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Sustainable Sediment solutions for
the Danube - Black Sea system



D1.5 HARMONIZATION OF SAMPLING PROTOCOLS FOR THE QUANTITATIVE AND QUALITATIVE ANALYSIS OF SEDIMENTS

02/08/2025



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Abstract	<p>This output presents an updated and harmonized framework for sediment sampling, aimed at improving the quantitative and qualitative analysis of sediments from large and complex aquatic ecosystems, in particular from the Danube River Basin. It builds on initiatives from previous projects, such as the SIMONA project, but also on ISO standards for sediment and water sampling (e.g., the ISO 5667 series). The document brings methodological improvements based on the authors' practical field experience, on inter-institutional collaboration between several Danube countries, and on the scientific literature consulted. The sediment and water sampling strategy, site selection, methods and equipment, and sampling frequency, as well as the transport and stabilization of collected samples, are addressed from a field practice perspective, including updated recommendations on the investigation of chemical, physical, biological, and microbiological properties of sediments, with a special focus on emerging pollutants such as microplastics. The focus is on taking into account hydromorphological variability and biological indicators and on improving monitoring in areas affected by anthropogenic activity and climate change. By promoting consistency and comparability between monitoring efforts, this document supports more effective sediment</p>

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Executive Summary

Due to its diverse morpho-hydrological characteristics, anthropogenic pressures, and transboundary governance needs, the Danube River presents unique challenges for sediment monitoring. In this context, harmonized protocols are critical to ensure data consistency, comparability, and integration across national and regional monitoring programs

The D1.5 - Harmonization of sampling protocols for the quantitative and qualitative analysis of sediments is focused on creating a harmonized sediment monitoring and management framework by enhancing the sampling protocols for suspended and bottom sediment and for water samples incorporating quantitative and qualitative assessment strategies. Thus, the document will help in improving the sediment management in the Danube River, which is the objective of WP1.

This output was built on both previous SIMONA project initiatives (https://dtp.interreg-danube.eu/uploads/media/approved_project_output/0001/49/a478eafbee66d5b1ad8f7ad86f830e4a2b05d18b.pdf) and on standards that refer to sampling, such as ISO 5667, including ISO 5667-12, 5667-17, and 5667-15, among others. However, due to the integration of practical knowledge gained in over 15 years of fieldwork focused on the Danube, as well as insights from recent scientific literature and ongoing EU projects, the document is not a simple adaptation of information from the previously mentioned studied documents. It introduces updates that address emerging challenges such as microplastic pollution, invasive species monitoring, and the effects of dredging on sediment dynamics and ecosystem health.

The protocol covers several dimensions: types of investigations (chemical, physical, biological, microbiological), site selection, equipment choice, sampling strategy, frequency with which sampling could be done depending on the type of sample and the location from which it is taken, as well as protocols for sample transport and preservation. Special attention is paid to the practical limitations and specificities of sampling in complex aquatic systems, including deep channels, deltaic environments, confluence areas, and dredged areas, such as the Sulina Channel and the Sulina Bar. The updated protocols support sampling that reflects the dynamic behavior of sediments under conditions of climate and flow variability so that it takes into account not only the season in which it is done but also the climatic events that occur at a given time in the location to be monitored.

Beyond monitoring traditional pollutants in sediments and water, deliverable D1.5 proposes an extension of sediment monitoring to include emerging contaminants such as pharmaceuticals, PFAS, and microplastics. It provides guidance on how to adjust sampling and analysis techniques depending on the type of pollutant, sediment characteristics, and ecosystem sensitivity. In addition, recommended adaptations are presented regarding biota sampling, ballast water monitoring, and the use of environmental DNA (eDNA) to detect changes in biodiversity and the possible presence of invasive species.

Last but not least, harmonized and field-validated, this protocol aims to harmonize practices in the field of sample collection and preservation among project partners. This allows for more efficient sustainable sediment management, which directly contributes to the objectives of the Water Framework Directive (WFD), the EU Biodiversity Strategy 2030, and the International Commission for the Protection of the Danube River (ICPDR) and supports ecological health, safe navigation, and cooperation in transboundary waters in the Danube River Basin.



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ABBREVIATIONS

DRB	Danube River Basin
DWSR - `Danube-Black Sea`	Deep-Water Shipping Route "Danube-Black Sea
eDNA	Environmental DeoxyriboNucleic Acid
ICPDR	International Commission for the Protection of the Danube River
MPs	Microplastics
WFD	Water Framework Directive



Section 1 Introduction

Hydro-Geomorphological Dynamics of the Danube Delta Ecosystem

The Danube River Basin (DRB) is the most international river basin in the world covering territories of 19 countries. With an area of 803,260 km², the DRB is the second largest river basin in Europe` (Danube River Basin Management Plan/ICPDR-Update 2021), being home for approx. 79 million of people. The DRB is distinguished not only by its size and the number of countries it covers but also by its varied landscapes and significant socio-economic diversity.

