LABPLAS

Horizon 2020
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Land-Based Solutions for Plastics in the Sea

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D2.1 Harmonisation of Sampling Methods

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PROJECT INFORMATION

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List of participants:

Nº	Participant name	Acronym	Country	Type
1	UNIVERSIDADE DE VIGO	UVI	SPAIN	HES
2	UNIVERSIDADE DA CORUÑA	UDC	SPAIN	HES
3	Bundesanstalt fuer Gewaesserkunde	BfG	GERMANY	RTO
4	LABORATORIO IBERICO INTERNACIONAL DE NANOTECNOLOGIA	INL	PORTUGAL	RTO
5	KATHOLIEKE UNIVERSITEIT LEUVEN	KUL	BELGIUM	HES
6	GEOMAR HELMHOLTZ ZENTRUM FUR OZEANFORSCHUNG KIEL	GEOMAR	GERMANY	RTO
7	NATIONAL OCEANOGRAPHY CENTRE	NOC	UNITED KINGDOM	RTO
8	SORBONNE UNIVERSITE	SU	FRANCE	HES
9	OPEN UNIVERSITEIT NEDERLAND	OUNL	NETHERLANDS	HES
10	LEIBNIZ INSTITUTE FOR BALTIC SEA RESEARCH	IOW	GERMANY	RTO
11	ASSOCIACAO PARA O DESENVOLVIMENTO DO ATLANTIC INTERNATIONAL RESEARCH CENTRE	AC	PORTUGAL	RTO
12	UNIVERSIDADE FEDERAL DE SAO PAULO	UNIFESP	BRAZIL	HES
13	BASF SE	BASF	GERMANY	LE
14	TG ENVIRONMENTAL RESEARCH	ER	UNITED KINGDOM	SME
15	CONTACTICA S.L.	CTA	SPAIN	SME
16	STICHTING EGI	EGI	NETHERLANDS	Non-P
17	STICHTING RADBOUD UNIVERSITEIT	RU	NETHERLANDS	HES

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Executive summary:	This report corresponds to Deliverable 2.1 Harmonisation of sampling methods acquiring sampling instruments, planning of sampling campaigns and selection and sub-sampling of sediment cores, resulting from Task 2.1. of the LABPLAS project. It covers the description of the harmonised sampling methods for every environmental compartment to ensure comparable techniques.

Version	Date	Comments
1	11.10.2021	Initial version
2	16.11.2021	Revised final version
3	10.02.2023	Reviewed with changes to address comments by the PO and reviewer

Disclaimer

The views and opinions expressed in this document reflect only the authors' views, and not necessarily those of the European Commission.

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ABBREVIATIONS AND ACRONYMS

Abbreviation / Acronym	Description
CLSM	Confocal Laser Scanning Microscopy
CTD	Conductivity-Temperature-Depth profiler/sensor/sonde
DWTP	Drinking Water Treatment Plant
MSFD	Marine Strategy Framework Directive
MUC	Multi-corer device
SEM	Scanning Electron Microscope
SML	Surface micro layer
SMNP	Small, micro-, and nano- plastics
ULW	Underlying water
WWTP	Waste Water Treatment Plant

1 Introduction

Plastic is pouring from land into our oceans at a rate of nearly 10 million tonnes a year. Once in the sea, plastics fragment into particles moving with the currents and ocean gyres before washing up on the coastline. The smaller the size the higher the risk posed by these particles to organisms and human health. Because small, micro- and nano-plastics (SMNP) cannot be removed from oceans, proactive action regarding research on plastic alternatives and strategies to prevent plastic from entering the environment should be taken promptly. The LABPLAS project is a 48-month project whose vision is to develop new techniques and models for the detection and quantification of SMNP. Specifically, LABPLAS will determine reliable identification methods for a more accurate assessment of the abundance, distribution, and toxicity determination of SMNP and associated chemicals in the environment. It will also develop practical computational tools that should facilitate the mapping of plastic-impacted hotspots and promote scientifically sound plastic governance.

This deliverable deals with the harmonisation of sampling methods and the planning of the first sampling campaign to ensure comparable techniques among all partners across sampling. The harmonisation of methods took place via many different meetings in which the different participants discussed in detail the sampling methods. A meeting in the laboratories was not possible due to the restrictions connected to the covid pandemic. For sample preparation methods please refer to deliverable 3.1.

