze the samples

Sampling of Technology 2 retentate:

1. Collect the retentate according to the technology providers specifications
2. Transfer the sample to an opaque container and store it in the fridge
3. If the whole of the sample is too large to be stored homogenize the solution and store a representative subsample
4. If sample preparation cannot ensue within 24 h, freeze the samples

An overview of the planned sample types and volumes for each sampling point can be taken from Table 3.

Table 3. Sampling points with planned sample volumes and sample preparation, an x indicates that the sample will be processed.

		Type of sample at the sampling points:		
Sampling point	**Sample volume**	**Solids on filter: TSS > 2 µm**	**Solids on filter: colloidal fraction 20 nm – 2 µm**	**Water sample (filtrate)**
S1	7.2 L	X		X (< 2 µm)
S2	7.2 L	X	X	X (< 20 nm)
S3	4.0 L (8 x 0.5 L)	X	X	X (< 20 nm)
S4	4.0 L (8 x 0.5 L)	X	X	X (< 20 nm)
S5	4.0 L (8 x 0.5 L)	X	X	X (< 20 nm)
S6	4.0 L (8 x 0.5 L)	X	X	X (< 20 nm)

Blanks:

In addition to collecting samples, it is necessary to collect suitable blanks. For this purpose, test runs, executed before regular operation, using tap or rainwater can be performed, within these test runs samples can be collected according to the described sampling procedure. It may be sufficient to determine blanks for one of the setups – either a) or b). The configuration of the technologies and the availability of either tap or rainwater needs to be discussed with the technology providers and the WWTP operators.

5. Sample preparation

The initial sample preparation for TWP and leachables is a two-step filtration. Afterwards the retained solids will be further prepared for TWP analysis. The filtrate will be further prepared for leachable analysis.

Before filtration all filters need to be dried and weighed in (tara) and clearly labelled. This way it becomes possible to determine the mass of the retained solids on the filters. Afterwards, the filters need to be rinsed to limit the contamination of our samples by water soluble substances from the filter.

The filtration protocol uses a standard vacuum filtration setup.

Filter preparation:

1. Take the approximate number of filters you may require and place each in an unequivocally numbered storage box. Use a water-, heat- and freezeproof marker for this
2. Weigh the filters and note down the exact weight together with the filter number
3. Attach the filters to the vacuum filtration apparatus one after another and rinse each with approximately 20 mL of type 2 water

4. Shake the filters to dry them off a little bit and place them inside of their storage box again and close it

Two-step vacuum filtration:

1. Make sure enough clean glass bottles and Petri dishes are available for sample storage
2. Prepare all labels and make sure that the writing and the labels are water- and freezeproof
3. Make sure that enough 2 µm filters (membrane filter, cellulose acetate, 47 mm diameter) are available for the first filtration round
4. Rinse the sampling apparatus and all used glassware with a generous amount of type 2 water and discard the water afterwards
5. Homogenize the sample thoroughly
6. Place one filter membrane centrally inside of the filter tray
7. Load an aliquot of the sample into the funnel and start filtration
8. When the filtration flask is filling up transfer the filtrate to a suitable storage container
9. When the filter membrane is clogged discard the remaining liquid and replace the filter. Place the clogged filter in a clean plastic Petri dish labelled with the respective number of the filter
10. Continue with the filtration until the sample has been completely filtered
11. Determine the volume of the filtrate by weighing it and note it down, so that later mass concentrations can be determined

Afterwards a second filtration step for the recovery on NPs will be performed using 20 nm Anodisc filters. The use of the Anodisc filters works according to the same procedure just employed.
After vacuum filtration using the Anodisc filters, solid phase extraction (SPE) for leachable enrichment and conservation should ensue directly. 500 mL of the filtrate remaining after SPE needs to be frozen as backup sample.
To obtain blanks, it is necessary to repeat the two filtration steps with 4 L of type 2 water used in the laboratory for rinsing. Afterwards SPE will be performed, too.

SPE for leachable enrichment and preservation:
Pre-requisites:

- 100 g of sample (water) needs to be weighed into a pre-rinsed glass bottle
- All samples will be doped with 10 µL of an internal standard solution
- The influent and effluent samples will be prepared two times, one set will be spiked with 50 µL of a doping solution
- SPE cartridges and sample bottles need to be labeled unequivocally
- The sample hoses need to be pre-rinsed using type 2 water

SPE cartridge conditioning procedure:

1. Place the cartridges in the SPE module
2. The vacuum does not need to be connected; conditioning ensues using gravity
3. Administer 3 mL of methanol to each cartridge using an adequate, clean pipette
4. Administer 2.5 mL of type 1 water before the methanol from the previous step elutes completely
5. Administer another 2.5 mL of type 1 water before complete elution of the water

6. Close the valves when only about 1 cm of water remains visible in the cartridge
7. The cartridges are now ready for SPE

SPE procedure:

1. Plug the conditioned cartridges into the SPE module
2. Homogenize the samples by giving the bottles a good shake for 15 s
3. Connect the labelled cartridges to the respectively labelled sample bottles
4. Turn on the vacuum pump
5. Set the drip rate to approximately 1-2 Hz using the knobs
6. The drip rate must be monitored throughout the SPE run and corrected if necessary
7. Tilt the sample container to completely empty it, afterwards lift the sampling hose to guarantee complete sample transfer
8. Check again that all sample bottles were connected to the correct cartridges, if not correct labelling
9. Disconnect tubing
10. Dry the SPE cartridges carefully using a gentle stream of nitrogen gas
11. Make sure the label on the cartridges is intact and place them in the freezer at -24 °C
12. Rinse the SPE tubing with at least 1 L of type 2 water

<u>Drying and weighing in of the filter membranes:</u>

The filters containing the formerly suspended solids also need to be prepared for shipment and analysis. To this end they will be dried and weighed following the following protocol:

1. Place the filters individually in glass Petri dishes
2. Label the filters unambiguously again if the original labelling faded
3. Spread the Petri dishes evenly in the heating cabinet
4. Set the temperature of the oven to 75 °C
5. After 4 h take out one representative filter from the bottom of the cabinet and weigh it
6. Wait another 4 hours and weigh it again
7. Only take out all the filters once the weight of the filter does not change anymore
8. When constant weight for the reference filter is reached wait 4 more hours
9. Afterwards weigh all filters, note down the weights of them and store each filter individually inside of one clearly labelled Petri dish each

6. Shipment

After sample preparation, the samples and blanks need to be shipped to be analyzed in the laboratory. The Petri dishes containing the filters need to be sealed and labelled properly. Afterwards they can be shipped at room temperature. The SPE cartridges should be shipped frozen to prevent degradation. 200 – 500 mL of ultrapure water used in the laboratory needs to be shipped frozen in a glass-bottle as solvent blank.

7. Analysis

<u>Leachables:</u> Once the SPE cartridges arrive in the laboratory they will be kept frozen. Before instrumental analysis can commence, the SPE cartridges will be eluted. The eluate will be filtered, transferred to a vial and stored in the freezer until analysis. Leachable quantification will be performed using liquid chromatography (LC) coupled to a triple quadrupole mass spectrometer (MS) operating in multiple reaction monitoring (MRM) mode. Finally, the

obtained concentration will be corrected for the SPE enrichment factor to obtain the actual sample concentration. The leachables listed in Table 4 are intended to be measured in the collected samples.

Table 4. Leachable substances to be investigated.

Full name	Sum formula	Cas number	Abbreviation
Diphenylguanidine	C13H13N3	102-06 -7	DPG
Hexamethoxymethylmelamine	C15H30N6O6	3089-11-0	HMMM
Benzothiazole-2-sulfonic acid	C7H5NO3S2	941-57-1	2-SO3H-BTH
N-Cyclohexyl-N´-phenylurea	C13H18N2O	886-59-9	
Dicyclohexylamine	C12H23N	101-83-7	
Dibenzylamine	C14H15N	103-49-1	
Cyclohexylurea	C7H14N2O	698-90-8	
Triphenylguanidine	C19H17N3	101-01-9	
N-Hexyl-N´-phenylurea	C13H20N2O	1142-07-0	
N-Butyl-N´-phenylurea	C11H16N2O	3083-88-3	
N-(1,3-Dimethylbutyl)-N′-phenyl-p-phenylendiamin	C18H24N2	793-24-8	6PPD
N-(1,3-Dimethylbutyl)-N′-phenyl-p-phenylendiamin-quinone	C18H22N2O2	n.a.	6PPD-Q
Benzothiazole	C7H5NS	95-16-9	BTH
Methylbenzothioazole	C8H7NS	120-75-2	2-Me-BTH
Mercaptobenzothiazole	C7H5NS2	149-30-4	2-SH-BTH
Hydroxybenzothiazole	C7H5NOS	934-34-9	2-OH-BTH

<u>TWP:</u> For TWP analysis two extraction approaches will be used. One is based on acid digestion for the quantification of Zn [25,26] and the second one is based on extraction using methanol for the quantification of organic markers [27]. Additionally, there are separate approaches for handling of the colloidal fraction (< 2 µm and > 0.02 µm) and the suspended matter fraction (≥ 2 µm).

<u>TWP suspended matter fraction (≥ 2 µm):</u>

1. Zn analysis:
   For the first approach based on Zn, approximately half of the filters will be taken, and the attached solids will be transferred to a 50 mL centrifugation tube for density separation. Solids present in the Petri dish will be transferred as well. The used filters will be weighed to determine the amount of solid remaining on the filter. After density separation, the light fraction containing TWP will be transferred into a crucible for incineration. The remaining soot will be digested using microwave assisted digestion, where a mixture of HNO_3 and H_2O_2 will be used for digestion. The generated solution will then be diluted with ultrapure water as preparation for inductively coupled plasma mass spectrometry quantification of Zn. With the obtained Zn concentration, it becomes possible to determine the TWP concentration of the overall sample.
   To this end the mass of Zn present after incineration will be determined using the obtained concentration and digestion volume. The TWP mass that must have been

present in sample before incineration can be calculated based on a conversion factor equivalent to the mass fraction of Zn in tyres. Now the proportion of TWP of the analyzed subsample mass can be determined and should be representative for the mass fraction of TWP of the overall sample. Using total sample mass and TWP mass fraction it becomes possible to determine the mass of TWP in the sample and factoring in the filtered volume enables the determination of mass concentrations. By comparison of mass concentrations before and after a technology reduction efficiency assessment can be performed.