The Danube river flows approximately 2,783 km from the confluence of the Breg and Brigach tributaries to the Black Sea at Sulina port. Sediment transport in the Danube results from natural processes such as erosion and flooding, which significantly increase sediment loads during high discharge periods (Habersack et al., 2013). For instance, major floods in Romania between 1980 and 2010, particularly in 2006, caused significant sediment deposition in riverbeds and floodplains (Marin, 2017; Jugaru Tiron et al., 2009).

At the end of a journey, the Danube River collected waters from a vast catchment area that covers more than 8% of Europe. The river that has been shaping, for more than 16,000 years one of the most beautiful and ecologically significant deltas in Europe and the world: the Danube Delta, recognized as one of the planet's great wetland regions.

The Danube Basin is divided into three major parts: the Upper Danube, Middle Danube, Lower Danube, including the Danube Delta. The Upper Danube originates in Germany's Black Forest and flows to the Austria-Slovakia border (Habersack et al., 2013). The Middle Danube extends from the Devín Gate to the Iron Gate II on the Romania-Serbia border, while the Lower Danube flows from the Iron Gate II to the Danube Delta and the Black Sea (Schmid et al., 2023).

The current **morpho-hydrographic configuration** of the Danube Delta is the result of continuous interaction between the river and the Black Sea during the **Holocene epoch**. At the beginning of the Holocene, as sea levels rose to approximately current levels, a Danube gulf formed. At the mouth of this gulf—between the Jibrieni promontory to the north and the Murighiol-Dunavăț promontory to the south—the initial **Letea-Caraorman barrier ridge** emerged. The sediments that formed this ridge were transported by marine currents from northern areas near the mouths of the Dniester, Southern Bug, and Dnieper rivers.

The **oldest branch** of the Danube, the **Sfântu Gheorghe branch**, flowed into the sea at the southern end of this barrier, forming the **first deltaic structure**, known as **St. George Delta I**.

Later, as sediment deposition blocked this branch, a second branch—the **Sulina branch**—began to develop and gradually took over more water and sediment flow, building its own delta: the **Sulina Delta**.

Simultaneously, in the south, a **secondary delta** (Cosna Delta) formed due to the Dunavăț branch. Over time, the Sulina Delta eroded, while the **Chilia branch** (to the north) and the **new course of the Sfântu Gheorghe branch** (to the south) formed their own deltaic systems: **Chilia Delta** and **St. George Delta II**.

The **Chilia and Sfântu Gheorghe deltas** were built primarily from Danube sediments, while additional material carried by coastal currents accumulated north of the Chilia branch, forming the **Jibrieni formation**.

The **modern morphology** of the Danube Delta was significantly shaped by the last sea-level rise of the Black Sea, which triggered intense sedimentation, resulting in increased **meandering and branching** of the main channels.

The **morphological evolution** of the Danube Delta has been governed by a delicate balance between sediment supply, hydrological variability, marine processes, and anthropogenic alterations. The development of multiple delta lobes—St. George, Sulina, Chilia, Cosna—reflects changing sedimentation regimes and hydrological pathways throughout the Holocene. In recent decades, sediment dynamics have been increasingly altered by **human interventions** such as dam construction, channel regulation, and dredging operations (Habersack et al., 2016). These modifications have led to **sediment deficits**, altered deposition-erosion balances, and disrupted natural delta-building processes, particularly in the lower Danube basin.

Sediments are not only central to the physical structuring of fluvial and deltaic environments, but they also play a critical role in ecosystem functioning. They serve as both carriers and reservoirs of nutrients, pollutants, and organic matter, influencing water quality, benthic habitat availability, and biological communities. In the context of the Water Framework Directive (WFD) (WFD, 2000/60/EC), sediments are recognized as a key supporting element for assessing hydromorphological conditions and for interpreting the ecological status of water bodies through their interactions with biological quality elements (European Commission, 2003).

Despite their importance, sediment sampling and analysis remain heterogeneous, with varying protocols, analytical standards, and data quality objectives. This lack of harmonization hampers the comparability, reproducibility, and integration of sediment data at both national and international scales. The lower Danube basin, as a transboundary zone shared by Romania, Serbia, Bulgaria, and Ukraine, illustrates the need for sediment monitoring efforts, especially given the sediment's role in floodplain connectivity, delta stability, and contaminant transport.

