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Danube Hazard m³c

Internal document

Protocol for sampling, storage and transport during monitoring in the pilot regions

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1 Introduction

The present document provides protocols for sampling of river waters, wastewater samples, atmospheric deposition samples, soil samples and suspended particulate matter (SPM) samples for chemical analyses planned within the Interreg Danube Hazard m3c project. The document delivers instructions for sample identification, handling, preservation and transport. The cleaning procedures for the vessels used for different type of the analysis are also described. The parameters for chemical analysis are provided for a particular sample matrix, as well as the contact persons who are responsible for receiving the samples for the analysis.

The selection of the pilot regions and of the specific sampling points in each of them are described in the Deliverable D.T1.2.1 "Final selection and description of pilot regions" and in the Deliverable D.T1.2.2 "Methodological approach for the measurements in the pilot regions and final selection of substances", respectively.

2 Scope of the monitoring

The scope of the monitoring within the project Danube Hazard m^3c covers a range of micropollutants, which will be determined in samples from different environmental and anthropogenic matrices by four laboratories. The specific analysed substances, following the the most important contaminants set by the water framework and its daughter directives (Directive 2000/60/EC, 2000; Directive 2008/105/EC, 2008; Commission Directive 2009/90/EC, 2009; Directive 2013/39/EU, 2013; Commission Directive 2014/101/EU, 2014) and selected specific contaminants as well as the responsible laboratories for the analysis are summarized in Table 1.

Table 1: Substances analysed within the project Danube Hazard m^3c, sample matrices and responsible laboratories.

Compound class	**Analytes**	**Analytical laboratory performing the analysis**	**Sample matrix**
Hg and other metals	Hg, Cd, Ni, Pb, Cr, Zn, As	JSI	River water SPM Atmospheric deposition Wastewater Soil
16 PAH		NARW	River water Atmospheric deposition Wastewater
16 PAH		UBA	SPM Soil
PFAS	PFOS, PFOA (others are depending on the winner lab. Proposed other forms: PF4C – PF12C, PF4S – PF10S)	Wessling Hungary Ltd	River water SPM Atmospheric deposition Wastewater Soil
4-ter Octylphenol			
Nonylphenol			
Bisphenol A			
Tebuconazole			
Diclofenac			

Compound class	Analytes	Analytical laboratory performing the analysis	Sample matrix
Carbamazepine			
Metolachlor (incl. Metabolites)			

SPM – suspended particulate matter, PAH - Polycyclic aromatic hydrocarbons, PFAS - Per- and polyfluoroalkyl substances

In addition, all partners responsible for monitoring in pilot regions must perform regular sampling and analysis at their own premises to determine the suspended solids content in river samples at the location where turbidity probes are installed. Such sampling is needed at low flow but even more importantly at high flow conditions (grab samples or autosampler) to calibrate turbidity against suspended solids. This is essential to be able to estimate reliable SPM river loads and thus builds a very important basis for the subsequent modelling.

3 Transport and contact persons

Table 2 and Table 3 report the contact details of the persons responsible for the sampling in each pilot region and for the analysis of the different chemical parameters, respectively.

*Table 2: Contact persons responsible for the **sample collection** and addresses for the pre-cleaned empty bottles delivery*

Pilot region	Contact person	Addresses for the delivery of empty bottles	Email, Telephone
Ybbs	Oliver Gabriel	Umweltbundesamt GmbH Probeneingang Spittelauer Lände 5 A-1040 Vienna Austria	Oliver.Gabriel@umweltbundesamt.at Tel: +43-(0)1-313 04/3681
Wulka	Steffen Kittlaus	TUWien - Forschungsbereich Wassergütewirtschaft 226-1 Lilienthalgasse 21, Objekt OD, 2 Stock 1030 Wien Austria Contact person for delivery: Ernis Saracevic, erni@iwag.tuwien.ac.at +43 (1) 58801-22660	steffen.kittlaus@tuwien.ac.at +43 (1) 58801 22636
Vit	Dimitar Mihalkov	Bulgarian Water Association 1 Hristo Smirnenski Blvd., 1046 Sofiq,	d.mihalkov@bwa-bg.com +359 885 508 305

		Bulgaria	
Koppány	Zsolt Jolánkai	Budapest University of Technology and Economics, "Vizi Közmü és Környezetmérnöki Tanszék" Műegyetem rkp. 3. KM 45 H-1111 Budapest Hungary	jolankai.zsolt@epito.bme.hu +36 1 463 2955
Zagyva	Máté Kardos	Budapest University of Technology and Economics, "Vizi Közmü és Környezetmérnöki Tanszék" Műegyetem rkp. 3. KM 45 H-1111 Budapest Hungary	kardos.mate@epito.bme.hu +36 20 5259163
Vișeu	Adriana Muntean	ABA Somes – Tisa, SGA Maramures, LCA Baia Mare str. Hortensiei, nr.2, Baia Mare, jud. Maramureş, Romania	adriana.muntean@sgamm.dast.rowater.ro +40 745 660 337
Someșul Mic	Alexandru Fekete	ABA Somes – Tisa, LRCA Cluj – Napoca, str. Vânătorului, nr.17, Cluj-Napoca jud.Cluj, Romania	alexandru.fekete@dast.rowater.ro +40 743 156 501

*Table 3: Contact persons responsible for the **chemical analyses** and addresses for the sample delivery*