2. Organic tyre wear markers:
   For the second approach based on extractable organic tyre wear markers the other half of filters and filtered samples will be weighed in. The particles will be transferred to vials to undergo ultrasonication assisted extraction using methanol as solvent. The suspended solids mass remaining on the filter will be determined by weighing the filter again. After extraction, the extract will be filtered and transferred to a new vial. Finally, LC-MS quantification in MRM mode ensues. Based on the obtained concentrations of tyre wear marker compounds like 6PPD-Quinone the amount of TWP in the sample can be determined.
   To this end, the total mass of the marker compound will be determined based on concentration and extraction volume. Based on a conversion factor the mass of TWP present in the extraction vial can be determined. Using the TWP mass fraction of the total solid sample that was weighed in back calculations on the overall TWP content of the weighed in sample is enabled. Finally, based on the total mass, comparable mass concentrations can be determined using the total filtrated volume.

<u>TWP colloidal fraction (< 2µm and > 0.02 µm):</u>
For the colloidal fraction, density separation will be omitted. One part of the anodisc filters will be subjected to analysis of organic tyre wear marker and the other part to Zn analysis.

1. Zn analysis: The filter sample is placed directly in microwave digestion tube. The following procedure is similar as described for the suspended matter fraction (> 2 µm).
2. Organic tyre wear markers: The filter sample is placed in a glass vial (20 mL). 5 to 10 mL of solvent (e.g., methanol) will be added to the glass vial and will be placed in an ultrasonic bath (25 °C) for 2 h. The extract will be treated and analyzed according to the suspended matter fraction (> 2µm).

5.2. Release-catch experiments for elimination technologies assessment

This section provides a protocol for the assessment of the efficiency of an elimination technology, specifically for a photocatalytic degradation nanocoating device. For elimination technologies, the goal is not to assess the capacity to catch plastic and remove it from the environment or inlet water, as explored in the other protocols presented in this document. However, these technologies can be combined with plastic collection technologies to degrade the plastics previously removed (in the backwash) or not collected (in the treated water) by them, in a multi-step treatment. This treatment approach will be used in INSPIRE, with a sequence of technologies (installed in a WWTP) that ends with a photocatalytic degradation for the particles remaining in the treated water, making the inclusion of this protocol appropriate here.

Several stages (Fig. 8) are involved in the determination of the plastic degradation efficiency of the microplastics (MPs) in the effluents of a WWTP. The stages include the following:

1. Collection and identification of MPs
2. Backwash of MPs
3. Elimination of MPs (in INSPIRE demo case by photocatalytic degradation with a nanocoating device)

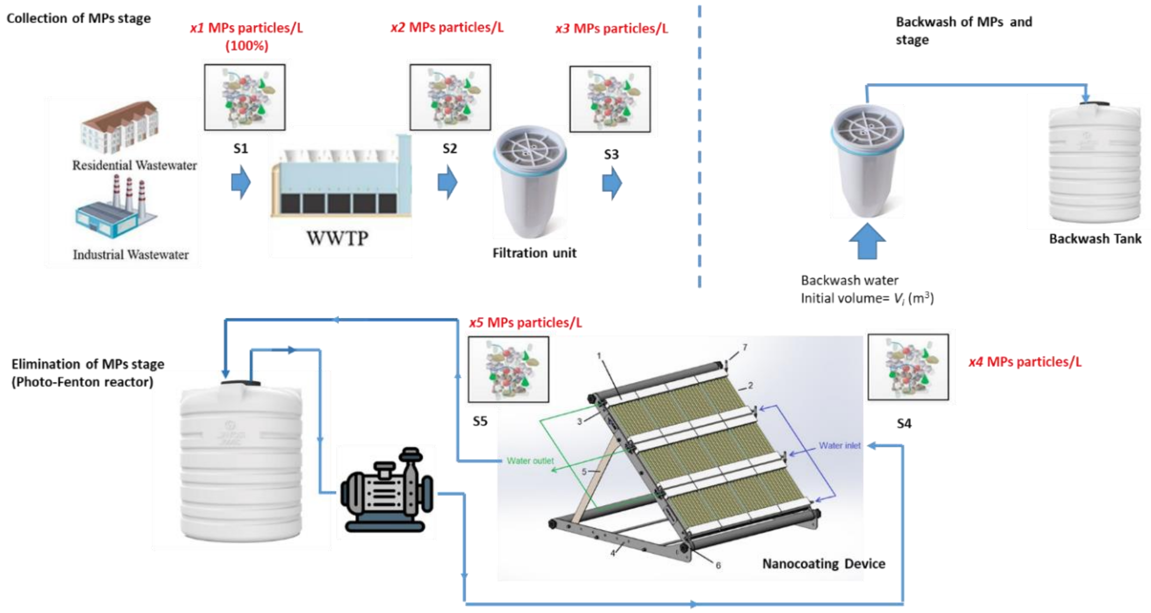

Fig. 8. Schematic representation of the collection and elimination processes of microplastic particles from WWTP effluents when the backwash is fed to a photocatalytic degradation nanocoating device.

The plastic degradation efficiency of the INSPIRE nanocoating device will be determined under controlled conditions at KTH (Section 6.2). The efficiency of this photocatalytic technology will be evaluated using release-catch experiments with a laboratory-prepared mixture of microplastic particles (Section 7.2). These tests will be performed with a specific amount and characteristics (sizes, polymer type, and shapes) of microplastic particles. The mimicked realistic sample is likely to be demo site specific due to the unique characteristics of the plastic pollution in each demo site. Therefore, the efficiency of the nanocoating device will be performed using a feed solution with characteristics similar to the real characteristics of the wastewater effluent at the selected WWTP (demo site in Slovenia).

MPs will be analysed using several methods to identify and quantify MPs by size range and plastic type. These methods include spectroscopic tools, like FTIR and Raman, as well as by GC-MS, Dynamic Light Scattering (DLS), optical microscopy, and Scanning Electron Microscopy (SEM). Raman and FTIR and, to some extent GC-MS, are used to detect and chemically identify the type of MPs, but do not provide information on particle size distribution (PSD). PSD analysis can be performed by DLS, particles size ranging from 0.3 nm to 10 µm, and SEM, for particle size > 10 nm, with the limitation that the algorithms consider the particles to be spherical. Homodyne measurement with autocorrelation is the basis of the widely used "photon correlation spectroscopy" (PCS) that was originally proposed by Einstein and Smoluchowski, who considered the liquid as a continuous medium where thermal fluctuations create inhomogeneities, resulting in density and concentration fluctuations (Fluctuation theory of light scattering [28,29]). The size of MPs can be also determined using the optical microscope (> 1µm; the resolution limit of standard optical microscopes is around 200 nm for microplastics, with super-resolution techniques like stimulated emission depletion (STED) being able to push the limit down to 50 nm) [30]. The overall quantity of MPs within a set size range will be reported as a mass fraction (in µg/l).

Step-by-step protocol for determination of plastic degradation efficiency of the nanocoating device:

1. Prepare a spiked feed solution (prototype solution) with a certain volume (m^3) containing the MP particles. A mixture of different polymers (e.g., PP, PE, PA, PVC) in the form of spherical solid particles and with different sizes (10 µm-300 µm) is selected as the model particles (test materials). A known number of MPs is added to a known volume of deionized water (treated or backwash water, m^3) to determine the MP load (number of MPs/m^3).
2. The sample is withdrawn at the inlet of the nanocoating device connected to the feed tank (with the prototype solution) to confirm the mean MP particle size and number of particles. The mean particle size of MPs can be qualitatively estimated using the standard optical microscope. The PSD analysis can be performed by DLS technique.
3. The prototype solution is circulated through the nanocoating device for certain time in order to make sure that most of the MP particles are trapped onto the surface of the designed photocatalytic material.
4. Samples are withdrawn at the outlet of the nanocoating device at time intervals. The mean MP particle size and the number of MPs are determined in similar way as mentioned in step 2.
5. The retaining (trapping) efficiency of the nanocoating device can be calculated using Eq. 3.

$$\%Retaining(\#) = \frac{\#(MPs\ at\ device\ inlet) - \#(MPs\ at\ device\ outlet\ )}{\#(MPs\ at\ device\ inlet)} \times 100 \qquad \textit{Eq. 3}$$

6. After the determination of the retaining efficiency, the circulation of the treated/backwash water is stopped. The filled nanocoating device containing the trapped MP particles is subjected to an artificial light (visible light) with intensity (mW/cm^2) similar to that of the demo site, for a certain exposure time (determined previously).
7. Samples of MP particles are collected, carefully washed with deionized water, and then air-dried for 24 hours prior to any further analysis. The degradation efficiency of the nanocoated materials is evaluated under controlled conditions using the designed nanocoating device. The MPs degradation is analysed using the following methods:
    - Optical microscope: this instrument can be used to determine the MPs mean particle size and the particle size distribution. Image analysis software (such as ImageJ from the National Institutes of Health, Laboratory for Optical and Computational Instrumentation (LOCI, University of Wisconsin)) [31] is used to estimate the average particle size from at least 400 particles. The degradation efficiency, *%Degradation(V)*, of the nanocoating device is qualitatively estimated using Eq. 4 and based on volume (*V*).

$$\%Degradation(V) = \frac{V(MPs)_i - V(MPs)_f}{V(MPs)_i} \times 100 \qquad \textit{Eq. 4}$$

- The surface morphology of MP samples after visible light exposure for the determined time interval is evaluated by SEM.
- Calculation of the carbonyl index (*CI*) that can be carried out from the spectra obtained using FTIR technique. The polymer type is needed in order to calculate the *CI*, which is particularly useful for the understanding of the oxidative processes that affect the polymer structural integrity over time and is an indicator of the degradation of the polymer. This can be carried out using the software developed by KTH called EmMa, the calculation of this index, facilitating rapid evaluations in research settings is automated [32]. The EmMa program, available in open access [33], is developed in Python, consisting of several parts: finding peaks and correcting baseline, integration of data, and sensitivity analysis with a user interface.