The sampling methods described in this deliverable are based on the JRC guidance document for monitoring marine litter within the implementation of the Marine Strategy Framework Directive (JRC 2013) with methodological advances targeting microlitter. For water sampling, the fraction escaping conventional Manta trawl nets (ca. 300 µm) or Continuous Plankton Recording devices are targeted by using pump-filter systems collecting down to 10 µm particles (refer to Section 3.1.1). An additional method intended to specifically sample the surface microlayer by using a Garret screen is also included (refer to Section 2.1.5). In addition, for air deposition, a microplastic input pathway not considered by JRC (2013), is also targeted. For sampling microplastics in marine biota, the EPHEMARE-BASEMAN joint harmonized protocol is considered (Bessa et al. 2019). All the procedures described in this deliverable will be validated and updated if needed according to the results from ongoing field sampling and laboratory analysis.

2 Sampling Methods

2.1 Water samples

2.1.1 Microplastics, 10 µm – 1 mm

Water sampling for smaller microplastics (MPs, 10 µm - 1 mm) will be conducted in the entire study area (case study 1: Greater North Sea with Elbe and Thames River Basins, and case study 2: Mero-Barcés River Basin) using a custom-built pump-filter system based on the design by Mintenig et al. (2017) for smaller particles. This consists of a high-pressure pump attached to an enclosed cartridge containing a 10 µm stainless steel mesh filter, with a water meter to determine volume. A prefilter with 1 mm allows to only sample smaller microplastics. One water sample will be retrieved below the water surface in the middle of the river from each sampling site.

2.1.2 Plastics, >330 µm

Microplastics floating at the sea surface will be sampled with triplicate neuston catamaran trawls at a towing speed of 2-3 knots for about 20 min each.

At the river surface, neuston trawls will be used stationary from a boat or a bridge.

The trawl has a mouth opening of 40 cm (height) x 70 cm (width) and is mounted to a 3-m long net with a mesh size of 0.3 mm, ended by a 30 x 10 cm^2 cod-end (Löder and Gerdts, 2015). Plastic particles will be picked from the collected material and stored in 96-well plates for subsequent experiments or polymer verification in land-based laboratories. A flowmeter will be fixed on the net for determining the water volume.

2.1.3 Nanoplastics

Water sampling for nanoplastics will involve sampling river and seawater with a water bottle of agreed composition and volume (1 l glass bottle). One sampling site will be chosen where several samples will be taken from different depths.

2.1.4 Stormwater and treated urban wastewater

Stormwater and treated urban wastewater sampling will be conducted in the Mero-Barcés river basin using automatic grab-samplers and flowmeters to prepare composites of flow proportional samples during dry weather (WWTP) and wet weather (stormwater and WWTP) conditions. At stormwater monitoring stations, a rain gauge and continuous turbidity and conductivity probes will be installed to seek correlations between microplastics and conventional stormwater pollutants (solids) during rainy events.

2.1.5 Water surface microlayer (SML) sampling

Sampling for the surface microlayer will be conducted from a small work boat on the upwind side of the vessel using a screen sampler (Garrett screen, stainless steel, mesh size 1.2mm) following applied practice (Garrett 1965). The Garrett screen takes advantage of the naturally high surface tension of water as it retains the SML samples between the meshes. Before sampling, the screen will be conditioned by several dippings in seawater and sampling bottles will be rinsed with the sample prior to collection. The sampling procedure follows the instructions given by Gašparović et al. (2014) (editors Cunliffe and Wurl 2014). Samples will be collected in deionized washed brown borosilicate bottles. After sampling, the screen sampler will be cleaned thoroughly with a jet of clean water derived from the ship's reverse osmosis processing plant. Reference samples from the underlying water (ULW) will be taken from the CTD at a depth of 2 m. Samples will be processed at GEOMAR for sizes, density, shapes, surface structures, aggregation status and biofilm composition/density of the plastic samples using different techniques (weighing, CLSM, SEM, optical light microscopy).

2.2 Sediment

2.2.1 Bulk sediment samples

The uppermost 15 cm of the sediment column will be retrieved with a Van –Veen grab sampler in the rivers Thames, Elbe and Mero-Barcés at the same location as water samples, to determine potential correlations between suspended and deposited microplastics at different locations, and assess microplastic accumulation in recent sediments. Grab samples will also be collected at the North Sea stations. During two large field sampling campaigns (winter 2021, and summer 2022), 24 locations will be sampled across the case study sites. Two sites in each case study will be sampled more frequently to allow more refined spatial and temporal-scale research questions to be addressed (2022-2023).