Parameter	**Contact person**	**Addresses for the sample delivery**	**Email, Telephone**
Hg and other metals	Radmila Milačič	Department of Environmental Sciences Jožef Stefan Institute Jamova 39 1000 Ljubljana SLOVENIA	radmila.milacic@ijs.si Tel:+386 1 4773560
Preparation, lyophilisation and distribution of solid samples	Sandra Kulcsar	Umweltbundesamt GmbH Probeneingang Spittelauer Lände 5 A-1040 Vienna Austria	Sandra.kulcsar@umweltbundesamt.at Tel: +43-(0)1-313 04/5277
16 PAH in solid samples	Sandra Kulcsar	Umweltbundesamt GmbH Probeneingang Spittelauer Lände 5 A-1040 Vienna	Sandra.kulcsar@umweltbundesamt.at Tel: +43-(0)1-313 04/5277

		Austria	
16 PAH in liquid samples	Carmen HAMCHEVICI	**Laboratorul National de Calitatea Apei – A.N. Apele Romane (ANAR)** Street Splaiul Independentei nr. 294, sector 6, 060031 Bucuresti, Romania	carmen.hamchevici@rowater.ro +40 755 063 869
PFAS and other organic substances	Gabor Bordos	WESSLING Hungary Ltd. **Környezetanalitika Mintaátvétel** 6 Anonymus st., Budapest 1045, Hungary	bordos.gabor@wessling.hu +36 20 335 8627

*SAMPLER BOTTLE QUANTITIES AND ADDRESSES ARE SUMMERISED IN Bottle Delivery.xlsx table uploaded in the owncloud directory.

Each laboratory provides to the partners responsible for sampling the empty bottles and containers, which need to be returned filled with the samples to be analysed. Such empty bottles can be sent via regular mail.

Bottles and devices required to generate the composite samples, as well as chemical compounds needed for preservation, must be procured by each partner, following instructions on material and cleaning provided by the analytical laboratories in this document.

The composite water samples for selected **(define which)** organic class contaminants must be sent via courier in boxes filled with dry ice and an express delivery must be selected, which ensures reaching the analytical laboratory within 48 hours, while for metals and … organic compounds **(define which)** delivery with a regular mail reaching the laboratory, performing the analysis, in 48 hours is adequate. Each partner responsible for sampling must buy the required boxes and dry ice. The adequate protection of the glassware is the responsibility of each partner, and should not left to the transportation companies. Recommended packaging include liquid foam packaging to secure the bottles firmly.

It is recommended to order the polystyrene foam box that tightly fits the bottles. Consider that all the volume of the box should be filled with dry ice. The density of dry ice is 1.4 to 1.6 kg/L.

Example:

6 glass bottles of 1 L (diameter 9.5 cm, height 25 cm, weight of the bottle + 1 L of water sample is about 1.5 kg) can be put into polystyrene foam box with inner dimensions: 27 cm x 32 cm x 22 cm. The volume of such box is 19 L.

Approximately 6 L is the volume of sampling bottles, so 9 L should be filled with dry ice (considering average density 1.5 kg/L, this means approximately 13.5 kg of dry ice).

The mass of such package for the transport by courier is 9 kg (for 6 glass bottles filled with 1 L of water) + 13.5 kg (for 9 L of dry ice) – together = 22.5 kg.

Based on the information of the DHL courier, which ensures the transport door to door in EU countries within 24 hours, the maximal allowable weight for one box on dry ice is 64 kg.

4 River water sampling, storage and transport

River monitoring aims to generate two types of composite samples, namely 6 composite samples representative of baseflow-midflow and 6 composite samples representative of high-flow events.

4.1 Baseflow-midflow samples

4.1.1 Sampling

Each of the six composite samples is composed of weekly grab samples collected during two months (Obviously at high flow events, the weekly grab samples will not be collected, therefore the composite sample of this period will be less in volume).

Grab sampling is performed in accordance with the standard ISO 5667-3 (2018) on sampling of rivers and streams [2].

Depending on parameters measured, polyethylene (PE) (transparent or amber), Teflon (for Hg and metal analysis) or amber glass bottles are used (See Table4). If the composite sample for organic class contaminants is collected, metallic or stainless-steel bucket should be used while for metal contaminants PE bucket. Please note that if Teflon and PE bottles are provided for composite sampling, the actual collection of the river sample can be done, using the same **glass** bottle for each parameters. Please ensure that the glass bottles used for sampling are cleaned properly according to the descriptions in *section 9*. At the lab, all composite sample can be filled from this bottles.

If, for preservation purposes, the bottles used for sampling are filled with acidic solution*, the content must be discarded, and the bottle must be rinsed three times with river water. Mount a vessel in a telescopic holder and immerse an open-mouthed bottle of sampling system into a laminar flow stream approximately 30 cm below the river surface (ISO 5667-3; 2018). The inlet of the sampling bottle should face the direction of the river flow. **Warning: consider that the bottles should be filled maximum to 85% of the bottle volume. Otherwise, they can break during the sampling and/or transport!** Please put all bottles into plastic ZIP bags and stick the labels on the bottles and on the ZIP bags.

***Safety warning: Use protective nitrile or other gloves and protective eyewear when emptying bottles with preservation liquids, to avoid accidents**

These grab samples must be immediately transported in cooled conditions to the partner's premises, in order to be frozen if possible within 4 hours since sampling.

WARNING: when samples are taken by wading into a river, account should be taken of the possible presence of soft mud, quicksand, deep holes and swift currents. A wading rod is essential to ensure safe wading. By probing ahead, a person who is sampling can estimate the shape of river bottom. A safety line should be attached to a secure object on the bank for support. The use of life jacket is strongly recommended. At least two people should work together on river sampling.