5.3. Release-catch experiments for technologies deployed at freshwater abstraction points

This third subsection for the technologies installed in urban infrastructure focus on technologies deployed at freshwater abstraction points (Fig. 9), which can be connected to DWTPs or industrial facilities, such as power plants. The main difference from the two previous subsections lies with the release of the test materials still in an open area, in conditions similar to those described in section 4.1.1. (Controlled release of non-plastic items or plastic items recovered from the environment - Release-catch experiments for floating plastic collection technologies). Since the test materials recovery at the technology water outlet is more like what was described in section 5.1.1. (Assessment of technologies deployed inland - Release-catch experiments with plastic particles), the protocol here presented resulted from the combination of these two protocols. This protocol is applicable for macro-, meso- and large microplastics.

The INSPIRE protocol includes the following steps:

1. **Selection and preparation of tagged test materials**
   The same recommendations given in section 4.1.1. apply here.
   Considering that the freshwater abstraction points are usually natural environments, such as rivers or lakes, the test materials selected need to take that into account. In this case, this protocol can be used in combination with the same test materials proposed for section 4.1.1., namely the materials described in sections 7.1 (Plastic litter items recovered from the environment) and 7.3 (Non-plastic items that mimic plastic behaviour). In some scenarios, the freshwater might be stored in a specific reservoir not opened to a freshwater body. Only in a scenario like this, other test materials can be used in the release-catch experiment, namely the ones described in section 7.2 (Laboratory-prepared mixtures of plastic particles).

2. **Preparation of the site for the release-catch experiments**
   The same recommendations given in section 4.1.1. apply here.

Fig. 9. Schematics of release-catch experiments for technologies at freshwater abstraction points.
Legend: 1 – potential locations for the release point, 2 – collection from the technology and around it, 3 – collection after the technology, 4 – collection of plastic litter not intercepted, 5 – collection of plastic litter in the treated water.

3. **Release of tagged materials at release point**
   The same recommendations given in section 4.1.1. apply here.

4. **Observations and materials recovery**
   As it was recommended in step 4 of section 4.1.1., during and after the release of the test materials, it is recommended that key locations have observers positioned in order to record the test materials transport along the path for both water and riverbanks and to catch these test materials: at the release point, at the technology and after the technology (Fig. 9).
   However, more similarly to section 5.1.1, the water treated by the technology should be sampled and analysed for the presence of the tagged plastic litter. For this last fraction, the recommendations given in step 4 of section 5.1.1 apply. The water flow at the technology outlet must be recorded and reported as part of the experimental conditions for the release-catch experiment.
   All these fractions need to be assessed separately and, therefore, should be stored in different containers or bags labelled accordingly.
   Example for technology code PP and release-catch experiment number 01 for that technology:
   - PP01_TL_IC – Test materials intercepted and inside the collection net/cage – used to calculate *%PR*, part of *%I* and *%C*.
   - PP01_TL_ILO – Outside loss: test materials intercepted but lost outside of the technology (trapped elsewhere) – used to calculate part of *%I* and part of *%L*.
   - PP01_TL_ILP – Retaining loss – used to calculate part of *%I* and part of *%L*.
     - PP01_TL_ILP-O – Retaining loss during operation: Test materials intercepted but not retained (found in the treated water).
     - PP01_TL_ILP-E – Retaining loss when emptying collection net/cage: Test materials intercepted but not retained during the step to retrieve them from inside the collection cage/net.

- PP01_TL_E – Test materials not intercepted – used to calculate *%E*.

While the retaining loss can be reported as only one value for section 4.1.1., it is relevant to report the two separate subfractions of the retaining loss when applying this protocol, since the one during operation (PP01_TL_ILP-O) give us the fraction of plastics in the treated water. This can be also reported as *%L(treated water)* in order to separate it from the other losses. In section 5.1.1, the only loss is the "retaining loss during operation", with the outside loss being null and the "retaining loss when emptying collection net/cage" being non-applicable.

5. **Analysis of the quantity and characteristics of the materials collected by fraction**
   The same recommendations given in section 4.1.1. apply here.

6. **Repetition of the release-catch experiment for replicate data and in different scenarios**
   The same recommendations given in section 4.1.1. apply here.

7. **Analysis and reporting of the technology removal efficiency under different scenarios**
   According to Chapter 8.

6. Laboratory or pilot scale validation

While in the two previous sections, we propose protocols for the assessment of actions or technologies deployed in the field (*in-situ*), in full scale, in this section we provide some guidelines to test the technologies at laboratory or pilot scale. At laboratory scale, the technology is validated, succeeding proof of concept, and demonstrating the viability to advance to the scale up and testing in more relevant environmental conditions. After that, at pilot scale, the technology prototype readiness is demonstrated, preceding its deployment in the field.

<u>The technology readiness level (TRL) is classified as listed below</u> [34]<u>:</u>
TRL 1 – basic principles observed
TRL 2 – technology concept formulated
TRL 3 – experimental proof of concept
TRL 4 – technology validated in lab
TRL 5 – technology validated in relevant environment (industrially relevant
environment in the case of key enabling technologies)
TRL 6 – technology demonstrated in relevant environment (industrially relevant environment in the case of key enabling technologies)
TRL 7 – system prototype demonstration in operational environment
TRL 8 – system complete and qualified
TRL 9 – actual system proven in operational environment (competitive manufacturing in the case of key enabling technologies; or in space)

Performing the release-catch experiments in a controlled setting, allows some advantages such as:

- Testing the experimental design with well-defined and reproducible operational conditions, such as the same water flow and water quality between different experiments.
- The relationships between the different variables and the specific mechanisms can be isolated to be studied and to test hypotheses.
- Precise measurements on the test materials transport and accumulation.
- Possibility to use camaras to record the full experiment and later analyse the images for the plastic transport in a channel for example, detecting where the items are getting trapped.
- All the test materials can be recovered, with no expected subsequent impact to the environment.
- Validation of test materials selected before they are used in the field, which is particularly relevant for non-plastic items to simulate plastic behaviour (section 7.3).
- Possibility to release biota and non-plastic items during the experiments, to test the possible change to the efficiency due to the presence of these materials (e.g., other litter) or the bycatch of biota such as fish and vegetation (e.g., reeds or tree branches).
- The timeframe and resources needed for the experiments can be reduced, allowing a higher replicate number and, therefore, the results confidence and precision will increase.
- More tests can be executed with adjustments in settings and compositions so the best performing setup can be found.
- If the experimental results are combined with computational modelling, less complex models and shorter simulation runs can be used.

However, the release-catch experiments in controlled settings also bring limitations and disadvantages such as:

- In case of a laboratory setting, the experiments may need to be downscaled due to the limitations on the scale that can be assembled and performed in the laboratory. This has implications on the technology size and test materials size and number/mass. Accordingly, it requires changes to the operation conditions, such as the water flow, distance between release-point and technology, among others.
- In pilot scale, the technology can be tested under conditions more similar to the field conditions, even using the technology and test materials at full scale. However, the experiment will be limited by several simplifications on the environmental conditions, such as the effect of sediment and subtract of the river channel or the water quality in a WWTP. These simplifications can lead to significant deviations between the results obtained at controlled settings and the ones expected in the field.

Taking this into account, we recommend that laboratory or pilot scale testing of the release-catch experiments precedes field release-catch experiments. Two main types of settings were identified for these release-catch experiments in controlled settings: i) hydraulic flume or wave basin for floating plastic collection technologies (Section 4.1.1), and ii) spiked artificial water fed into technologies deployed inland (Section 5.1) and/or into elimination technologies (Section 5.2). One of the main goals of these experiments is to validate the test materials selected before field release-catch experiments, under the assumption that the technology readiness was already demonstrated.

6.1 Tests in hydraulic flume or wave basin

Before performing release-catch experiments under the protocol described in section 4.1.1, the test materials, as well as the technology, can be first tested in a hydraulic flume or wave basin.

A hydraulic flume is a scaled-down artificial channel, with a pumping system, wave generators, and usually transparent walls, that is used in the field of hydraulic research. It is used to study e.g., the water flow in channels with different types of cross-sections, current patterns, sluices and other type of gates, fish passages, erosion and sediment transport, among others. A down-scale version of the technology can be tested in this setting to test different hypotheses under highly controlled conditions and measurements.

Gallitelli et al. [35] conducted experiments in a laboratory flume to study how submerged plastics are transported in the river water column and how vegetation can influence this and the plastics behaviour. In another study, Leone et al. [36] tested in an experimental flume facility the effect of changing parameters (e.g., flow velocity) on the proportion of biota items caught by two generic, non-commercial, and custom-made plastic cleanup mechanisms.

As mentioned, the experiments performed in a hydraulic flume may require a downscale, making these experiments relevance restricted to the study of relationships between the different variables and to isolate specific mechanisms to be studied. Thus, the use of hydraulic laboratory wave basins might prove to be more interesting in the scope of the plastic removal efficiency assessments.

A hydraulic laboratory wave basin (also called as pool or tank) is an artificial structure bigger in width than a flume, but also used for research. It is equipped with a wave generator capable of generating different types of waves. As an example, at Deltares (NL) institute, the Delta basin has 50 x 50 m and 1 m depth, while the Scheldt flume has a total length of 110 m, 1 m width and 1.2 m depth. These basins are specifically utilized to perform controlled tests with physical scale models, serving as important tools for major coastal engineering projects. Frequently tested topics include the stability of structures built along coastlines (i.e., groin, jetties, breakwater and seawall) and the water pressure and wave force on them, wave run-up and wave overtopping on dykes and quay walls, wave penetration in ports, turbulence, and erosion control.

Because of their bigger dimensions, the wave basins might enable the test of the plastic removal technologies in full scale (depending on the size of the technology). This is particularly relevant since it provides an option to test the technology and test materials selected before *in-situ* experiments to assess the plastic removal efficiency. Some tests that can be explored include:

- Validation of test materials, particularly for those identified in section 7.3. Experiments can be conducted with the natural materials alternative to plastic and repeated with the plastic items that they are simulating, to validate that they reproduce the plastic behaviour.
- Validation of the size range and type of materials that the technology can collect, including experiments with test materials that they were not designed to target.
- Identification of loss points in the technology (i.e., weak points where plastic gets trapped or escapes through, reducing the plastic removal efficiency).
- Test of the technology after improvements are made to its design.