2.2.2 Sediment core samples

To test the hypothesis of increasing plastic amounts in marine sediments/compartments with time, sediment cores will be sampled and analysed. These results (timelines) of microplastic contamination will eventually feed discussions on the onset of the Anthropocene epoch, still to be formalized in the geological time scale and will be described in an extra report/deliverable.

Sediment cores were collected in the Baltic Sea during previous expeditions from the IOW. Up to eight short (45 cm) sediment cores were obtained with an intact sediment-water interface using a multi-corer (MUC) device. LABPLAS will rely on cores sampled at the Landsort Depp, Eastern Gotland basin and the Gulf of Finland (up to 270 m deep). These undisturbed sediments of the deep Baltic Sea basins allow excellent temporal resolution and the potential establishment of microplastic temporal trends over the last century.

In the North Sea, sediment cores will be obtained with an intact sediment-water interface using a MUC identical to that used in the Baltic Sea sampling. Cores will be sliced onboard every 0.5 to 1 cm to be used as single or composite samples. Samples will be stored in Al foil or glass jars previously ashed at 500°C. In the shore-based laboratory, sediment samples will be freeze-dried and weighed (0.001 g). For sample processing, the same procedure as for the sediment grabs will be used i.e. oxidation of organic matter and density separation.

2.3 Atmospheric deposition

The atmospheric microplastic deposition will be evaluated through passive sampling using bulk (dry and wet) deposition samplers at two selected sites in each case study (representative rural, urban, industrial, and motorway). Samples will be collected in glass vessels (plastic containers should be avoided). Each atmospheric deposition sampling will be carried out for 1 month approximately (4-6 weeks) seasonally (spring, summer, autumn and winter) within the second year to estimate the spatial and seasonality variability. The atmospheric deposition fluxes and the microplastic transport will be studied. At the moment, tests are running to determine which instrument is the best to use. The deliverable will be updated depending on the results.

2.4 Biota

Biota from three representative feeding guilds will be collected depending on the availability at three selected sites within the project-studied rivers (Elbe, Thames and Mero-Barcés) and in the North Sea, once per year and over two years. Sampling and analysis for zooplankton and bivalves (described below) follow the EPHEMARE-BASEMAN recommendations for biota projects to fulfil standardization procedures (Bessa et al., 2019).

2.4.1 Zooplankton

Zooplankton will be collected from surface waters by using a hand-held zooplankton net (200 µm), or from the water column by using bottom-to-top vertical tows with a standard SCOR WPII plankton net (200 µm). If zooplankton is not available (i.e. in fast-flowing rivers), benthic invertebrates will be collected.

2.4.2 Bivalves

Bivalves e.g. mussels or Asian clams will be collected by hand just below the water level in the three rivers or obtained from National authorities. Bivalves will be collected in the North Sea using surface sediment grabs and sieving the sediment through a 1 mm mesh. Specimens will be processed and analysed as pooled samples to ensure sufficient tissue.

In the Thames river basin, a special permit is necessary to sample invasive species. Thus, a variety of benthic invertebrates, including bivalves, will be sieved from sediment, representing a sediment invertebrate community.

2.4.3 Fish

Planktivorous pelagic fish (e.g., *Clupea* sp., *Scomber* sp., *Merlangius* sp), or benthic detritivores (e.g. *Limanda* sp., *Pleuronectes* sp., *Platichthys* sp.) will be obtained commercially, in cooperation with national authorities or from sport fishing organisations. The exact species collected during each survey will depend on availability in the catch. The samples will be processed by removing the gut within a clean lab environment. If possible, the guts and tissues will be analysed separately to distinguish between ingestion and bioaccumulation. All guts and a subsample of tissues will be analysed (e.g., all fish from one site only). The sampling is based on the JPI-Oceans projects EPHEMARE-BASEMAN agreement for biota.

2.5 Interaction between microplastics and zooplankton

The interactions between microplastics and zooplankton will be studied by collecting surface samples in the marine areas of the Elbe and Thames estuary using a Manta net (300 µm). These samples will be analysed at the same time and using the same methods as the samples previously collected in 2019 in the Mediterranean Sea. Plastic and zooplankton will be sorted, counted and digitally imaged with a ZooScan digital scanner. Image post-processing will be performed with the Zooprocess and plankton Identifier software that enumerates and gives a large set of morphological parameters for each plastic and zooplankton item.