4.1.2 Storage

Each partner holds in the freezer the plastic bottles/containers provided by the laboratories responsible for the chemical analyses in the project. Samples for metals and organic contaminants in plastic bottles must be deeply frozen at -20°C.

For PAH and the rest of the organic substances, partners should keep the glass bottles in cool condition (2-4 °C) and not frozen.

After each weekly sampling, an aliquot of the fresh sample is poured into the bottle/container in the freezer and if necessary a proportional amount of stabilizing compound is added. If sufficient space is available in the partner's freezer a duplicate of this cumulative sample will be generated (in case the first one breaks during storage or transport).

Metals: Due to the carbonate precipitation issues from the start of the next possible* composite samples the dissolved samples should be filtered and acidified in labs of the partners, making the collection. Only specified filters** and acids*** should be used to avoid contamination. Each partner has to procure the specified filters, while the acids used are provided by JSI. Whole water samples should be acidified as previously, but also suprapure acid to be used, provided by JSI.

*Depending on the procurement time of the specified glass fibre filters.

** Filters to be used: Sartorius Ministart (Hydrophillic) RC25 17765-Q A500 SART Syringe Filter, Nonsterile: 28 mm diameter, 0.45 um pore size.

*** Acids to be used: Carlo Erba SuperPure nitric acid RS 67-70%, tested by JSI.

PAH samples only collected for Total PAH analysis in total samples. From December 2021, no Dissolved PAH fraction is measured!

4.1.3 Required volumes and bottles

Table 4 reports which volumes and bottles are needed for the composite samples (to be kept in the freezers during two months) and an indicative estimation of which volume of water shall be added each week if the composite sample is composed of 8-week samples. The indicated volume has to be adjusted if the number of weeks deviates from 8. The table also reports the total final amount of compounds needed for the preservation of the samples (if applicable).

Table 4: Volumes, bottles and preservation compounds required for the composite samples of river water at baseflow-midflow conditions

Parameter	Volume and bottle material for the composite sample	Volume and bottle material for the weekly sampling	Compounds for preservation
Metals and Hg	0.16 L of whole water 0.5 l Teflon bottle	~ 20 ml x 8 weeks Glass	0.16 mL of HCl s.p. (30%) or 0.16 mL of HNO3 s.p. (65%) (add proportional aliquot after weekly addition of fresh sample!) Frozen
Metals (for analysis in filtered samples)	0.16 L of filtered water 0.5 l Teflon or PE bottle	~ 20 ml x 8 weeks Glass	Filtered in partners lab using filters specified above. 0.16 mL of HCl s.p. (30%) or 0.16 mL of HNO3 s.p. (65%) (add proportional aliquot after weekly addition of fresh sample) Frozen

16 PAH	1 L of whole water Amber glass The inner surface of plastic cups must be covered with aluminium foil to avoid contamination from plastic material	~ 120 ml x 8 weeks Glass	Cooling (2-4 °C) and not frozen max 2 months
PFOS, PFOA	250 ml, PE	~ 28 ml x 8 weeks Glass	Frozen for composites, otherwise cooling: max 6 days
Diclofenac, Carbamazepine Bisphenol A	1 L dark glass	~ 120 ml x 8 weeks Glass	Cooling (2-4 °C) and not frozen max 2 months
4-tert-Octylphenol Nonylphenol	1 L dark glass	~ 120 ml x 8 weeks Glass	Cooling (2-4 °C) and not frozen max 2 months
Metolachlor (incl. Metabolites) Tebuconazole	2x40 ml EPA vial	~ 2x4 ml x 8 weeks Glass	Cooling (2-4 °C) and not frozen max 2 months
~~Backup sample if the glass would break during freezing~~	~~1 L PE bottle~~	~~~ 100 ml x 8 weeks Glass~~	~~Frozen~~

4.1.4 Transport

At the end of the two months, the deeply frozen bottles/containers are sent to the respective laboratories via express courier transport in boxes of polystyrene foam filled with dry ice or ice batteries.

The receiving laboratories should measure sample temperatures at delivery. In case of temperatures > 8°C, the laboratory should contact the sender, who should reconsider his approach for sending.

4.2 High-flow samples

4.2.1 Sampling

Each of the six composite samples is composed of samples collected during one high-flow event. Samples shall cover rising and descending stages of the hydrograph and shall be collected as much as possible in a flow-proportional manner. Samples can be collected either manually or via auto-samplers controlled by flow/water level and turbidity.

If samples are collected manually, grab sampling is performed in accordance with the standard ISO 5667-6 on sampling of rivers and streams [2]. For sample collection glass bottles can be used for all contaminants. Depending on parameters measured, polyethylene (PE) (transparent or amber), Teflon or amber glass bottles are used for composite collection and transport. If the composite sample for organic class contaminants is collected, metallic or stainless-steel bucket should be used while for metal contaminants PE bucket. If, for preservation purposes, the bottles used for sampling are filled with acidic solution, the content must be discarded*, and the bottle must be rinsed three times with river water. Mount a vessel in a telescopic holder and immerse an open-mouthed bottle of sampling system into a laminar flow stream approximately 50 cm below the river surface. The inlet of the

sampling bottle should face the direction of the river flow**. <u>Warning</u>: consider that the bottles should be filled maximum to 85% of the bottle volume. Otherwise, they can break during the sampling and/or transport!** Please put all bottles into plastic ZIP bags and stick the labels on the bottles and on the ZIP bags. These grab samples must be immediately transported in cooled conditions to the partner's premises.