Brambini et al. [11] and Dommergues et al. [10] conducted model tests for a plastic interception technology in the Concept Basin (220 x 4 m, 3.6 m depth) at the Maritime Research Institute of the Netherlands (MARIN), which were used to better understand the behaviour of the system and to calibrate dynamic analysis models and Computational Fluid Dynamics (CFD) models. In these studies, the technology tested was a floating boom, to be placed perpendicular to the main ocean plastic flow, to intercept and concentrate plastic litter to a point where it can be extracted, shipped and processed in a cost-effective way. The boom was tested at 1:5 scale. During the tests, the parameters measured were the three Degree of Freedom motions of the boom, the loads in the mooring lines, dynamic pressures on the screen (for the rigid case) and the plastic capture efficiency (reported as catch probability for different rising velocities and current velocities). For the plastic capture efficiency assessment, plastic spheres were selected as test materials, which here considered as both relevant to the plastic particles found in the Pacific Ocean and easy to model [10]. Camaras were placed to record different angles and the behaviour of all plastic particles: if they were caught or entrained, how they were entrained, and how they behaved after being entrained. The plastic spheres had the following described characteristics:

- Diameters between 5 mm and 15 cm (at model scale), equivalent to 2.5 cm and 75 cm at full scale.
- Their rising speeds were calibrated by filling them with either fresh or salt water, with different densities, so they would match the rising velocity of the ones found in the Great Pacific Garbage Patch. Their rising speeds were ranging from 4.5 to 27 cm s^{-1}, equivalent to 10 to 60 cm s^{-1} at full scale (faster than the rising speeds of most plastics found in the Pacific).
- For each sphere size, 4 rising speeds were considered. The inside water was tainted in different colours to easily identify them.

6.2 Experiments with spiked artificial water

Before performing release-catch experiments under the protocols described in Section 5.1, the test materials, as well as the technology, can be first tested in a laboratory setting. in INSPIRE, this is expected to occur with the test materials identified in Section 7.2.2 (Tyre wear particles) for a release-catch experiment version, at the laboratory, of the protocol described in Section 5.1.2 (Tyre wear and tyre wear leachables reduction assessment). In Section 5.2 (Release-catch experiments for elimination technologies assessment), we already described a protocol for a release-catch experiment with spiked artificial water, since this assessment will be performed at KTH facilities and not at the WWTP.

For these experiments, artificial water, with characteristics similar to the one predicted for the *in-situ* test, can be spiked with the test materials and stored in an adequate agitated container. Alternatively, a real water sample can be filtered (to remove existing particulate matter in suspension) and spiked. Then, the technology under assessment can be fed with the spiked artificial water in the container. At the outlet of the technology, the treated water is stored in a second container. The treated water, as well as the materials removed/reduced/eliminated by the technology or left inside the first agitated container can be assessed, allowing to determine all the plastic fractions and the estimation of the plastic removal efficiency (or reduction efficiency / degradation efficiency), according to the methodology established in Chapter 8.

This approach provides a laboratory determined plastic removal efficiency assessment, which should be later compared with the results obtained in the *in-situ* experiments. The main advantages of performing the laboratory assessment previous to the field assessment, lie in the possibility to validate the test materials, and to perform more experiments and test different individual variables to optimize the technology and predict the *in-situ* outcomes.

This approach has been used to assess not only new technologies but also existing technologies that were never assessed before for their plastic removal efficiency. As an example, Rajala et al. [37] studied the microplastic removal by coagulation/flocculation followed by settling, in a WWTP secondary effluent matrix. For test materials, polystyrene spheres with diameter of 1 µm (red and fluorescent) or 6.3 µm (yellow and non-fluorescent) were selected due to the smaller microplastics sizes not being commonly assessed in WWTP studies. The wastewater effluent was collected from a secondary effluent from a municipal WWTP in Finland and was spiked with the particles, with non-spiked effluent as experimental blank. In another study, Miranda et al. [20] tested the behaviour of microplastics in a laboratory scale direct contact membrane distillation (DCMD) system used to produce drinking water from seawater. This study included the evaluation of the microplastics removal efficiency and the impact of their presence on the treatment process performance. As test materials, unplasticized PVC particles with 159 ± 43 µm mean diameter were used to spike filtered (1.2 µm pore size) real seawater.

7. Test materials for release-catch

Release-catch experiments will be conducted following the protocols described in Chapter 4 and Chapter 5 to assess the efficiency of the plastic removal solutions in INSPIRE. As identified in Section 8.3, the first step will lie in the selection of test materials, which need to be representative of the litter observed at the deployment location (assigned to a technology) and consider the plastic litter type and size class being targeted by the technology. These test materials will be plastic litter items (macroplastic) or particles (meso- and/or microplastic) (Table 5). The protocols can also be used with other non-plastic test litter items (i.e., glass, metal, paper), to assess the litter removal efficiency. However, this chapter focus on listing plastic litter items or alternative natural materials which simulate an identical behaviour to plastic in the environment (e.g., buoyancy).

Table 5. Plastic classification based on size.

Standard classification	Size	Size class	Size class code
Macroplastics (2013 MSFD TG ML Guidelines for floating litter items)[12]	> 50 cm	> 50 cm	A
	30 – 50 cm	10 – 50 cm	B
	20 – 30 cm		
	10 – 20 cm		
	5 – 10 cm	2.5 – 10 cm	C
	2.5 – 5 cm		
Mesoplastics	0.5 – 2.5 cm	0.5 – 2.5 cm	D
Large microplastics (ISO/TR 21960:2020)	1 mm – 5 mm	1 mm – 5 mm	E
Microplastics (ISO/TR 21960:2020)	1 – 1000 µm	300 – 1000 µm	F
		200 – 300 µm	G
		20 – 200 µm	H
		1 – 20 µm	I
Nanoplastics (ISO/TR 21960:2020)	< 1 µm	< 1 µm	J

The plastic test materials that will be used during these experiments should be diverse in shape (e.g., fragment, filament, beads), size, polymer types (e.g., PE, PP, PS, PET), to mimic the plastic litter items that can be observed in the environment. Having a diversity of items enables the evaluation of any difference in the technology efficiency estimated, which could be biased if only a limited number of parameters are tested. Nevertheless, it is necessary to have an alignment between the technology (e.g., limitations on size targeted), test materials (environmental relevance at the location), and environmental compartment targeted (i.e., floating versus non-floating litter). Therefore, a first step towards the selection of the test materials is to identify whether the technology targets floating and/or non-floating litter and the size range that it can remove. After, based on the baseline pollution levels assessment (Section 3.1, built on INSPIRE WP1 observations for macro-, meso- and microlitter), a group or groups of materials will be selected, representative of the plastic litter accumulated at the location where the technology is or will be deployed.

Considering the diversity showed by the protocols in Chapters 4 and Chapter 5 and the ethical considerations identified in section 7.4, the test materials are organized according to the following classification:

- **Plastic items recovered from the environment** – applicable to macroplastics, mesoplastics and large microplastics (down to pellets), and to protocols 4.1.1, 4.1.2 and 5.3.
- **Laboratory-prepared mixtures of plastic particles** – applicable to large microplastics, microplastics and nanoplastics, and to protocols 5.1.1, 5.2 and 5.3.
- **Non-plastic items that mimic plastic behaviour** – applicable to macroplastics, mesoplastics and large microplastics (down to pellets), and to protocols 4.1.1, 4.1.2 and 5.3.

In section 7.5, we include recommendations on the amount (number or mass) to be release during the release-catch experiments.

7.1 Plastic litter items recovered from the environment

One of the options identified for test materials is the collection of plastic litter on the riverbanks nearby the deployment location during the assessment of the baseline pollution levels. From the total amount of litter collected, a representative subsample should be selected and used for the release-catch experiments. For this step, a similar approach to Blettler et al. [7] is recommended, where macroplastic items were collected within an area 20 km around the place where the technology was tested later. A total of 3376 macroplastics were collected by hand, in transects located 2 m above the water line, and transported to the laboratory where they were washed, classified item by item, and grouped into 33 types. From these items, a total of 52 plastic items were tested in quintupled: the 33 types found in the sampling campaign and 19 under a variety of "conditions" (e.g., bottles empty, partially and fully filled with water) and polymer composition, including floating and non-floating items.

For the plastic efficiency assessments which will be done within the scope of INSPIRE, we will follow a similar approach by:

1. Collecting the test materials from previously conducted sampling campaigns in the tested areas of the use cases, and drying the items
2. Labelling (sample code) and listing the litter sample items. Label following the sample code system in place: Plastic research sample codes.docx; accessible for internal use. Characteristics listed applying the template "INSPIRE *campaign code* - 5 Macrolitter analysis dataset", as per the system that was established in WP1: Sample_replicate code, Operator name, Operator email, Particle/item code, J-code, J Name, Plastic (yes/no), Analysis instrument/method, Instrument match (%), Polymer type, Lenght (mm), Width (mm), Size Class, Mass (g), Colour, Transparency, Shape, and Photo/s label.

For example (each field corresponds to a column in the Excel spreadsheet "Macrolitter" of INSPIRE BE07 - 5 Macrolitter analysis dataset):

- BE07_RC_01R1_083: item number 083 collected in Belgium campaign number 07 during a river cleanup (RC) in area 01 (Temse area 1), bag R1 (replicate 1).
    - J-code: J225
    - J name: Plastic food containers made of hard non-foamed plastic
    - Plastic: Yes
    - Analysis instrument/method: Trinamix
    - Instrument match (%): "High"
    - Polymer type: Polystyrene (PS)
    - Length (mm): 75

- Width (mm): 55
- Size Class: 5 – 10 cm
- Mass (g): 6
- Colour: White
- Transparency: Opaque
- Shape: Cylindrical (yogurt pot)
- Photo/s label: BE07_RC_01R1_083

3. Selecting the test materials from the samples collected to represent the main types of plastic litter observed at the test location, tagging them if applicable, and storing them in a dry and dark place until the experiments are conducted. The number of items stored by type of items should consider the number of experiments that are planned and the replicates' number per experiment.
4. Labelling (fate code) and listing the test materials. After the items are selected, a second sample code needs to be attributed to the item, such as PP01_TL_T_001 – example of label for technology code PP, release-catch experiment 01, item 001 of the total released (T). After the release-catch experiment, if the item is retrieved back, a third sample code will be attributed, such as PP01_TL_IC_010 – example of label for technology code PP (Patje Plastic), release-catch experiment 01, item 010 of the total intercepted and inside the collection net/cage (IC).