Moreover, sediment-related processes are increasingly influenced by climate change, which alters precipitation regimes, increases the frequency of extreme hydrological events, and shifts sediment transport patterns. Combined with ongoing anthropogenic pressures, this creates complex challenges for sediment management, requiring not only improved monitoring but also adaptive and integrative management frameworks.

The Danube River, as a complex transboundary ecosystem, requires a unified approach to water quality monitoring, especially in the context of the Water Framework Directive (WFD, 2000/60/EC) and regional efforts coordinated by the International Commission for the Protection of the Danube River (ICPDR), accompanied by a homogenous approach to water quantity.

Therefore, an effective WFD monitoring program must give a central role to biological assessment, complemented by chemical and hydromorphological data for an integrated understanding of the status of the water body. This ecological and interdisciplinary approach is fundamental for modern and sustainable management of aquatic resources. The Water Framework Directive 2000/60/EC (WFD) is the European Union's main legislative instrument for the protection and sustainable management of water quality resources, its fundamental aim being to **achieve "good status"** for all bodies of surface water.

Section 2 Sampling strategy

The Danube River is a complex, dynamic ecosystem with a high spatial and temporal variability. This diversity and dimension imposing special requirements in terms of sampling, whether biological, physico-chemical or hydromorphological.

The SUNDANSE project primarily focuses on understanding and managing sediment dynamics in specific sections of the Danube River. By studying the movement, composition, and environmental impact of sediment in these areas, the project aims to support sustainable river basin management and inform relevant policy decisions. Some clarifications should be considered, such as:

- The documentation, studies and methodologies proposed in ISO 5667–12:2017; ISO 5667–17:2008; ISO 5667–15:2010; ISO 5667–6:2017; and ISO 5667–1:2023 are considered. Some of these methodologies were implemented by previous projects, e.g. SIMONA. A list of standards and methods on sampling, sample treatment and analyzing methods that are usually used for sediments are presented in Annex 5.
- The upgraded recommendations generally have a technical character of application resulting from the experience expertise of the partner teams in SUNDANSE consortium, the experience resulting from 15 years of research on the Danube and other surface aquatic ecosystems as well as from the consultation of literature on similar topics.
- To bring added value to the project; e.g. in SIMONA project the biological assessment of water status based on community monitoring of benthic aquatic organisms was not included.

It is important to note that this document outlines proposed upgrades to be implemented during sampling activities within SUNDANSE. Building on ISO standards and SIMONA sampling protocols, it introduces technical improvements based on in-field experience across various Danube locations (see Table 1).

While some examples presented are specific to the Danube River and its basin, the upgraded protocol is designed to be applicable to any large and complex aquatic ecosystem exhibiting similar hydromorphological and ecological characteristics.

The protocols are designed to enhance methodological consistency, increase comparability between pilot sites, and support predictive sediment modeling in later stages of the project. These harmonized methods are crucial to support SUNDANSE's objective of sustainable and effective sediment management. The types of investigation performed within SUNDANSE (chemical, physical, biological and microbiological determinations) from a sediment quality point of view are presented and the specific conditions which may influence each investigation are given in Section 3.

- The criteria for selection of sampling sites are presented in Section 4. These criteria take into consideration the specifics of each type of areas (river areas, connecting canals and small canals, interconnected lakes and ponds, delta).
- The specific conditions for the selection of the sampling methods and equipment are presented in Section 5, together with the sampling frequency.

In the following, solutions are discussed and proposed according to the following methodology:

- Separate tables will identify the methods described in ISO 5667–12:2017; ISO 5667–17:2008; ISO 5667–15:2010; ISO 5667–6:2017; ISO 5667–1:2023 and SIMONA project - Sediment quality sampling protocol for hazardous substances in surface waters in a first column.
- Column 2 includes the observations and practical problems encountered during the research activities in the Danube ecosystems and those associated with the Danube that require clarifications and completions in the practical application of the methods used so far.
- Column 3 includes, in short, the upgrade proposals resulting from the previous experience of the SUNDANSE partners as well as from the consultation of international scientific literature, if applicable.
- The proposals are described extensively by the tables to be able to distinguish their details.
- The proposals are mentioned in different tables, depending on the issue to ensure the distinction between the different technical and methodological areas.
- Considering the complexity of the studied issues and the need to accumulate correct and complete data, proposals are made to cover the methods of sampling, conservation and research specific to complex ecosystems such as the Danube River (see Table 1). These proposals include the collection of sediment, water, and biota samples, as well as direct observations of ecosystem quality.
- Maintenance dredging is essential to ensure navigational access between the Danube River and the Black Sea, particularly via the Sulina Canal, as outlined in the DALIA Project. Of the three branches of the Danube that connect to the Black Sea, Chilia, Sulina, and Sfântu Gheorghe, Sulina is the primary navigable route. Although more than half of the water flow from Ceatal Tulcea is directed toward the Chilia branch, the Sulina Canal was historically chosen and maintained as the official navigable channel due to its suitability for maritime traffic.
- The Sulina Canal has distinct characteristics that set it apart from the other branches. It has been fully regulated and developed for inland-maritime navigation along its entire 71 km length. Engineering interventions, such as the straightening of meanders, have enhanced its navigability and enabled partial self-cleaning through natural water flow. Depths in the canal are maintained between 7.32 m and 18 m, and dredging operations are carried out more frequently here than on other branches.
- A key area requiring constant attention is the Sulina Bar, where the canal meets the Black Sea near the town of Sulina. This area is particularly challenging due to dynamic sediment transport and the formation of shifting sandbanks. As the only deep-water outlet of the Danube accessible to seagoing vessels year-round, it serves a strategic role in international navigation. To maintain safe navigation,