In case that the events are sampled via auto-samplers, the bottles contained in the device will be made of glass. Once the event is over, automatically collected samples must be retrieved as soon as possible and brought to the partner's premises.

***Safety warning: Use protective nitrile or other gloves and protective eyewear when emptying bottles with preservation liquids, to avoid accidents**

WARNING: when samples are taken by wading into a river, account should be taken of the possible presence of soft mud, quicksand, deep holes and swift currents. A wading rod is essential to ensure safe wading. By probing ahead, a person who is sampling can estimate the shape of river bottom. A safety line should be attached to a secure object on the bank for support. The use of life jacket is strongly recommended. At least two people should work together on sampling of river samples.

4.2.2 Storage

Each partner shall generate as quickly as possible upon sampling the composite samples and deliver them to the analytical laboratories. The composite samples must thus not be frozen, but only cooled until they are dispatched. If required, stabilizing compounds must be added to the samples during the generation of the composite samples.

Metals: Due to the carbonate precipitation issues from the start of the next possible* composite samples the dissolved samples should be filtered and acidified in labs of the partners, making the collection. Only specified filters** and acids*** should be used to avoid contamination. Each partner has to procure the specified filters, while the acids used are provided by JSI. Whole water samples should be acidified as previously, but also suprapure acid to be used, provided by JSI.

*Depending on the procurement time of the specified glass fibre filters.

** Filters to be used: Sartorius Ministart (Hydrophillic) RC25 17765-Q A500 SART Syringe Filter, Nonsterile: 28 mm diameter, 0.45 um pore size.

*** Acids to be used: Carlo Erba SuperPure nitric acid RS 67-70%, tested by JSI.

PAH samples only collected for Total PAH analysis in total samples. From December 2021, no Dissolved PAH fraction is measured!

If sufficient space is available in the partner's freezers, a duplicate of this composite sample will be created (in case the first one breaks during transport).

4.2.3 Required volumes and bottles

Table 5 reports which volumes and bottles are needed for the composite samples. No estimation can be provided on the amount needed for each sample, because it strongly depends on how many samples are collected to cover each event. The table also reports the total final amount of compounds needed for the preservation of the samples (if applicable).

Table 5: Volumes, bottles and preservation compounds required for the composite samples of river water at high-flow conditions

Parameter	Volume and bottle material for the composite sample	Volume and bottle material for the sampling during the high-flow event	Compounds for preservation
Metals and Hg	160 mL of whole water 0.5 l Teflon bottle	Glass	0.16 mL of HCl s.p. (30%) or 0.16 mL of HNO3 s.p. (65%) (add proportional aliquot after weekly addition of fresh sample!)
Metals (for analysis in filtered samples)	160 mL of filtered water 0.5 l PE or Teflon bottle		Filtered in partners lab using filters specified above. 0.16 mL of HCl s.p. (30%) or 0.16 mL of HNO3 s.p. (65%) (add proportional aliquot after weekly addition of fresh sample) Frozen
16 PAH	1 L of whole water Amber glass The inner surface of plastic cups must be covered with aluminum foil to avoid contamination from plastic material		Cooling (2-4 °C) and not frozen max 1 week
PFOS, PFOA	250 ml, PE		Frozen for composites, otherwise cooling: max 6 days
Diclofenac, Carbamazepine Bisphenol A	1 L dark glass		Cooling (2-4 °C) and not frozen max 1 week
4-tert-Octylphenol Nonylphenol	1 L dark glass		Cooling (2-4 °C) and not frozen max 6 days
Metolachlor (incl. Metabolites) Tebuconazole	2x40 ml EPA vial		Cooling (2-4 °C) and not frozen max 2 months
~~Backup sample if the glass would break during freezing~~	~~1 L PE bottle~~		~~Frozen~~

4.2.4 Transport

As soon as the composite samples are ready, they are sent to the respective laboratories via express courier transport in boxes of polystyrene foam filled with dry ice, or ice batteries.

The receiving laboratories should measure sample temperatures at delivery. In case of temperatures > 8°C, the laboratory should contact the sender, who should reconsider his approach for sending.

4.3 Determination of suspended solids

At each sampling of river water (both weekly sampling at baseflow-midflow conditions and targeted sampling at high-flow events), samples will be taken for the determination of suspended solids.

Each partner will analyse the content of suspended solids in these samples in its own laboratories or organize the analysis at own costs. This must be carried out for the whole monitoring year and if possible started already in advance, in order to have a sufficient dataset to calibrate the turbidity-suspended solids curve.

5 Atmospheric deposition sampling, storage and transport

Bulk (wet and dry together) deposition will be sampled throughout a year, leading to 4 samples. Each partner can choose between two options:

- A) Each composite sample is generated by the cumulative addition of each rainfall event for 3 consecutive months.
- B) Each composite sample is generated by the cumulative addition of each rainfall event for 1 month (one in each season).

The following protocol applies to both options A) and B).

5.1 Sampling

The design of the device required for sampling of atmospheric deposition as well as the criteria to select an adequate location for installing such devices are described in the deliverable D.T1.2.2 “Methodological approach for the measurements in the pilot regions and final selection of substances”.

Each device will be equipped with a 2 L glass bottle. As soon as possible upon each rainfall event, the bottle with the sample will be transported under cooled conditions to the partners’ premises and a new empty bottle will be installed in the device.