2.6 Blank samples and replicates

When working with microplastics in small size scales it is crucial to quantify false positives on samples to allow robust and reliable numbers to be reported. To avoid false-positive microplastic particles, blank samples will be run concomitantly to environmental samples to allow potential identification of contamination routes during for example sampling, transport and processing. Microplastic particles in blanks will be analysed in terms of shape, size, colour and polymer type and are then compared with particles in environmental samples.
Replicates will be taken during fieldwork. It is planned to take several sediment samples. Replicates for water samples (plastics 10 µm - 1 mm) can be taken in the laboratory with a subsample. For smaller microplastics, the system is closed and will not open during fieldwork. Thus, no control is needed. For larger plastics, several samples will be taken.

3 Sampling Instruments

3.1 Water samples

3.1.1 Microplastics 10 µm - 1 mm

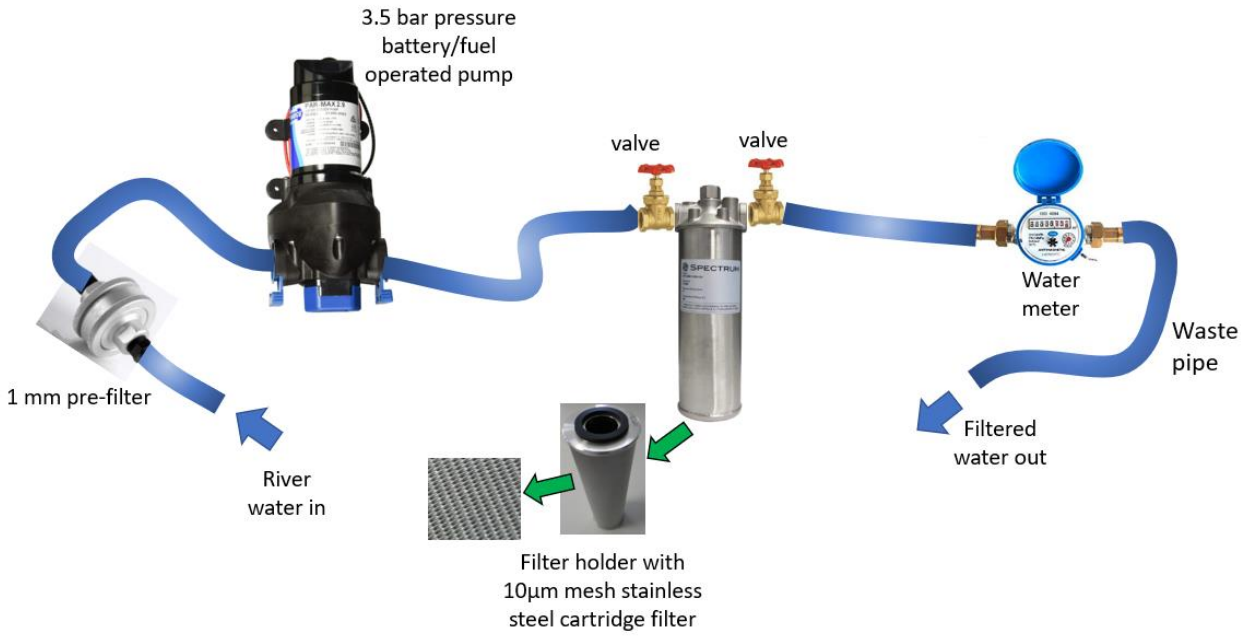

Figure 1a: Diagram of how to sample smaller microplastics (10 µm - 1 mm) (A. Horton).