The following "fate codes" can be used for the same item PP01_TL_*insert fate*_*insert number* during the release-catch experiment:

- **PP01_TL_T_*insert number*** – Test material released at release point – used to calculate *%T*.
- **PP01_TL_IC_*insert number*** – Test material intercepted and inside the collection net/cage – used to calculate *%PR*, part of *%I* and *%C*.
- **PP01_TL_ILO_*insert number*** – Outside loss: test material intercepted but lost outside of the technology (trapped elsewhere) – used to calculate part of *%I* and part of *%L*.
- PP01_TL_ILP – Retaining loss – used to calculate part of *%I* and part of *%L*.
    - **PP01_TL_ILP-O_*insert number*** – Retaining loss during operation: Test material intercepted but not retained (found in the treated water at the outlet of the technology).
    - **PP01_TL_ILP-E_*insert number*** – Retaining loss when emptying collection net/cage: Test material intercepted but not retained during the step to retrieve them from inside the collection cage/net.
- **PP01_TL_E_*insert number*** – Test material not intercepted – used to calculate *%E*.

Therefore, three item codes are expected to be used for a test material item used for a release-catch experiment: when it is collected from the environment, when it is released, and when it is retrieved.

Even though the technology deployment locations are expected to be cleaned (of litter) before a release-catch experiment (as described in Chapter 4 and Chapter 5), it is expected that the test materials will mix with other litter floating, in suspension in the water or on the riverbanks at the location. **Therefore, the items selected to be released should be tagged before the release-catch experiment.** They should be tagged distinctively (e.g., with different colours) for each release-catch experiment, in the scenario of not being able to retrieve back all the items and having several experiments taking place on the same day/week. In addition, the items can be tagged according to their release point, specifically downstream/upstream (section 4.1.2) or e.g., next to the riverbank or in the middle of the channel (sections 4.1.1 and 5.3). Different tagging methods can be used, such as waterproof paint, coloured tapes or markers. Before the INSPIRE release-catch experiments implementation phase, different tagging methods (e.g., different types of paint) will be tested and validated.

Fig. 10. Plastic pellets pollution at Londenhaven (Port of Rotterdam) in August 2024 (Photograph by Mariana Miranda).

Specifically, for mesoplastics and large microplastics (such as pellets), similar considerations to those identified above for macroplastics should be taken, including on tagging the items/particles selected to be released. However, for large microplastics, instead of using number of items, the release-catch experiments can account for dry mass of these items, if it is not realistic to count the number of pellets for example. In this case, the mixtures of large microplastics can be reported as a separate table with labels for each group of items, listing their characteristics, the mass (after tagged) or number, and corresponding percentage of the total amount of material to be released. PP01_TL_T_001 – example of label for technology code PP, release-catch experiment 01, group 001 of the total released: PE pellets, average diameter size 4 mm, 400 g, 60 % of the total mass (among other characteristics).

Plastic pellets (also known as nurdles) are raw materials used in industry to produce objects made of plastic. Assessing the removal efficiency of the technologies for this type of plastic material is instrumental, due to the well-known pellets loss in industrial ports, and will be explored in INSPIRE, particularly in port areas nearby where pellets are produced and shipped (e.g., Port of Rotterdam, Fig. 10). The pellets' polymer used in the release-catch experiments can be diverse, including e.g., PE, PP, PS and acrylonitrile-butadiene-styrene (ABS) [40], and be representative of the pellets found at the demo site locations.

Concerning the test materials required for the protocol described in section 4.1.2 (Release-catch experiments for floating macroplastic detection using remote sensing techniques), the same diversity of plastic items can be used as for the protocols described in sections 4.1.1 (Release-catch experiments for floating plastic collection technologies) and 5.3 (Release-catch experiments for technologies deployed at freshwater abstraction points), but restricted to macroplastics. However, as explored in its dedicated section (4.1.2), both tagged and non-tagged test materials should be used in separate release-catch experiments. It is necessary to test non-tagged test materials to avoid any biased detection due to the colour used to tag them. Furthermore, experiments with floating plastic, but also floating non-plastic litter, should be considered, since the remote sensing techniques can be also validated to distinguish the plastic from non-plastic items. For this, all the most relevant litter classes that cover floating macrolitter should be included, which are identified and listed in the JRC Technical Report "Floating Macro Litter in European Rivers – Top items" [41]. In this report, a general top 20 items list in the European Seas is defined, with these top items being estimated to cover 96.8 % of the total items found (the top 10 estimated to cover 86.0 %). Additionally, the report also includes top items lists by regional seas (North-East Atlantic, Mediterranean, Black, and Baltic).

7.2 Laboratory-prepared mixtures of plastic particles

For the protocols described in sections 5.1.1, 5.1.2 and 5.2, the release-catch experiments will be performed with the untreated and treated water having a confined flow, making it possible to use laboratory-prepared mixtures of plastic items or particles as test materials. For the protocol described in Section 5.3, it is only ethically responsible to use the test materials described in this section when there is no connection to the natural environment, such as when the water to be spiked with the test materials is confined in a basin not open to a natural water body.

Therefore, for the plastic removal technologies deployed inland (Chapter 5), such as those deployed in a wastewater treatment plant, a laboratory-prepared mixture with a known number of particles on the micro or nano sized range can be used. The water to be treated by the technologies during the release-catch experiments will consist of a spiked water: water with a known amount (dry mass or number of particles) of laboratory-prepared mixture of plastics.

The laboratory-prepared mixture of plastics can be composed by one or more plastic types that cover a defined range of particles sizes. The type of plastics that can be used fall into the three categories identified in the following subsections:

- Conventional and commonly observed polymers,
- Tyre wear particles, and
- Biodegradable polymers.

If feasible, the particles can be tagged to enable faster quantification. Different options of tagging are explored in the material categories (Sections 7.2.1, 7.2.2, 7.2.3).

As described before (section 7.1 and also in Chapter 5), the test materials to be released need to be labelled and characterized before and after the release-catch experiment. The labelling system described in the previous section for macrolitter is applicable here, with two "fate codes" for the same group of test materials, before and after the release-catch experiment. For each release-catch experiment, different groups of test materials can be used together in the same laboratory-prepared mixture, being essential to report the percentage of mass of each material group that is used in preparing the mixture of the different groups.

Furthermore, the characterization system in place for INSPIRE must be used, including some of the fields established in the template “INSPIRE *campaign code* microlitter dataset”:

- Characteristics to list for a group of particles: Institution (prepared mixture), Operator name (prepared mixture), Operator email, Polymer type, Material supplier, Particles mean diameter (or length and width) (µm), Particles size class, Particles dry mass (mg), Percentage of the total amount (mass) of materials to be released, Particles colour, Particles transparency, Particles type, Particles shape, Photo/s label.

For example (each field corresponds to a column in the Excel spreadsheet):

- EC01_TL_T_001: example of label for technology code EC (EcoPlex), release-catch experiment 01, laboratory-prepared mixture group 001 of the total released (T) of two groups (PE and PP).
    - Polymer type: Polyethylene (PE)
    - Material supplier: (supplier name and batch number if available)
    - Particles mean diameter (µm): 200 (the particle size distribution should be reported if available)
    - Size Class: 20 – 200 µm, 200 – 300 µm, 300 – 1000 µm (Vocabulary: EMODnet micro-litter size classes http://vocab.nerc.ac.uk/collection/H03/current/)
    - Dry mass (g): 2
    - Percentage of the total amount (mass) of materials to be released: 60 %
    - Colour: Colourless (Vocabulary: EMODnet micro-litter colour classes http://vocab.nerc.ac.uk/collection/H04/current/)
    - Transparency: Transparent/translucent (Vocabulary: EMODnet micro-litter transparency classes http://vocab.nerc.ac.uk/collection/H06/current/)
    - Particles type: Granules (Vocabulary: EMODnet micro-litter types http://vocab.nerc.ac.uk/collection/H01/current/)
    - Shape: Cylindrical (Vocabulary: EMODnet micro-litter shapes http://vocab.nerc.ac.uk/collection/H02/current/)
    - Photo/s label: EC01_TL_T_001

7.2.1 Conventional and commonly observed polymers

A first plastic type category consists of conventional and commonly observed polymers of environmental microlitter, which can be obtained from different plastic suppliers in different forms: powder, foil, rod, tube, fibre, fabric, coil, disk, balls, or granules. The test materials characteristics can be selected to better fit the study, such as:

- Polymer type
- Size (i.e., diameter, length, thickness)
- Structure
- Form and shape
- Purity and/or grade
- Formula, molecular weight, and additives (e.g., HDPE, LDPE or UHMW PE; additive free polymer, extrusion grade, injection moulding)
- Colour and transparency
- Density

The selection of the polymer(s) used will be based either on their relative importance considering global plastic production [20] (Table 6) or on previous assessments from litter reports in the area [8]. Moreover, different size classes will be used for the particles, depending on the relevant size range to the removal technology [8].

If different polymers are used, tagging can be used to differentiate them. That can be achieved with different fluorescent colours (e.g., Nile red after sample processing, [8,42]), using textile dyes [42], or by using self-fluorescent polymers [43].