Today, in the context of full-scale Russian military aggression, occupation, and blockade of a number of Ukrainian Black Sea ports by Russia, the load on shipping and Ukrainian ports on the Danube has increased significantly. Unfortunately, these same factors determine the inability to conduct high-quality monitoring of areas within the Ukrainian part of the Danube Delta.

The sharp growth in the capacity of seaports and other infrastructure facilities in the Danube region directly determines the increase in the number of ship passages through the canal and dredging works in its water area. The DWSR "Danube-Black Sea" has a total length of 170.36 km and consists of four sections:

- 1). a sea approach channel, 3.30 km long and 85 m wide at the bottom, which passes through the sea bar in the area of the Bystre estuary;
- 2). sea — Vylkove, 19.051 km long, with a bottom width of 60 m at the Bystre estuary and 120 m at the Starostambul and Kiliya estuaries;
- 3). Vilkovo — Izmail Chatal, 95.415 km long, with a natural bottom width of 120 m;
- 4). Izmail Chatals — Reni, 54.36 km long, with a width of at least 120 meters.

The most active economic activity to maintain the depths of the shipping channel in working condition is characteristic of the first two sections, where there is active accumulation of suspended sediments.

In the future, after the end of the war, it will be necessary to resume hydromorphological and ecological monitoring of sediments in the dredging and ship passage areas, in particular in the sea-Vylkove area.

Dredging small canals that provide water supply to the inland lakes in the Danube Delta.

- The canals are located in sensitive areas and monitoring must be carried out and planned.
- The biological population is very dense and preliminary determinations are mandatory.
- During the dredging works, the important small canals that ensure the supply and drainage of water to the inland lakes in the transition areas (lagoons, deltas, estuaries) must be targeted, some of them being known as an integral part of the UNESCO heritage (e.g. the Danube Delta Biosphere Reserve).

A sampling form was developed starting from the form proposed by SIMONA project (see ANNEX 4).

TABLE 1 RELEVANT STANDARD SECTIONS FOR THE SAMPLING STRATEGY

Clarifications ²	Practical implementation details required	Upgrade proposals
<p>Sampling strategy</p>	<p>In SIMONA sampling protocol and ISO standards, no particular cases are specified, such as:</p> <ul style="list-style-type: none"> - Methods of study to determine the environmental impact resulting from dredging activities for maintaining the navigability of navigable channels for large rivers (e.g. the Danube) where pre-dredging, dredging and post-dredging studies are required. - There is no information on the dredging activities for the canals that provide water access to the lakes in the deltaic areas and that risk being eutrophicated in the absence of a water flow. - There are no mentions of continuous hydromorphological and physico-chemical monitoring before and after dredging in representative points. 	<p>The sampling activities will be adapted according to the complexity of the aquatic ecosystem influenced by dredging activities of different types. Dredging activities should be classified into different categories such as:</p> <ul style="list-style-type: none"> - Maintenance dredging of access from the Danube to the sea and vice versa (see example of the Sulina canal - <i>DALIA project (Reference)</i>). - Maintenance dredging of the fairway depending on the specificity of the sectors: <i>Middle and Lower Danube, maritime sector of the Lower Danube³, etc.</i> - Dredging small canals providing water supply of the inland lakes of the Danube Delta (under strict environmental regulations and approvals, allowed only for ecological restoration purposes, e.g. reconnecting isolated lakes to the river system, according to <i>EU Biodiversity Strategy 2030; EU Water Framework Directive (WFD), ICPDR</i>).