5.2 Storage

There are two options here:

- The partner (or the person, who will arrange the collection) holds the large collection bottles in the freezer the bottles/containers, which will be provided by the partners responsible for the sampling. Samples must be deeply frozen at -20°C. Please use specific glass bottles (which can be frozen) and keep all the samples in one collection bottle (risking glass breaks, therefore prepared for the handling of the break and save the sample)
- Do not freeze the large glass bottle, but keep it in cool conditions. If the partner arranging the collection has the chance to proportionally divide the rainwater samples after each event, can store rainwater for metals analysis separately, adding nitric acid according to Table 6.

After each sampling, all the sampled water is poured into the large bottle/container in the freezer/refrigerator and if necessary a proportional amount of stabilizing compound is added (If collection is organised offsite, the added chemicals can be neglected). Duplicates are not necessary in this case as there are multiple locations, therefore the lost sample can be substituted by one of the closest location form modelling purposes.

5.3 Required volumes and bottles

Table 6 reports which volumes and bottles are needed for the composite samples (to be kept in the freezer either three months or one month). The table also reports the total final amount of compounds needed for the preservation of the samples (if applicable).

Table 6: Volumes, bottles and preservation compounds required for the composite samples of atmospheric deposition

Parameter	**Volume and bottle material for the composite sample**	**Volume and bottle material for the sampling of atmospheric deposition**	**Compounds for preservation**
Metals and Hg	0.5 L of whole water 0.5l Teflon bottle	2 L Glass	0.5 mL HCl or HNO3 (or add proportional aliquot after each addition of fresh sample)
16 PAH	1 L of whole water Amber glass The inner surface of plastic cups must be covered with aluminum foil to avoid contamination from plastic material		Cooling (2-4 °C) and not frozen max 2 months
PFOS, PFOA	250 ml, PE		Cooling (2-4 °C) and not frozen max 2 months
Diclofenac, Carbamazepine Bisphenol A	1 L dark glass		Cooling (2-4 °C) and not frozen max 2 months
4-tert-Octylphenol Nonylphenol	1 L dark glass		Cooling (2-4 °C) and not frozen max 2 months
Metolachlor (incl. Metabolites) Tebuconazole	2x40 ml EPA vial		Cooling (2-4 °C) and not frozen max 2 months
~~Backup sample if the glass would break during freezing~~	~~1 L PE bottle~~		~~Frozen~~

5.4 Transport

At the end of each three-months period (option A) or at the end of each sampling month (option B), the deeply frozen bottles containing the composite samples has to be defrosted at ambient temperatures, portioned to the bottles sent by the labs (add preservation chemicals if needed), and sent to the respective laboratories via express courier transport. Samples should be either sent after refreezing the samples (in case of Teflon or plastic bottles or sent in isolated box using cooling packs to keep the temperatures below 8 °C.

6 Wastewater sampling, storage and transport

The monitoring programme of the project foresees the sampling of raw municipal wastewater, of treated wastewater, both municipal and industrial, and of wastewater in mining sites.

The deliverable D.T1.2.2 "Methodological approach for the measurements in the pilot regions and final selection of substances" indicates the number of samples to be generated for this matrix in each pilot region.

6.1 Sampling

Each composite sample shall be generated by sampling during a week, and the result shall ideally be flow-proportional. The same procedure must be repeated three times in a year to obtain three composite samples for the same sampling point.

The sampling can take place either manually or via auto-sampler. One daily sample has to be mixed from 24 hourly samples.

6.2 Storage

Option 1. If there is a possibility, storage has to be solved on the WWTP site by local personnel (A qualified professional is needed). In this case, after each day, each bottle from autosamplers has to be homogenized (using a mixer), and the required amount of wastewater should be poured into a 10 l collection bottle (50 ml from each sample bottle). The large bottle has to be frozen below -20 °C. After each day, the same amount should be poured onto the frozen sample, which makes up 8.4 l by the end of the week. Partners have to transport the frozen samples to the labs, where it has to be defrosted and distributed to the final transport bottles, ensuring the preservation, using the prescribed chemicals. (Defreezing at ambient temperatures might take a long time as the volume is large)

Option 2. In case the local freezing and storage are not possible, autosampler bottles have to be exchanged for the new empty bottles at the end of each 24 hours. Full bottles have to be transported to the lab, where the necessary aliquots have to be decanted (after stirring up the wastewater with suspended matter) to the final collection bottles. Preservation chemicals have to be added, and samples have to be deep-frozen.

If sufficient space is available in the partner's freezer, a duplicate of this cumulative sample will be generated (in case the first one breaks during storage or transport) (this possibility only applies in the second storage option).

If autosampler is used, a blank sample should be used to check the autosampler contamination due to inadequate cleaning /initial contamination of the sampler parts. Run the autosampler with MilliQ

water, and pour it to a glass bottle. Send it with the waste water samples to the three labs. In case of WWTP own autosampler is used, the blank is not necessary, as it is already in equilibrium.

6.3 Required volumes and bottles

Table 7 reports which volumes and bottles are needed for the composite samples. The estimation of the volume added for every single day is based on the fact that daily mixed-samples are available for seven consecutive days. The volume might thus differ if a different sampling approach is followed. The table also reports the total final amount of compounds needed for the preservation of the samples (if applicable).