7.2.2 Tyre wear particles

Two reference tyre wear particles (TWP) to be used as test materials will be provided by DELVEC to FRE for in-house evaluation of technology performance:

a) Sample of processed industrial tyre wear micro- and nanoparticles
b) Sample of DELVEC Flame Spray Pyrolysis (FSP) own-made tyre-wear simulating nanoparticles

The processed industrial TWP sample is prepared as follows:

1. A quantity of off-shavings from an industrial tyre cold retreading facility is obtained.
2. This is passed through a cascade of sieves down to approximately half a millimetre to filter out macro-tyre wear.
3. The resulting fine powder is then frozen using liquid nitrogen and mechanically crushed to further reduce the size.
4. After evaluating the sample size frequency distribution via Dynamic Light Scattering (DLS), it is verified that it contains micro and nano sized particles.
5. The sample is then packaged and shipped.

The FSP TWP sample is prepared as follows:

1. The DELVEC FSP reactors are setup with a carbonizing flame and a secondary stage doping with metals, to simulate the composition of tyre wear. Using supersonic oxygen streams, the high temperature residence time of the nucleated particles is kept low, ensuring a size distribution in the 10 – 100 nm range.
2. The material is characterized using X-Ray Diffractometry, Raman spectroscopy and Electron Paramagnetic Resonance spectroscopy, with the size distribution obtained using DLS.
3. The particles are packaged and shipped with no further treatment after collection from the reactors.

7.2.3 Biodegradable polymers

Biodegradable polymers, such as natural or synthetic biodegradable polymers [44], could be an alternative to the test materials categories mentioned or complementary to their use, e.g., polyhydroxyalkanoate (PHA) [45][46].

Similarly to the non-biodegradable plastic test materials, the biodegradable test materials characteristics must still be in accordance with the technology's aim as mentioned in section 8.3.

7.3 Non-plastic items that mimic plastic behaviour

As an alternative option to using plastic items recovered from the environment (7.1), we suggest the use of natural non-plastic test materials which simulate the plastic behaviour. Similarly to the plastic test materials, the natural non-plastic items must mimic the plastic items characteristics as much as possible and be diverse in shape, size, and colour. Furthermore, the items must be selected according to the test aims, particularly regarding their size and density. The items' density (g cm^{-3}) should enable them to float or be neutrally buoyant, aligned with the compartment targeted by the solution. For example, for the protocol described in section 4.1.2 (Release-catch experiments for floating macroplastic detection using remote sensing techniques), some floating items that could be selected include:

- Wood: Shaped into different forms (e.g., sphere, straws, cubes)
- Fruits: Oranges, walnuts

In INSPIRE, we will explore the potential use of alternative materials to plastic as test materials for the release-catch experiments. As a first step, already some relevant materials have been identified (Table 7). Before the use of any of these materials in the field (protocols 4.1 and 5.3), these will be tested and validated according to the recommendations provided in Chapter 6.

Similarly to what is described in section 7.1, the test materials selected to be used during the release-catch experiments must be labelled. In this case, with only two labels with the "fate codes" for each item, for release and catch.

Table 6. Common polymers to be potentially used as test materials during release-catch experiments.

Polymer (abbreviation)	Size Class	Natural / Synthetic	Density (g cm^{-3})	Commercially available	Biodegradability in the marine environment	Potential toxicity	References
PE	Macro to nano	Synthetic	0.914 - 0.965 (LDPE and HDPE)	Yes	Some biodegradation under limited conditions	Low	[47–50]
PET	Macro to nano	Synthetic	1.20 - 1.45	Yes	Non-biodegradable	Low	[48,49,51]
PP	Macro to nano	Synthetic	0.90 - 0.91	Yes	Non-biodegradable	Low	[48,49,51,52]
PS	Macro to nano	Synthetic	1.05	Yes	Non-biodegradable	Low	[49,53,54]
PVC	Macro to nano	Synthetic	1.44 - 1.48	Yes	Non-biodegradable	High	[49,55]
PUR	Macro to nano	Synthetic	1.31	Yes	Non-biodegradable	High	[49]

Table 7. Potential natural materials, which simulate plastic behaviour in aquatic environments, to be used during catch-release experiments.

Material	Size	Natural/ synthetic	Density (g/cm^3)	Commercially available	Shape	Biodegradability in the marine environment	Toxicity	References
Blueberry seeds	Micro	Natural	0.8 - 1.2	Yes	Seed	Yes	Low	[59,60]
Coconut *(Cocos nucifera L.)*	Macro	Natural	1.07	Yes	Seed	Yes	Low	[61,62]
Coconut	Micro	Natural	1.40	Yes	Fibber	Yes	Low	[63,64]
Wood	Macro	Natural	1.5	Yes	Fibber	Yes	Low	[56]
Pumice Stone	Macro	Natural	0.85	Yes	Fragment	ND	Low	[65]
Coir	Macro	Natural	1.15 - 1.45	Yes	Fibber	Yes	Low	Reviewed by [66]
Avocado seeds	Macro	Natural	1.19	Yes	Fragment/pellet	Yes	Low	[67,68]
Cork	Macro	Natural	0.12 - 0.24	Yes	Fragment	Yes	Low	[69]
Walnut shell powder	Micro	Natural	0.48	Yes	Pellet	Yes	Low	[59,70,71]

ND: information not determined or available.

7.4 Ethical considerations

The release-catch experiments have the potential to create ethical issues and environmental impact due to the release of test materials in the environment, in particular in riverine natural environments. Because of this, the test materials were grouped into the three groups (7.1, 7.2, and 7.3) in this chapter, with already some actions and restrictions described to mitigate their potential impact and ethical issues, providing less environmental harmful options to the release of plastic (and non-plastic) litter in the environment.

According to article 36 of the Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 on waste, the “Member States shall take the necessary measures to prohibit the abandonment, dumping or uncontrolled management of waste, including littering”. Therefore, the release of litter in the environment is classified as a minor offence in most countries or even a crime, depending on the EU country. More recently, with the adoption of the Directive (EU) 2024/1203 of the European Parliament and of the Council of 11 April 2024 on the protection of the environment through criminal law, it was established that “Member States should [...] ensure that unlawful waste management constitutes a criminal offence where such conduct concerns hazardous waste in a non-negligible quantity, or it concerns other waste and such other waste causes or is likely to cause substantial damage to the environment or human health”, with plastic waste being listed.

With the present document, we identified the following mitigation actions as required to comply with EU law and reduce/eliminate the environmental impact of the release-catch experiments:

- Laboratory or pilot scale experiments can be performed previously to the *in-situ* experiments to validate the technologies, protocols and test materials and predict potential risks, minimizing the number of experiments done in real environmental settings and reducing accidental loss of tested materials.
- The use of new litter is restricted, being strongly recommended to only release this category of test materials (Section 7.2) if it is possible to ensure that they are recovered back. Because in riverine environments or ports is highly difficult to control every condition to guaranty this, these test materials should not be used for the protocols described in Chapter 4 and in most cases for the one described in Section 5.3.
    - For these protocols (Chapter 4 and Section 5.3), the use of plastic (or non-plastic) litter is limited to items recovered from the environment (Section 7.1) or to natural non-plastic items with high degradation in the environmental compartments affected, as demonstrated by relevant literature (Section 7.3).
- In all the protocols concerning the release-catch experiments, described in Chapter 4 and Chapter 5, measures are indicated to capture back the litter not collected by the solutions during the test phase. Examples include the use of nets installed *in-situ* for the duration of the release-catch experiment (Chapters 4 and protocol 5.3) or collection of the treated water followed by micro- and nanoplastics analysis (e.g., in wastewater treatment plants) (Chapter 5). If test materials are found in the treated water, this water cannot be discharged into the environment and should be adequately treated (e.g., via additional treatments such as ultrafiltration).
- The required local and national authorities will be contacted for permission before any *in-situ* experiments takes place, to ensure that the national and regional regulations are respected. Based on any existing restrictions not identified beforehand, the protocols and test materials will be adapted to comply with.

- All technologies deployed will be assessed for their environmental impact to identify their potential positive and potential negative effects in the environment (water surface and water column, sediment and biota), from the installation and operation (as part of INSPIRE T1.4), which will cover the period of the release-catch experiments.

In INSPIRE, annual ethics reports are in place and will be prepared to identify the ethics issues in the project and to describe the procedures on how to handle information, data protection, AI use, among others, according to current ethics standards and best practices as per the European Commission (D7.4 – Annual Ethics Report 2024). In the first report (D7.4), the *in-situ* release-catch experiments have been already identified as a potential issue due to the release of the test materials in the environment, which will be further investigated in the upcoming months, as well as the required actions to tackle it.

The following notice and/or board will be placed at each use case and testing site to indicate that a test is being conducted. It will be positioned at both the beginning and the end of the suspected transect to clarify to citizens that no criminal or harmful for the environment activity is taking place. This notice will inform them that the testing is for research purposes and that all materials will be recovered without any impact on the environment. An indicative notice will be as follows and it will be written in English and in the local language of the test site.

NOTICE: Scientific Study in Progress

Environmental Research in Action

This area is part of an **ongoing scientific study** conducted by the **INSPIRE project** that is funded by the European Commission under grant agreement No 101112879, to develop innovative solutions for plastic-free rivers. Our team is currently performing controlled tests to assess the efficiency of plastic removal technologies.

What You Need to Know:

- ✓ All test materials used are carefully selected and will be fully recovered after the experiment.
- ✓ This research complies with environmental regulations and has been approved by the relevant Authorities.
- ✓ We will minimize and mitigate any potential harm to wildlife or the ecosystem during these trials.

Why This Matters:

Plastic pollution is a major environmental challenge. Our research aims to develop effective methods to **prevent and remove plastic waste from water bodies**, contributing to cleaner rivers and a healthier environment.

Questions? Contact us at inspire-project@vliz.be
or visit our website: https://inspire-europe.org/

Thank you for your cooperation and support in protecting our environment!

7.5 Considerations on released amount (mass/number) and diversity of test materials

A suitable amount, i.e., mass or number, of test materials to be released for each release-catch experiment needs to be selected. Two different methods are proposed for this decision: i) test materials amount aligned with the baseline pollution levels, or ii) test materials amount representative of a worst-case scenario of litter accumulation in the environment.