² According to ISO 5667–12:2017; ISO 5667–17:2008; ISO 5667–15:2010; ISO 5667–6:2017; ISO 5667–1:2023; SIMONA project - Sediment quality sampling protocol for hazardous substances in surface waters

³ [Manual on Danube Navigation - viadonau](#)

Section 3 Types of investigation

The sampling methodologies used in the previous studies comply with the provisions of the ISO 5667 – 12 standard as well as those of the associated standards. Following the accumulation of experience in the previous studies of the REXDAN team from UDJG as well as from the experience of the SUNDANSE project partners, as it results from international scientific articles with sampling in large rivers and streams, some details related to the specificity of these surface ecosystems are useful (see Table 2).

In the purpose of this deliverable, *'chemical'* refers to a broad spectrum of organic and inorganic pollutants, but not limited to volatile organic compounds (VOCs), semi-volatile organic compounds (SVOCs), and other xenobiotics typically analyzed using GC-/LC-MS techniques. The nature of these substances, whether dissolved or particulate, can influence sampling strategies and introduces specific analytical challenges, particularly in the context of emerging contaminants and microplastics (MPs).

The international standard referenced in this deliverable, along with the SIMONA sampling protocol, already provides a comprehensive classification of chemical compounds and outlines established procedures for their sampling, preservation, and preparation. Building on this foundation, the present update aims to incorporate recent developments and complementary perspectives, rather than reiterating the existing protocols.

Relevant considerations regarding the role of MPs as vectors for chemical pollutants, highlighting the dual nature of contamination (dissolved vs. particulate), are addressed in dedicated sections of this deliverable.

TABLE 2 CORRESPONDING TO THE CHAPTERS OF THE STANDARDS RELATING TO THE TYPE OF INVESTIGATION

Clarifications ⁴	Practical implementation details required	Upgrade proposals
Chemical investigation	<p>The procedures do not contain details about particular cases such as:</p> <ul style="list-style-type: none"> - Analysis of emerging pollutants (microplastics, pharmaceuticals, some per- and polyfluoroalkyl substances – PFAS and pesticides, etc.); - Plastics (micro and macro) that are pollutants in themselves but also transport vectors for other types of organic or inorganic pollution & biological pollution. 	<p>The sampling plan must take into account some specificities such as:</p> <ul style="list-style-type: none"> - Sampling conditions in the case of analysis of emerging pollutants; - The particle sizes for which the categories of micro and nanoplastics are considered. - Considering the differences in density, it is necessary to distribute the sampling areas (water or sediment) on longitudinal section and depth. - The sampling phase and the sample preparation phase must simultaneously take into account both the composition of the microplastics and the type of associated pollutants, which are adsorbed on the surface of the microplastics or in association due to the cavernous structure of the particles. - Sampling protocols should recommend processing of approximately 1,000 liters of water per sampling event in riverine conditions to obtain representative MP concentrations.
Physical investigation	<p>Important parameters such as pH and temperature are included in physical determinations. In the determinations that take place, there are differences that have natural or anthropogenic causes that can affect the accuracy of the results.</p> <p>There are no mentions regarding the monitoring of the physical parameters per depth column</p>	<p>To determine the temperature at the level of complex aquatic ecosystems of the dimensions of the Danube, it is recommended to take into account some aspects such as:</p> <ul style="list-style-type: none"> - For temperature, there are variations in the confluence areas with different tributaries, which affect the temperatures recorded in key locations. - The seasonal dependence is affected by variable flows (variation from 1 to 8) which affects the recorded temperatures and, directly, the solubility of some pollutants, the development of biological systems in water and sediment, etc.

⁴ According to ISO 5667–12:2017; ISO 5667–17:2008; ISO 5667–15:2010; ISO 5667–6:2017; ISO 5667–1:2023; SIMONA project - Sediment quality sampling protocol for hazardous substances in surface waters