Table 7: Volumes, bottles and preservation compounds required for the composite samples of wastewater

Parameter	**Volume and bottle material for the composite sample**	**Volume and bottle material for each daily sample**	**Compounds for preservation**
Metals and Hg	1 L of whole water 1 L PE bottle	Glass	1.0 mL of HCl s.p. (30%) or 1.0 mL of HNO3 s.p. (65%) (add proportional aliquot after addition of each single sample)
16 PAH	1 L of whole water Amber glass The inner surface of plastic cups must be covered with aluminium foil to avoid contamination from plastic material		Frozen for composites, otherwise cooling: max 1 week
PFOS, PFOA	250 ml, PE		Frozen for composites, otherwise cooling: max 6 days
Diclofenac, Carbamazepine Bisphenol A	1 L dark glass		Cooling (2-4 °C) and not frozen max 2 months
4-tert-Octylphenol Nonylphenol	1 L dark glass		Cooling (2-4 °C) and not frozen max 2 months
Metolachlor (incl. Metabolites) Tebuconazole	2x40 ml EPA vial		Cooling (2-4 °C) and not frozen max 2 months
Backup sample if the glass would break during freezing	1 L PE bottle		Frozen

6.4 Transport

As soon as the composite samples are ready, they are sent to the respective laboratories via express courier transport in boxes of polystyrene foam filled with dry ice, or accu packs (labs to decide about the procedure). Temperatures should be below 8 °C upon arrival.

7 Soil sampling, storage, transport, and pre-processing

In each pilot region 10 composite soil samples will be created and analysed. Four major land use classes will be covered: woodland-shrubland, agriculture with annual crops, agriculture with permanent crops, and grassland.

The determination of the exact sampling locations in each pilot region will be carried out by the partner BME via GIS methods, following the procedure described in the deliverable D.T1.2.2 "Methodological approach for the measurements in the pilot regions and final selection of substances". Based on this theoretical localization, each project partner may adjust the actual sampling points according to accessibility.

7.1 Sampling

Each of the ten composite samples is made of 20 samples each. Each of the 20 samples is composed in turn of 1-5 subsamples, to be taken close to each other at one location.

Sampling preferably should take place by the end of winter 2021 (February-March), but latest till 1st November 2021 due to the absence of crop cover on the fields and to logistical bottlenecks later in the year in the UBA laboratory. Sample collection is carried out with a metal auger (Pürckhauer, Edelman, or other). Agricultural samples must be taken from the upper 30 cm; forest and grassland samples from the upper 10 cm. Plant residues/forest litter has to be removed in advance. Samplers should be carefully cleaned between the ten composites to avoid cross-contamination according to the cleaning protocol described in section 9.

Due to the SOP in each pilot region finally **10 composite soil samples** will be created, stored in one submitted Rex glass (from Umweltbundesamt) – which means a total weight of between 1 or two kilogram and shipped to Umweltbundesamt again. Following the detailed monitoring strategy outlined in the SOP **for each partner in consequence this means to guarantee a proportional share of each of the 20 spots (e.g. 50g each) (composed by 1-5 subsamples) in each of the resulting 10 composite samples**. Please do not submit larger sample quantities not yet weighted to the Umweltbundesamt!
The content of the 10 Rex glasses will then be processed by Umweltbundesamt and aliquots shipped to the responsible laboratories.

For more details, see deliverable D.T1.2.2 "Methodological approach for the measurements in the pilot regions and final selection of substances").

7.2 Storage

In each pilot region, the 10 composite samples should be collected and generated if possible during consecutive days and in a period of time as short as possible, so to limit the time required for sample storage. Until the last sample has been collected, the others will be stored under dark and cool conditions in sealable glass jars.

7.3 Transport to UBA

As soon as the composite samples are ready, they are sent to the UBA laboratory via express courier transport in boxes of polystyrene foam filled with accu-packs or other cooling agent. Glass jar are provided by UBA lab.

7.4 Pre-processing and distribution

The laboratory at UBA will pre-process the soil samples and lyophilize them. Subsequently, it will divide the lyophilized samples into proportional and homogeneous aliquots. One aliquot will be kept by the UBA laboratory to perform the analysis of PAH, whereas the other aliquots will be sent to the other analytical laboratories in charge of chemical analyses in soil samples. The other analytical laboratories shall provide UBA with containers for receiving the lyophilized soil samples.

7.5 Required volumes and bottles

Table 8 reports the amount of soil to be collected for each sample and the total amount of soil contained in the glass jar for the final composite sample to be sent to the UBA laboratory.

Table 9 reports which amounts of lyophilised soil are required to carry out the analysis of different compounds.

Table 8: Amounts of soil to be collected in each sample and total amount of soil in the final composite sample

Number of samples pro composite sample	**Amount of soil in each sample**	**Total amount for the composite sample**
20 samples	50 g	1 kg
20 samples made of 5 sub-samples each	50 g	5 kg

Table 9: Amounts of lyophilised soil required for analysis of different compounds

Parameter	**Minimum required amount of lyophilised soil (g dry matter)**
Metals and Hg	5 g (PE)
16 PAH	50 g (Amber PE)
PFOS, PFOA	100g (LOQ= 0.01 mikrog/kg) or 350 g (LOQ of 0.002 mikrog/kg)
Diclofenac, Carbamazepine Bisphenol A	
4-tert-Octylphenol Nonylphenol	
Metolachlor (incl. Metabolites) Tebuconazole	

8 Suspended particulate matter sampling, storage, transport, and pre-processing

In selected sub-catchments of the pilot regions in Austria and Hungary, the responsible project partners will also pursue the generation of samples of suspended particulate matter.

8.1 Sampling

The collection of SPM will be carried out using the auto-samplers installed for the collection of water samples during high-flow events. During specific events, such devices will be utilized for this purpose instead.