For the first option, the total amount of materials to be released corresponds to an environmentally relevant load, established based on previous observations (i.e., monitoring or sampling/observation campaigns) at the solution deployment location. This is the recommended approach since it enables to assess the solution in a realistic scenario, being representative of the solution deployment location. However, when opting for an environmentally relevant amount of test materials, it should be also considered that these items/particles should cover not only all the main plastic litter categories expected to be present at the location but also the most common identified in the literature [7,41]. This implies the inclusion of some plastic items that might have not been observed in the sampling/observations campaigns but are commonly observed in other locations, increasing the comparability of the release-catch experiments. In the case of floating plastic litter, these items can be selected based on the JRC Technical Report “Floating Macro Litter in European Rivers – Top items” [41], which includes the following top plastic items categories:

- Pieces 2.5 cm > < 50cm, > 50 cm
- Bottles
- Cover / packaging
- Bags
- Polystyrene pieces 2.5 cm > < 50cm, > 50 cm
- Sheets, industrial packaging, plastic sheeting
- Foam packaging/insulation/polyurethane
- Other plastic/polystyrene items (identifiable)
- Crates and containers / baskets
- Synthetic rope

As a second option, high loads of test materials can be released to mimic a worse-case scenario, for example due to an increased anthropogenic pressure (e.g., high touristic season with increased littering) or due to extreme weather events (e.g., storms or floods, which often are associated with a high input of litter items to accumulation zones). Testing multiple loads of test materials will enable to assess the technologies’ responses to diverse capacities, providing a more in-depth assessment. Furthermore, the worse-case scenario tests can enable the researchers to identify any limitations in the technologies when high levels of particles/items are present in the environment. For example, in a worst-case scenario such as a flood after heavy rain, the technology could become saturated by the number of information received (e.g., for remote sensing technologies) or clogged (e.g., riverine and inland plastic removal technologies).

8. Plastic removal efficiency definition, data analysis and reporting

8.1 Plastic removal efficiency

The removal efficiency measures the amount of pollutant or other constituent, specifically plastic litter in this case, that has been successfully removed using the solution, divided by the total amount of pollutant input [72]. Typically, it is calculated based on the mass (or concentration) of the pollutant, although in the past it has also been calculated based on the number of particles or items removed (or load) [72].

When the removal efficiency is calculated based on mass (*%R(m)*), it can be calculated directly when the mass (*m*) removed is measured (Eq. 5) or indirectly using a mass balance if the measurement accounts for the mass that remains (Eq. 6). The first equation is used when analysing, for example, the pollutant particles retained on a membrane, while the second equation is frequently used when, for example, analysing the inlet flow and outlet flow for a certain pollutant. However, it has been pointed out in the literature that when using Eq. 6 (and Eq. 8), the use of reduction efficiency instead of removal efficiency would be a more correct terminology since it reflects better the balance between the measured values of input and output and because it was not assessed if the pollutant was removed, transformed or eliminated [72].

$$\%R(m)_{\ t=n} = \frac{m(pollutant\ removed)_{t=n}}{m(pollutant\ in\ system)_{t=0}} \times 100 \qquad \textit{Eq. 5}$$

$$\%Reduction(m)_{\ t=n} = \frac{m(pollutant\ in\ system)_{t=0} - m(pollutant\ in\ system)_{t=n}}{m(pollutant\ in\ system)_{t=0}} \times 100 \qquad \textit{Eq. 6}$$

When working with solid pollutants such as plastic, it should be taken into account that for both equations (Eq. 5 and Eq. 6) it is desirable to calculate with dry mass data (mass of the sample after the water content was removed), usually after having the sample in an oven (at low temperatures to avoid degradation) or desiccator, for the necessary time. In addition, extra steps might be needed to clean the plastic samples, such as digestion of organic matter or density separation to separate inorganics like sediment particles.

When working with data that accounts for the number (*#*) of particles or objects (Eq. 7 and Eq. 8), the principle of the two previous equations still applies, but no steps need to be taken to dry the items or particles (as the associated water weight does not bias the results) or to clean them. Nonetheless, for the removal efficiency assessment based on enumeration (*%R(#)*), it is even more critical to categorize the items/particles by size to have a more complete assessment.

$$\%R(\#)_{\ t=n} = \frac{\#(particles\ removed)_{t=n}}{\#(particles\ in\ system)_{t=0}} \times 100 \qquad \textit{Eq. 7}$$

$$\%Reduction(\#)_{\ t=n} = \frac{\#(particles\ in\ system)_{t=0} - \#(particles\ in\ system)_{t=n}}{\#(particles\ in\ system)_{t=0}} \times 100 \qquad \textit{Eq. 8}$$

The limitation with the calculation of the removal efficiency with the above mentioned equations (Eq. 5-8) is that these might not necessarily take into consideration the diversity of plastics in the environment, which have different size classes, are made from different polymers with different densities and shapes, have a variety of associated chemicals (additives and sorbed contaminants) and different colours. For an assessment of the solutions that ensures data comparability between different types of technologies/actions but also between different studies, we strongly recommend to not only quantify the plastic removed, but also characterize it. Some characteristics that we recommend analysing are size class and polymer type (and shape for macroplastics) (detailed in Chapter 7).

In manual cleanup activities with citizen engagement, the results are frequently expressed in volume of collected litter, particularly as number of bags of e.g., 10 L collected per activity. However, due to being a less precise measurement than mass or number of items of litter collected, we recommend that the litter collected during manual cleanups is accounted for by weighting the bags content after a drying period and by enumerating the items collected.

8.2 Litter removal efficiency

Since the manual cleanups and plastic removal technologies collect other litter that is not plastic, it is important to report this as part of the efficiency of the solutions, i.e., the **removal efficiency for litter (*%LR*)**, if the assessment includes other litter than plastics. As part of the protocols for quantifying the amount (mass or number) of plastic collected by a technology or manual cleanup, it is expected that the amount of non-plastic litter can also be determined, following the steps below when doing a mass assessment (aligned with task 4.1 and D4.1 – optimisation and efficiency of the INSPIRE solutions):

1. Recovery of the litter collected by a solution
    - m_{wet} (total): total wet mass collected
2. Separation of litter from non-litter in the sample collected
    - m_{wet} (non-litter): wet mass of the non-litter fraction (e.g., algae, reeds)
    - m_{wet} (total litter): wet mass of the litter fraction
        - m_{wet} (litter) = m_{wet} (total) - m_{wet} (non-litter)
3. Cleaning, drying and classification of the litter fraction. The two litter fractions should also be separated into subfractions according to size classification (nano, micro, meso or macro, Chapter 7, Table 5).
    - m_{dry} (non-plastic litter): dry mass of the non-plastic fraction (e.g., metal, paper, glass, wood)
    - m_{dry} (plastic litter): dry mass of the plastic fraction
    - m_{dry} (total litter) = m_{dry} (non-plastic litter) + m_{dry} (plastic litter)
4. If the m_{dry} (environment total litter) and m_{dry} (environment plastic litter) are known (in the case of a release-catch experiment, Chapter 4 and Chapter 5), then m_{dry} (total litter) and m_{dry} (plastic litter) can be used to estimate the *%LR*(*m*) and *%PR*(*m*).

8.3 Release-catch experiments

Here we explore the concept of release-catch experiment to assess and quantify the efficiency in a standardized and controlled way. The main principle is that a known amount of selected test materials is released at the technology implementation location, and is then caught back, being identified and quantified.

Release-catch experiments offer a method for calculating plastic removal efficiency when the total quantity of plastic pollution present in the environment is unknown. Therefore, they allow to overcome several limitations that result from analysing the plastic removal based only on field observations, such as:

- The amount of plastic recovered by the solution can be determined (impacted by the analytical limitations of the lowest size that can analysed), but the amount of plastic in the environment can only be estimated based on previously conducted sampling and/or observation field campaigns that assessed the plastic (and litter) pollution baseline levels.
- Potential high variability of plastic litter (quantity and characteristics) due to weather and hydrologic conditions and due to different plastic input trends (including seasonality). This could lead to an over- or underestimation of the *%PR*.

As the solutions provided within INSPIRE target different plastic size ranges and are based on different mechanisms and principles, the goal was to create an approach that is flexible, modular, and interoperable. This INSPIRE approach is based on the same fundamental concepts (explained in this section), but uses different protocols (sections 4, 5 and 6) combined with different test materials (Section 7).

In general, a release-catch experiment consists of two main steps:

i) Test materials selection: The test materials should be selected to be representative of the litter found in the location where the technology is going to be or is already implemented. It should also take into account the limitations of the plastic litter being targeted, such as size range and buoyancy.

ii) Release-catch experiments: The application of the adequate protocol involves releasing the selected materials at a specific location (release point), either upstream or before the inlet. After enough time, these materials are then captured at the technology and its surroundings, including the release point, downstream, outlet and other designated 'loss points'.

These release-catch experiments are not applicable for all solutions explored in INSPIRE, such as the manual cleanup activities. Furthermore, we also propose a different approach for the assessment of the technologies that target the removal of litter on riverbeds. The protocols and/or recommendations for these solutions are detailed in their dedicated sections (4.2 and 4.3).

The schematic below (Fig. 11) exemplifies a release-catch experiment for a technology deployed in a river, for example. In this theoretical example, 100 plastic items were selected to be representative of the diversity of plastic litter found at the location. These plastic items are then released upstream from the technology deployment location (admitting the normal flow of the river downstream) at the release point. After the adequate time, considering the hydrologic and weather conditions, the technology is inspected and 45 plastic items inside its collection cage/net are retrieved. In parallel, 40 items did not intercept the technology and are found downstream or at the release point, being collected back with nets deployed in the location for this goal. Finally, 15 items are found either i) trapped outside of the technology collection cage/net (ILO: outside loss), or ii) released back into the environment (ILP: retaining loss) during the operation of the technology because they pass through (ILP-O, found at the outlet) or during the step when the plastic is retrieved from the collection cage/net (ILP-E).