	<p>correlated with the water flow velocities at depth.</p>	<ul style="list-style-type: none"> - Temperature is directly influenced by anthropogenic factors such as: areas that use very large amounts of cooling water (e.g. Cernavoda Nuclear Power Plant) with an impact on very important areas. It is recommended to monitor the temperature in the depth column as well. - pH is variable and depends on the geological structure of the riverbed, temperature and flow; therefore, pH monitoring is very important and reflects the overall quality of surface ecosystems. It is recommended to monitor the pH in the depth column as well.
<p>Biological and microbiological investigation</p>	<p>Biological determinations require differentiated treatment depending on the sampling areas, the anthropogenic impact of communities, industry and agriculture that must be specified in the sampling plan.</p> <p>Since the Danube is the main source of drinking water, the specific conditions of certain microbiological species with aggressive potential are not detailed in the present protocols.</p> <p>The protocols do not clearly present the ways to determine the role of biological and microbiological transport vector of pollutants such as microplastics.</p> <p>On the river-sea confluence areas, the presence of invasive species that reach freshwater areas coming from other climate zones can be recorded.</p>	<p>During a long period of research on the Danube, including the pre-delta and deltaic area, the following ideas regarding biological investigations have emerged:</p> <ul style="list-style-type: none"> - Along the Danube there are several urban and rural communities associated with the presence of industrial and agricultural activities. In these areas, guidelines and planning are needed to include populated and opposite banks, areas of ship traffic and those without traffic, areas of confluence with tributaries with different biological loads. - Longitudinal sampling is necessary in these areas to have accurate information about biodiversity and its presence, in different stages of development, in water and sediment. - It is necessary to adapt the sampling areas on navigable and maintained canals, to ensure navigability in other areas as well. - For correct sampling in the standard, a layer of at least 10 cm of sediment is recommended, and geomorphological monitoring is necessary before sampling to highlight: the structure of the riverbed (sand, gravel, etc.), the unevenness of the riverbed bottom to orient itself towards areas with important level differences where the accumulation of sediments and their stability are ensured. - For certain types of solid pollutants (e.g. microplastics), adapted sample preparation methods are required to consider the possible biological and microbiological load of particulate matter.

<p>There is no mention of the importance of accessing reporting information on the timing and quantities of water and ballast sediment.</p> <p>There are no mentions regarding the correlation of biotic components and sediments with the ballast water of ships.</p> <p>No particularities are mentioned regarding the possibility of fixing potentially invasive alien species on the hulls and relocating them to the sediment.</p>	<ul style="list-style-type: none"> - The analysis of microplastics in macroinvertebrates and fish has a crucial role in understanding the impacts of plastic pollution on aquatic ecosystems and food webs. - For biological samples, special attention should be paid to areas of heavy ship traffic (e.g. maritime Danube) where ships coming directly from the Black Sea can carry potentially invasive organisms on the hull. - Ichthyofauna and/or macrozoobenthic sampling must consider the behavior of the species: autochthonous, allochthonous, anadromous, potamodromous or invasive species as well as the preferential living and feeding environment: pelagic, bentonophagous or mixed. - In difficult-to-access habitats such as the Danube River, eDNA analysis is an innovative and efficient method for monitoring macroinvertebrate and fish species - Monitoring of invasive species. - We recommend mentioning in the standard references to international regulations on ballast water management that could favor the introduction of non-native species (e.g. "<i>International Convention for the control and management of ships' ballast water and sediments, 2004</i>"). - It is recommended to adapt the sampling protocols according to the situation mentioned by introducing an additional activity of sampling from hulls at the entry of ships from the sea to the river or navigation channels (e.g. at Sulina).
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For chemical determinations in sediments:

- There are important differences in depth, in the same sampling area, as well as in longitudinal and cross sections. These differences arise from variations in depth, current velocities, and flow rates, as well as from the pronounced lateral heterogeneity observed across certain cross-sections — for instance, in some areas of the Danube, depths can range from 1 meter to 30 meters within the same transect. On these sections there are important unevenness, most of them natural, but there are also numerous anthropogenic interventions that produce sediment accumulations, with conditions of accumulation of pollutants in layers of important dimensions.
- The Danube bed has different geological compositions depending on the areas, sometimes on the same longitudinal and cross sections; hence the need to adapt the sampling methods.
- In all the above cases, an orientation made by bathymetry is mandatory because other types of observation are without result.
- Considering the need to integrate the experimental phases (sampling – preparation – analysis) it is necessary to take into account some aspects related to: the role of microplastics (MPs) as vectors that MPs, due to chemical affinity, polarity and polarizability, as well as the cavernous/irregular structure; These properties require analysis, respectively sampling and differentiated preparations for organic or inorganic compounds. For example, in the case of the analysis of the presence of heavy metals adsorbed on the surface of MPs, it is recommended to use a sampling system lined with a non-metallic material, but not plastic. Moreover, in riverine environments such as the Danube River, harmonized microplastic sampling protocols should recommend the processing of approximately 1,000 liters of water per sampling event. This volume is necessary to capture a representative load of microplastics, particularly in flowing freshwater systems where particle concentrations are typically low and spatially variable.

Usually, for the sediment sampling for microplastic analysis in Serbia are used the protocols ISO 5667:1 and ISO 5667:12. There is also a standardized methodology proposed by Fries et al. 2018. Briefly, sediment is collected using a metal trowel (<5 cm) across 3 [line transects](#) located 50 m from each other and 3 points should be sampled across each transect line to give a total of 9 sampling points per beach in a square-like formation. Care should be taken when digging up the sediment to avoid mixing deeper layers with the surface layer. Between sampling points the trowel should be rinsed with Milli-Q water (0.22 µm) to avoid cross-contamination (João Frias, 2018).