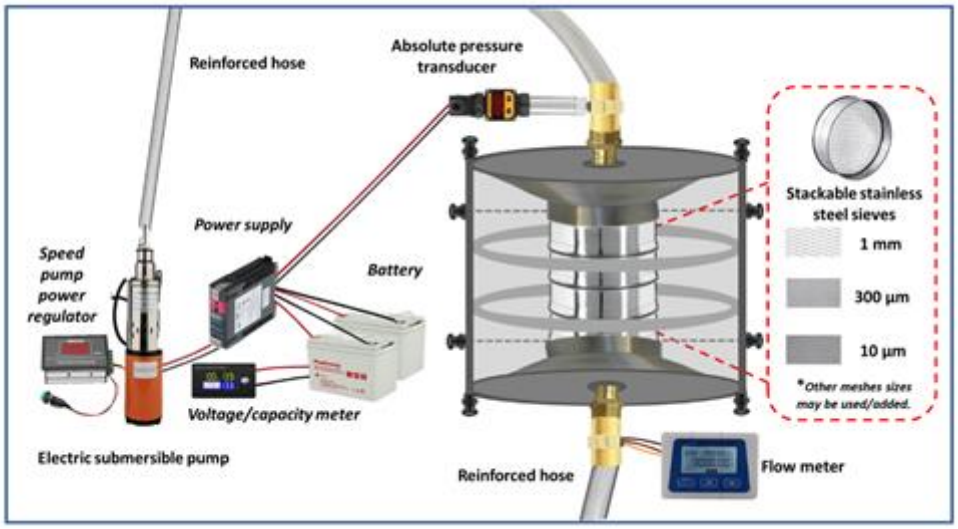

Figure 1b. Diagram of how to sample smaller microplastics (10 µm - 1 mm) according to ASTM D8332 (S. Muniategui).

2.1.2 Plastics >330 µm

Figure 2: Manta net for sampling plastics > 330 µm from the water surface (left side: river sampling, right side: catamaran trawl for the North Sea) (photos: Hydro Bios).

3.2 Sediment

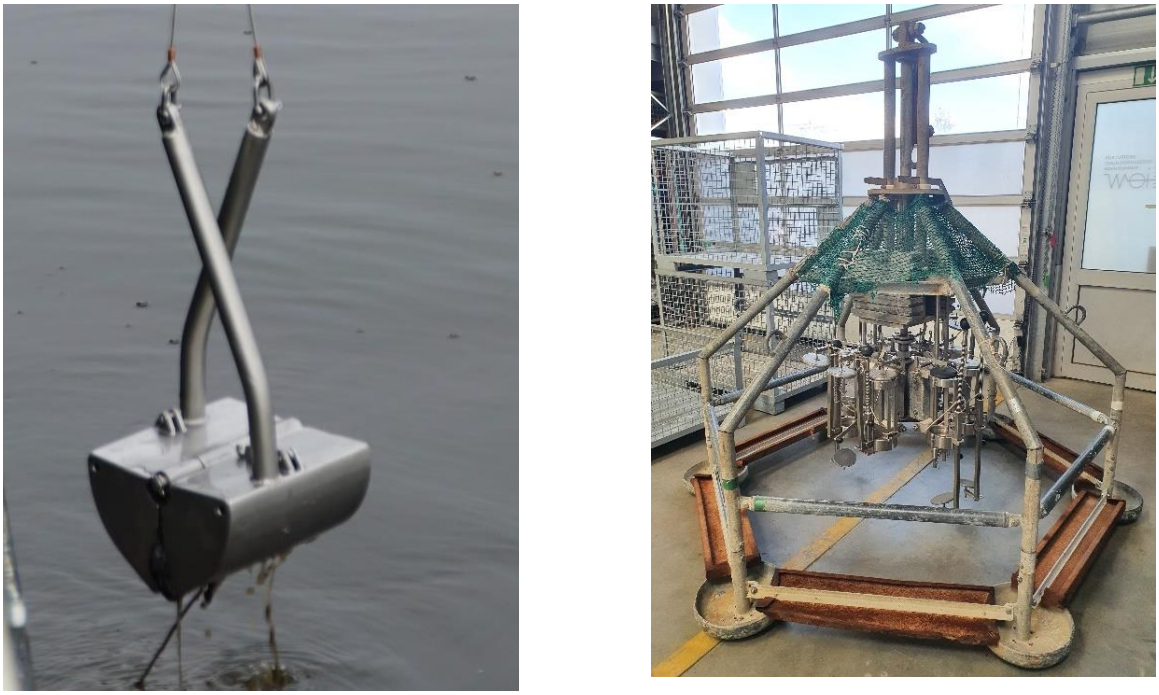

Figure 3: Van-Veen grabber for sampling sediments in rivers (left side) (F. Stock). Multi-corer for sampling in the Baltic and North Sea (right side) (photo A. Frahm, IOW).

3.3 Atmospheric deposition

Figure 4: Depobulk total deposition sampler (photo UDC).