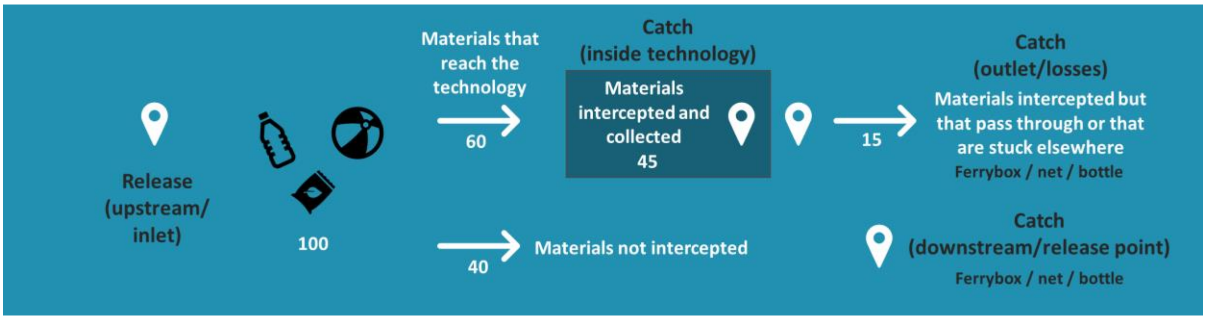

Fig. 11. Schematic of a release-catch experiment with example of enumerated plastic items.

The plastic removal efficiency, *%PR*, of the technology can be estimated based on the fractions of the total released test materials (Eq. 9 and Eq. 10):

$$\%T = 100 = \%I + \%E \ = \%I \times (\%C + \%L) + \%E \qquad \textit{Eq. 9}$$

$$\%PR = \%I \times \%C \qquad \textit{Eq. 10}$$

Where, for a *%PR(#)* assessment based on number of items:

- *%T* is the percentage of total plastic litter released (example 100 items, 100%)
- *%I* is the percentage of plastic litter intercepted (example 60 items, 60%)
  - *%C* is the percentage of plastic litter collected from the total intercepted (example 45 items, 75%). To avoid confusion between *%C* and *%PR*, *%C* was named as **technical efficiency**
  - *%L* is the percentage of plastic litter that passed through or is trapped outside, and is lost from the total intercepted (example 15 items, 25%)
- *%E* is the percentage of plastic litter that is not intercepted by the technology and stays in the environment (example 40 items, 40%)

When estimating the *%PR* based on the plastic litter fractions is recommended that at least the *%I* or *%C* is also reported. Therefore, in the example given, the *%PR(#)* is 45, with *%I(#)* = 60. This means that, regarding the number of items, 45% of the plastic litter tested is being effectively removed from the environment with the technology and that 60% of the litter is intercepted and 75% of it is removed (*%C* obtained through Eq. 10).

The assessment and analysis of the *%I* and *%L* fractions are particularly relevant when trying to improve and optimize the technology. The *%I* can enable the identification of the best environmental or operational conditions to run the system, leading to higher interception of plastic litter and higher cost-efficiency when optimized to increase. While the *%L* fractions (ILO: outside loss; ILP-O and ILP-E: retaining losses) will assist in finding the technological weaknesses, leading to modifications to the technology design and/or operation in order to decrease them.

Estimating the *%PR* can be more challenging in some protocols. For example, if the focus is on the analysis of the plastic remaining in the treated water and not on the plastic litter retained inside the technology and collected from it. In this case, and for uniformization of the data reported, the retaining loss (the outside loss is null in protocols described in section 5.1) can be reported alone as mass or number of test materials if the interception and the other fractions are not assessed. **<u>The *%PR* must not be reported as a simple subtraction of the plastic litter percentage found in the treated water and the total plastic litter released during the release-catch experiment (100 %).</u>** In a theoretical example, 10 m^3 of water with 100 particles are fed into a technology and only 5 particles are found in the treated water. The *%PR* is, however, 80 % and not 95 %, because 80 particles are collected from the technologies filters and 15 particles are found at the feed reservoir (*%E* = 15): 100 % (100 particles released) = 85 % (85 intercepted particles, *%I*) + 15 % (non-intercepted particles, *%E*) = *%I* × (*%C* + *%L*) + *%E*, with *%C* = 94 and *%L* = 6.

8.3.1 Normalization of *%PR*

The basic principles of the release-catch experiments have been explained in the overarching Section 8.3. However, the time needed to reach a certain efficiency, and the environmental transport agents impact on it, are other important considerations when assessing the *%PR*.

Two different technologies can have the same *%PR* but at different time frames, which will impact litter removal amount (number or mass) over time and, consequently, the cost-benefit. Therefore, results obtained based on the general release-catch experiments should be normalized over time. This new assessment parameter, ***PR rate*** is calculated as a ratio of the number of items (Eq. 11) or mass (Eq. 12) removed over time of operation (excluding the time during which the system is turned off or under maintenance), with units h^{-1} or day^{-1} for Eq. 11 and kg h^{-1} or kg day^{-1} for Eq. 12. Similarly, other parameters, such as the *%I*, can also be calculated as an ***I rate*** (Eq. 13 and Eq. 14).

$$PR\ rate\ (\#) = \frac{\#\ PR_{\ t=n}}{t = n} \quad \textit{Eq. 11}$$

$$PR\ rate\ (m) = \frac{\mathrm{m}_{dry}\ PR_{t=n}}{t = n} \quad \textit{Eq. 12}$$

$$I\ rate\ (\#) = \frac{\#\ I_{\ t=n}}{t = n} \quad \textit{Eq. 13}$$

$$I\ rate\ (m) = \frac{\mathrm{m}_{dry}\ I_{t=n}}{t = n} \quad \textit{Eq. 14}$$

In addition to the concept of *PR* rate, we assume that **different *PR rates* need to be assessed for different operation scenarios** due to a non-uniform *PR* rate. We expect this due to the technology limitations on how much litter it can take before maintenance, i.e., close to the operation end time (just before the necessary maintenance period) the technology is expected to process less water and remove less litter over time because of membrane/mesh fouling, pore wetting, and/or mineral scaling or because less volume is available in the net/cage. Even though there is still plastic litter available to be collected, this technological limitation will lead to a stationary phase for the number of litter items or particles inside the technology, reducing its *PR rate* (tending to zero). In parallel, the amount of litter intercepted by the technology might not be constant over time and is dependent on the pollution sources input rate. Furthermore, when a technology is installed in a polluted area, and is working consistently to remove litter, the *PR rate* is expected to be the highest when the system is first deployed (exponential and/or linear phase, Fig. 12), being reduced over time until the stationary phase (Fig. 12) is reached, when less or no plastic litter is available to be collected, assuming that no extreme weather conditions or acute pollution events occur. Therefore, **it is recommended that the *PR rate* reported states the scenario in which it was assessed or, if enough data is available, the amount of PR is reported as a function** of number or mass of plastic litter over time (inside the technology for each period between maintenance or cumulative since deployment).

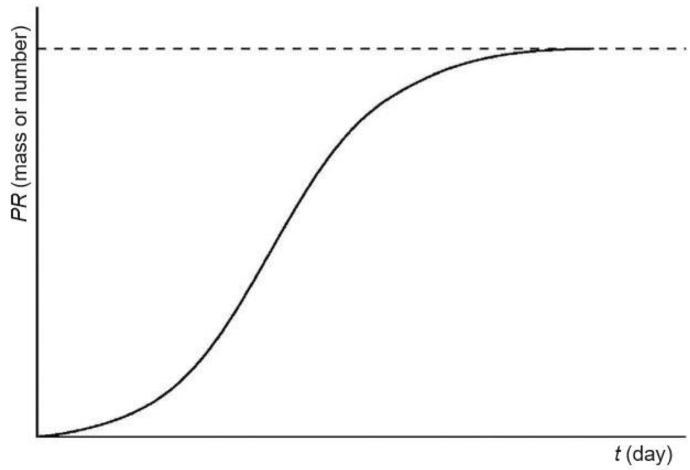

Fig. 12. Example of possible evolution of cumulative PR over time, plotted as a logistic function (t = 0 corresponding to the technology deployment).

Similarly, other environmental parameters, such as weather and hydrologic parameters, can be used to normalize the results. This would allow to compare the results between technologies deployed in different environmental conditions. Two important agents for the transport of plastic are the water and/or wind speed and direction. Thus, it is expected that these will impact the *%I* (and *I rate*).

8.3.2 Reporting

There are two main data outputs expected from the application of the protocols described in this deliverable: 1) an excel spreadsheet; and 2) a report. Templates for both will be created and available. It would be noted that, the plastic removal efficiency should be assessed by several release-catch experiments and, preferably, under different scenarios. Therefore, multiple excel spreadsheets are expected, which can be reported together.

Recommended metadata and data fields to include:

- Name and characteristics of the solution, including dimensions, horizontal coverage of the water/riverbank, plastic size range targeted (including mesh size used), active/passive (i.e., using electricity or not), among others.
- Name and contact information of the main person responsible for the assessment
- Date (YYYY/MM/DD), time (HH:MM) and location (GPS coordinates) of the assessment
- Field measurements: weather conditions (e.g., precipitation), hydrologic measurements (e.g., river flow rate), compartment (depth and/or area) targeted by the solution, distance between release point and solution, water flow and pumps operational parameters

- Operational parameters relevant to the release-catch experiments, such as maintenance time, time since first deployment or last cleanup, and if is working continuously or under a specific time frame
- Other solution specific parameters assessed (e.g., number of people that joined the manual cleanup activity)
- Characterization of the test materials: material type used (e.g., polymer), size range, shape, colour, transparency, mass, and J-code if applicable [12]. More information in Chapter 7.
- Release-catch experiment fractions, rates and other additional parameters determined as suggested in section 8.3.
- Inspection data (including photographs)
- Brief summary on the baseline pollution levels with reference to respective dataset, if available.

9. Perspectives

With the INSPIRE Deliverable 2.1, we established the concepts and terminology required for assessing the plastic removal efficiency, and we described the protocols and test materials for the upcoming release-catch experiments and the plastic removal efficiency related assessments.

As a next step, detailed plans are going to be prepared for the plastic removal efficiency assessment of each INSPIRE WP2 solution, based on this deliverable, on the results of the baseline pollution levels from Work Package 1, and on demo site observations. These plans will include the tests timeline, list the test materials with the particles/items selected, identify specific actions to be taken, among other required and relevant information. We expect that the protocols here proposed will be continuously improved during the preparation of the specific assessment plans for each solution, and with the acquired knowledge from the tests during their implementation. The direct outputs will be reported in INSPIRE deliverables 2.2 to 2.7.

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