- Atmospheric phenomena involving the transport of solid particles over long distances (volcanic eruptions, desert storms in the Sahara, Central Asia, etc.) can have an impact on the collected samples. Depending on the source of the weather phenomenon conditions, if the samples coincide with the course of an event, the samples could be contaminated with various chemical elements, including heavy metals. Before taking the samples, it is important to take into account the weather warnings regarding the development of events involving the transport and deposition of particles that have volcanic eruptions or arid areas as their source. The large surface area of some surface aquatic ecosystems (e.g. the Danube), specific atmospheric currents, the physical interactions of evaporated water and particulate-laden air, etc., lead to the accumulation of polluting particles in the Danube water. It is necessary to organize prior air sampling to determine the origin of pollution of large surface ecosystems. Samples from rainwater accumulations are needed to differentiate between the sources of pollution, especially those with heavy metals and microplastics. Considering the dynamic nature of riverine sediments, flow rate, flood events, and even the inland navigation can cause sediment resuspension which can significantly affect MP deposition patterns and contamination gradients. This supports the use of stratified or seasonal sampling strategies.
- The sensitivity of microplastics to UV radiation depends on their structure as well as their density, depending on which they are found on the surface of the water and are subject to UV degradation; Differentiated depth sampling and differentiated analyses are useful in order to determine the degree of potential degradation of microplastics caused by UV radiation.

- The recalculation of the number of MP particles per unit of sample material is carried out in accordance with methodological guidelines fragmentation and research data from various authors, based on the weight of wet or dry sediment. (Hanke & Ruiz-Orejón, 2023; JRC SCIENTIFIC AND POLICY REPORTS. Guidance on Monitoring of Marine Litter in European Seas, 2013)

At the same time, field and laboratory studies made by NECU have shown that the ratio of wet sample weight to dry sample weight for samples collected from shelf areas with different material-genetic types of sediments ranges from 1:1.3 to 1:10. At the same time, an increase in the ratio is usually observed for samples taken at different depths, degradation processes also yield nano plastics (<1 µm), which control the saturation of the surface layer of sediments with pore water, the volume of “silt” and the content of shell and detrital material.

- The maximum content of MPs, given its density, accumulation characteristics in bottom sediments, and period of active emission into marine waters, in most cases is formed in the surface layer of 1-2 centimeters. However, standard sampling techniques often lack the technical sensitivity needed to effectively target the most relevant sediment layers for microplastic interactions—especially those that are challenging to isolate and analyze using conventional protocols, such as the critical 0–5 cm vertical layer of sediments.
- These sediments can consist of both liquid mud and shell-detritus material, or exhibit other lithological characteristics, for which converting the MP content would be inaccurate.

Perhaps it is worth considering the option of recalculating the results to the weight of non-carbonate matter, assuming that it has an autochthonous genesis or a horizontal sampling area. The method of proportional recalculation of the number of MP particles in samples of different weights to a single reference mass also remains controversial. While reducing the number of particles to a single indicator seems logical, the feasibility of proportional increase remains questionable. This is especially true for rare MP categories (spherules, pyroplastics, etc.). Note on their potential presence and analytical limitations may future-proof the framework.

- Special attention must be paid to the chemical composition, including the presence of emerging pollutants, in the areas where water supply pumps are located for drinking water purposes in order to inform the structures responsible for water quality as well as the need to adapt treatment methods to the presence of certain chemical species with a negative impact on human health.
- In addition to adsorbed pollutants, microplastics often leach plasticizers, flame retardants, UV stabilizers, and other additives. Some of these are semi-volatile or prone to loss during handling; therefore, protocols may need to distinguish between leached additives and adsorbed external pollutants.
- River/sea confluence areas, such as lagoons and deltas, require specific means and methods in which the presence of certain chemical species must be determined not only in water and sediment but also in specific biota elements; The determinations must be adapted according to the sections of aquatic ecosystems: intermediate channels, lakes, flood zones, etc.
- Water and sediment quality studies must include flood zones where certain polluting chemical species are fixed and concentrated, affecting not only aquatic biota but also ornithofauna, as well as fauna dependent on flood zones.

For physical determination:

- The temperature of the Danube depends on natural or anthropogenic factors:
 - Season, flow, influence of tributaries.
 - Activities requiring significant amounts of cooling water (e.g. Cernavoda Nuclear Power Plant).
- Water temperature has a very high influence on the quality of surface aquatic ecosystems due to direct dependencies such as:
 - Dissolved oxygen concentration with direct impact on biota.