High-flow samples should be collected in a flow proportional manner from flood waves. At least 5 separate high flow samples should be gathered throughout the year to get statistically relevant information between flow-particulate matter and contaminant concentrations.

It is preferable to reduce residence time of sampled water in the auto-sampler to a minimum. Therefore it is desirable to collect high water samples from one flood wave if possible (At lower SPM concentration this will not work, so multiple flood waves should be sampled together). In order to prevent biological decomposition of the organic contaminant in the auto-sampler (especially in summer time), the sample collection should not take too long. Water should be collected in a time-integrated manner within a 3-day window, then cooled at low temperatures.

It would be desirable to collect samples of a large flood when intensive erosion occurs due to intensive rain events and intensive runoff.

For one sample it is necessary to collect **at least 155 g of dry matter.** As settles suspended solids contains also a large fraction of water, it is important that each partner should ensure that the dry matter is enough. This in practice would mean that around 20 % of the total volume of the 2l bottle is covered by settled sediment.

8.2 Decanting and transport to UBA

The SPM contained in the auto-sampler will be separated from the water phase by the responsible partner in its own laboratories by decanting the water from it slowly to prevent resuspension of settled SPM. SPM should be collected afterward into a 2l glass jar and immediately sent to the laboratory in charge of solid sample preparation (i.e. homogenization and lyophilisation), namely UBA.

Due to previous glass break issues and carbonate precipitation, cooling might be the appropriate way to store the probes until having sampled enough material and ship it to Umweltbundesamt.

The delivery will take place via express courier transport in boxes of polystyrene foam filled with dry ice. Each partner shall buy the glass jars needed in its pilot region.

8.3 Pre-processing and distribution

The laboratory at UBA will pre-process the SPM samples and lyophilize them. Subsequently, it will divide the lyophilized samples into proportional and homogeneous aliquots. One aliquot will be kept by the UBA laboratory to perform the analysis of PAH, whereas the other aliquots will be sent to the other analytical laboratories in charge of chemical analyses in SPM samples. The other analytical laboratories shall provide UBA with containers for receiving the lyophilized SPM samples.

8.4 Required volumes and bottles

Table 10 reports which amounts of lyophilised SPM are required to carry out the analysis of different compounds. Using these values as minimum reference, each partner shall try to gather a sample with a sufficient volume and dry matter content, so that after lyophilisation each analytical laboratory can obtain an aliquot sufficient for the analysis.

Table 10: Amounts of lyophilised SPM required for analysis of different compounds

Parameter	**Minimum required amount of lyophilised SPM (g dry matter)**
Metals and Hg	5 g (PE)
16 PAH	50 g (Amber PE)
PFOS, PFOA	

Diclofenac, Carbamazepine Bisphenol A	100g (LOQ= 0.01 mikrog/kg) or 350 g (LOQ of 0.002 mikrog/kg)
4-tert-Octylphenol Nonylphenol	
Metolachlor (incl. Metabolites) Tebuconazole	

9 Procedures for cleaning of bottles and other specifications for different compounds in aqueous samples

9.1 All glass collection bottles have to be cleaned in the following way

Reagents:

- Nitric acid HNO_3 (65% pro analysis - p.a.)
- Ultrapure water e.g. (Milli-Q) (> 18 M Ω cm), or HPLC reagent grade water

Cleaning procedure for glass bottles:

1) rinse the glass bottles thoroughly with tap water;
2) soak glass bottles with 10% HNO3 (65%) (v/v) for 24 hours and afterward rinse with ultrapure (Milli-Q) water, fill the bottle with 10 % HNO_3 and leave for 24 hours;
3) rinse the bottles thoroughly with ultrapure (Milli-Q) water (5 times);

9.2 Cleaning the bottles for Mercury and Metals

Sampling of water samples for mercury and other metal analysis:

1) put the gloves on
2) take the bottles out of the zip-locked bags
3) empty the Teflon bottles, which are filled with diluted acid
4) rinse them thoroughly with the water sample (3 times)
5) fill the bottle till the top with the sample (This is only applicable if only one sample is taken and no composite is collected) and acidify them immediately with 1 mL of HCl s.p. (37%) or 1 mL of HNO_3 s.p. (65%) per 1 L of water, so that final concentration is 1% v/v)
6) Put the sampler to the freezer.
7) put the bottles in PE zip-locked bags and store them for further analysis (if the sampling is taking place during the summertime, the samples should not be stored at room temperature) (This is only applicable if only one sample is taken and no composite is collected).

9.3 Polycyclic aromatic hydrocarbons (PAH)

Amber glass bottles to be used for samples dedicated to the analysis of PAH must be previously rinsed with HPLC-grade water.

In the laboratory of the partner doing the sampling, **careful thawing of the samples should be performed at controlled room temperature (4°C) or at room temperature**, in order to prevent loses of volatile PAH (e.g. Naphthalene). This procedure only applies to the atmospheric deposition samples (and maybe wastewater), which will be stored frozen and where the distribution to the smaller bottles will be arranged at the end of the collection period.

9.4 Other organic compounds

In the case of the glass sampling bottles used for the actual water sampling, the above procedure applies. (For specific cleaning in case if only on-time sampling is needed will be confirmed later by the winner lab.)

10 Sample identification and records

Sample bottles and containers must be clearly and unambiguously marked so that subsequent analytical results can be properly interpreted. When many sample containers are needed for a single sampling occasion, it is recommended to identify the containers by a code number. Sample labels should always be completed at the time of sample collection.