4 Sampling Campaigns

For case study 1, *The North Sea with Elbe and Thames river basins* (Figure 5), 18 sampling sites have been chosen. The river basins represent different characteristics: the Elbe River is highly industrialised, indicating multiple dispersed and point sources, while the Thames comprises both rural and significant urban influences; the final sink destination is the North Sea.

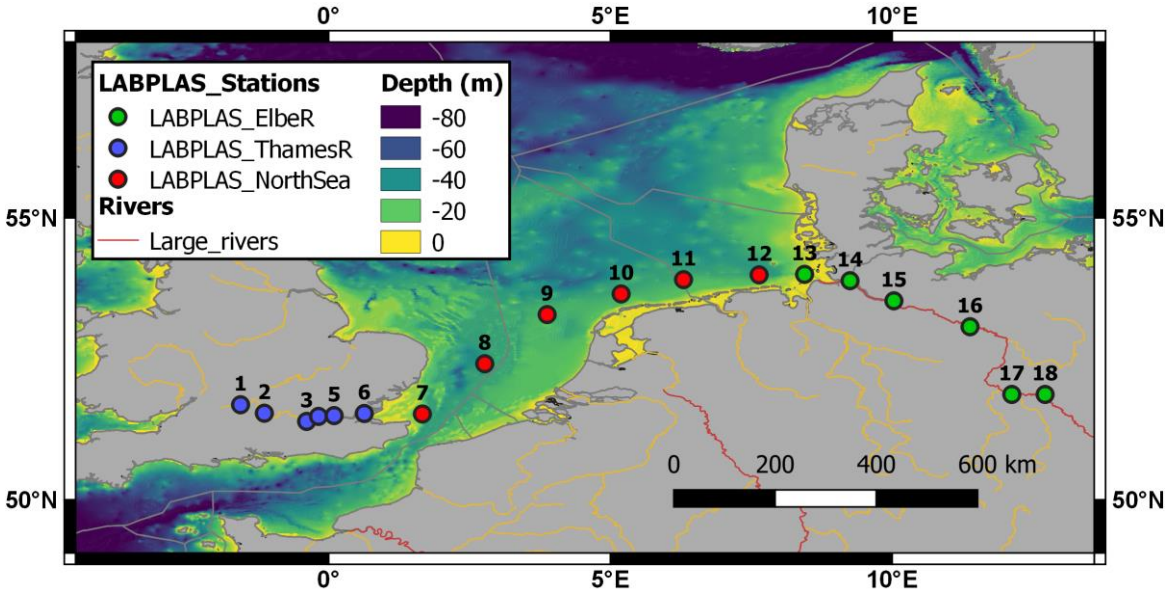

Figure 5: Study area 1 The North Sea with Elbe and Thames rivers basins

Sampling sites at the Thames river basin:

- 1: Lechlade (rural upper reaches)
- 2: Dorchester on Thames (rural with urban influence)
- 3: Upstream tidal limit (Hampton Court)
- 4: Downstream tidal limit (Richmond)
- 5: Central London (urban)
- 6: Thames estuary

Sampling sites at the North Sea:

- Sites 7, 10, 11, 12 coincide with sites previously sampled in the JPI Oceans project HOTMIC

Sampling sites at the Elbe river basin:

- 13: Elbe estuary (coincides with HOTMIC sampling site)
- 14: Central Hamburg (urban)
- 15: Elbstorf: Downstream tidal limit
- 16: Dömitz upstream tidal limit
- 17: Tributary: Mulde/Elbe confluence: industrial influence
- 18: Klöden: rural influence

Case study 2, the Mero-Barcés River basin, considers six sampling sites (Figure 6). The river basin located in the northwestern Iberian Peninsula is mainly rural but impacted by highway traffic; the final destination of the study area is a drinking water reservoir.