10.1 Labelling on the partner's premises

The labels on the samples collected and used at the partners' premises to generate composite samples must contain at least the following information:

- The name of the pilot region
- The name of the sampling location/station
- The date and precise time of sampling (with identification of time zone, preferable UTC)
- Matrix (e.g., whole water, wastewater, suspended particulate matter (SPM), soil, atmospheric deposition)
- Sample storage (cooled, deep-frozen, ...)
- Sample preservation (e.g., acidification with HNO_3, 1 mL/L ….)
- Sample thawing (cooled room, ambient room temperature) – where applicable
- Compounds to be analysed in the sample (metals, Hg, PAH, …)
- The person who carried out the sampling

Each partner is responsible for labeling (coding) the samples they handle.

10.2 Labeling of samples moved (delivered) between project partners

The labels on the final samples sent to the analytical laboratories must contain a unique code according to the following convention. Format of the code is:

AAA-BCC-NN/M

where

- AAA is identifier of sampling matrix (Put in first place, because lab and analysis method might be different).
    - RIV - River water

    - WW - waste water (either raw or treated)
    - AD - atmospheric deposition
    - SOL - soil samples
    - SPM - suspended particulate matter
- B is identifier for the country:
    - A – Austria
    - B – Bulgaria
    - H – Hungary
    - R – Romania
- CC is identifier for the pilot area
    - AW – Wulka
    - AY – Ybbs
    - BV – Vit
    - HK – Koppány
    - HZ – Zagyva
    - RS – Somesul Mic
    - RV - Viseu
- DDD is identifier for subcatchment/sampling station, deposition sampling station, WWTP or mining site
    - AWM – Wulka/Main river
    - AWE – Wulka/Eisbach
    - AWN – Wulka/Nodbach
    - AWA – waste water treatment plant A (not decided yet)
    - AWB – waste water treatment plant B (not decided yet)
    - AWW – deposition sampling station “Wiesen”
    - AWO – Deposition sampling station “Oslip”
    - HKK - Koppány / Törökkoppány
    - HKT - Koppány / Tamási
    - HZN - Zagyva area / Zagyva creek / Nemti
    - HZK - Zagyva area / Tarján-creek / Kisterenye
    - HZH - Zagyva area / Herédi-Bér creek / Heréd station
    - HZ6 - Zagyva area / Zagyva river / Hatvan station
    - HKL - Koppány area / Balatonlelle wwtp
    - HZS - Zagyva area / Salgótarján wwtp
    - ...

- NN is a 2-digit serial number starting with 01 in each sampling point and sampling matrix. If each sampling point is already defined by the previous code, 99 samples per sampling point will be enough. In the currently described system in the SOP (only pilot identification by coding) we will need more than 99 samples, because of the many river samples for suspended solids analysis for calibration of the turbidity-TSS-model.
- M is a one-digit number/letter: part of the sample differently preserved.
    - T - for PAH’s river water total phase analysis
    - D - for PAH’s river water dissolved analysis

For each pilot region, a separate excel file has to be filled and kept up-to-date by the PP responsible for that particular catchment. Minimally the following information has to be included in the excel file.

- Name of the pilot region
- The name of the sampling location/station
- The date and time (the period in case of composite samples) of sampling (with identification of timezone, preferable UTC)
- Matrix (e.g., whole water, wastewater, suspended particulate matter (SPM), soil, atmospheric deposition)
- Sampling method
- Sample storage (cooled, deep-frozen, ...)
- Sample preservation (e.g., acidification with HNO_3, 1 mL/L….)

To allow pre labeling of bottles reserved number ranges are to be used as follows:

River

- 01-10 low flow
- 11-20 high flow
- 21-30 blanks (autosamplers, pouring procedures)
- > 31 TSS-Analysis for turbidity-TSS-model calibration

See “Catalog_draft.xlsx” for more details. UBA has to have a separate excel file (or sheet at least) for both the soil samples and the SPM samples they get from the project partners for lyophilization and further analysis / delivery.

11 Reporting of the results

Each partner responsible for monitoring in the pilot regions will regularly document the sampling, preservation and storage, transport, and analysis of SPM in the river samples.

Reports of the partners must include:

- The weather conditions at the time of sampling
- The temperature of the water
- The flow condition of the water
- The appearance of the sample (colour of the water, suspended solids, clarity, odour)
- The type of sampling device used
- Information on any sample preservation
- Information on any sample filtration technique used
- Information on sample storage
- Information on the sample transport
- the exact coordinates of the sampling location with CRS, in case of soil samples for each sample of the composite sample

Each laboratory responsible for the chemical analyses will provide detailed technical reports, including the methods and procedures followed for sample preparation and analysis.

The analytical report must include:

- Measured quantity of the components in the samples
- Unit of measurement

- Method of measurement
- LOQ and LOD
- Indicate if measurement is below LOD

These documents must contain the detail of information to fulfil the requirements needed to serve as basis for scientific publications.

12 References

Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy.

Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008

on environmental quality standards in the field of water policy, amending and subsequently

repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and

amending Directive 2000/60/EC of the European Parliament and of the Council

Commission Directive 2009/90/EC laying down, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, technical specifications for chemical analysis and monitoring of water status

Directive 2013/39/EU of the European Parliament and of the Council 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy

Commission Directive 2014/101/EU 2014 amending Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for Community action in the field of water policy

ISO 5667-3:2018 Water quality — Sampling — Part 3: Preservation and handling of water samples