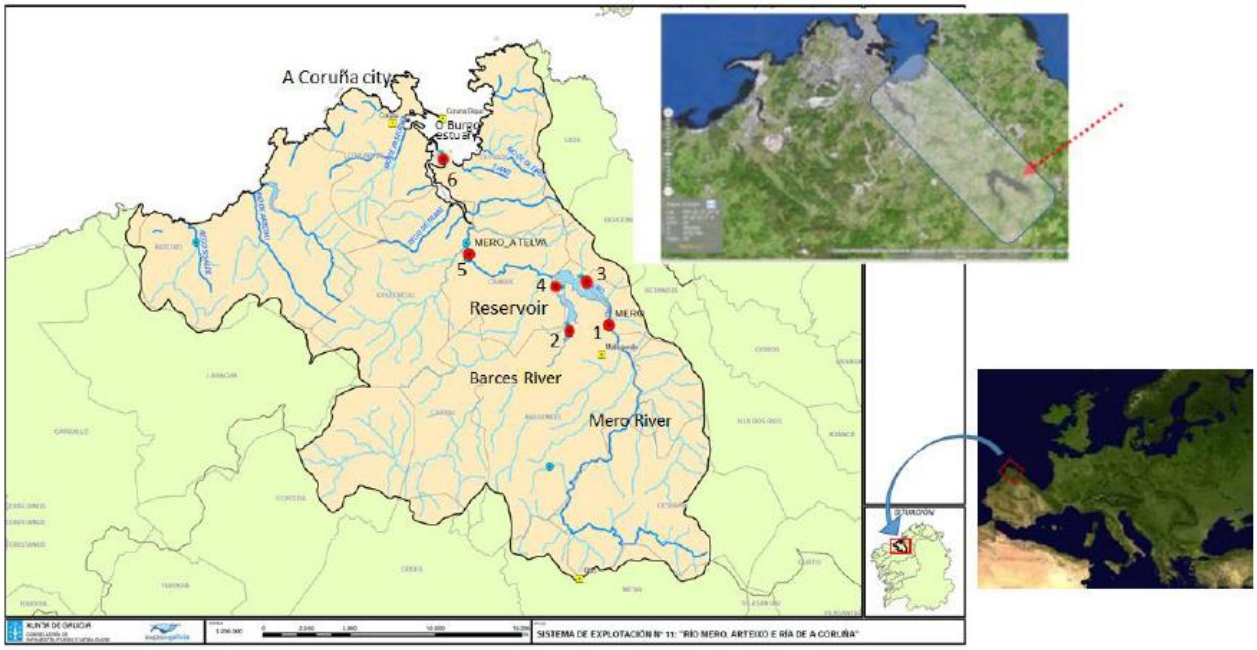

Figure 6: Case study 2: Mero-Barcés River Basin

Sampling sites at the Mero-Barcés River Basin:

- 1. Mero river upstream site: rural area, WWTP influence (1,600 inhabitants)
- 2. Barcés river upstream site: road runoff influence (A6 motorway), rural with urban influence, mining (lignite)
- 3. Abengondo-Cecebre reservoir: rural, road runoff influence (AP9 motorway)
- 4. Abengondo-Cecebre reservoir: dam
- 5. Mero river downstream site: urban, water catchment DWTP (drinking water treatment plant-catchment 2500 L/s for the city of A Coruña and its metropolitan area) (10 Km from the dam)
- 6. O Burgo estuary: urban, tidal limit.

In total, 6 sampling campaigns are envisaged. Two major sampling campaigns will take place twice within year 1 (winter: January 2021, and summer: July 2022) and will include six sites sampled for water, sediment and biota in each river system and the North Sea. The sampling on the North Sea depends on the availability of a ship. To not delay the project, the river basins will be sampled at the same time as planned even if/when the North Sea is sampled later.

Targeted sampling campaigns at two selected sites of interest in each river system for water, sediment and air, will take place seasonally within year 2 (autumn: October 2022, winter: January 2023, spring: April 2023, and summer: July 2023). These campaigns allow for spatial and temporal (seasonal) resolution analysis of microplastics within water, sediment and air samples, in addition to biotic interactions and bioaccumulation.

5 Selection and Sub-Sampling of Sediment Cores

LABPLAS will rely on sediment cores that are dated many decades back (2021-1900 AD). Cores are sampled using a Multi-corer (Oktopus Kiel; diameter 2.5 m, height 2.2 m, total weight 700 kg). The device samples a total of eight 45cm-short-cores (outside diameter 110 mm, inside diameter 100 mm, length 600 mm) and penetrates ~45 cm into sediments. Three sediment cores from the E Gotland basin, Landsort and Gulf of Finland have been selected. The cores will be checked for the integrity of the material. In addition, one extra core has been selected to test a method to minimise contamination. Finally, a long core (1 m) will be sampled to create a background contamination pattern for all subsequently analysed cores.

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