- The increase in temperature can favor the presence of invasive species with a negative impact on native species.
- Temperature directly affects the chemical speciation (ionic form) of some chemical species in which ionic solubility and dissociation are temperature-dependent.
- The rising temperature, associated with the reduced flow, favors eutrophication and reducing dissolved oxygen solubility, thus exacerbating hypoxic stress in the Danube's surface aquatic ecosystems.
- The pH of surface aquatic ecosystems is affected by both natural and anthropogenic causes:
 - Temperature, chemical composition of the geological bed, chemical composition of tributaries.
 - Large-scale human activities: industrial, agricultural, municipal wastewater discharges in large human agglomerations.
- pH causes multiple potential changes such as:
 - Influence of biota metabolism and reproductive capacity for sensitive species.
 - The variation of ions resulting from chemical species in which ionization is dependent on the pH value.
- Radioactive pollution:
 - Is due to both the presence of natural isotopes and those resulting from human activities
 - Since a significant number of people as well as livestock farms use the Danube as a source of drinking water and long-lived isotopes have a cumulative effect, well-defined research sections for radioactive emissions are required

For biological determinations.

Biological determinations in water and sediments provide important information on the quality of surface aquatic ecosystems, anthropogenic impact, pollution history as well as for determining measures to reduce pollution and prevent an increase in the impact on surface waters.

Information regarding **benthic macroinvertebrates**, **Phytobenthos**, **Fish fauna** and **Macrophytes** are already present in the available standards therefore will not be discussed further.

It is necessary to adapt the sampling methods as well as the sampling areas taking into account the structure of the riverbed associated with the probability of sediment accumulation (see ANNEX 3). The preparation of sampling periods is done, among other things, for complex ecosystems such as the Danube, through bathymetric predeterminations intended to:

- Locate unevenness and sediment accumulations for orientation and efficiency of sampling operations.
- The determination of sampling areas along the longitudinal section is based both on the study of sediments with biotic potential and on the strategic placement of samples considering specific currents and sediment accumulation zones influenced by the hydrodynamic and geomorphological characteristics of the Danube riverbed.
- For prior orientation on the geostructure of the riverbed bottom, to avoid sampling from hard areas and to comply with the standards that recommend sediment layers of at least 10 cm.

Biological and microbiological sampling is very important to directly or indirectly identify pathogenic aggressive systems stressors:

- The Danube is the source of drinking water for humans and animals for many millions of individuals.

- Lately, both epidemics and epizootics have been reported that have flowing water or certain particles with potential of transport vector properties as transport vectors.
- The analysis of **pathogenic microorganisms** is vital for the protection of public health, especially in areas used for recreation, water supply or fishing. The presence of fecal bacteria, viruses or other pathogens indicates potentially dangerous contamination, requiring rapid and effective interventions.
- This category also includes microplastics which, due to their irregular/cavernous structure, can transport microorganisms, potentially facilitating the spread of invasive species by providing novel habitats and vectors for their dispersal across aquatic environments.
- The identification of the possibility of transport by microplastics is done both through microbiology studies and through genetic determinations of the traces of some microorganisms in microplastics.
- These procedures require the adaptation of sampling methodologies as well as tailored sample preparation protocols. Given the high sensitivity of the analyses—such as microplastic quantification or trace contaminant detection—sampling equipment and storage containers must undergo rigorous sterilization according to specialized protocols to minimize contamination risk and preserve sample integrity.

The fluvial sampling areas present very large differences in terms of the quality of the biota and its biodiversity:

- There are areas with a strong anthropogenic influence: urban agglomerations with activity on the shore and with significant municipal wastewater emissions.
- Industrial areas with a history of impact (for example, the Mineral Port) where significant quantities of raw materials and materials **carried** by sea have been transported.
- Shipping areas that require dredging activities to maintain seaworthiness.
- Permanently maintained transport areas (e.g. Sulina Canal) compared to other canals that, although they have a higher flow rate (e.g. Chilia Canal), have a lower anthropogenic impact.
- The quality and diversity of the biota will be taken into account in correlation with the possibility of introducing alien species through the activities of ballast and deballasting ships (for example, in certain naval transport areas where maritime vessels can enter the river directly, such as the Sulina-Galati-Brăila river-sea area, there is the possibility of introducing invasive species from other continents that can endanger the native fauna).
- The areas of pre-deltatic and deltaic channels where the sediment structure is dependent on the distance from the Danube and the role of transport and water supply of the lakes inside the Danube Delta.
- Areas of confluence with tributaries where studies on the spatial influence of tributary waters on the receiver are required.

In case of native or migratory species and /or of species with characteristic habitats: deep sediment, bottom water, surface water, etc, differentiated procedures for ichthyofauna, macrozoobenthic sampling are required:

- The use of different and specific fishing methods/gear (e.g. gillnet, venty, electronarcosis) depending on the sampling area (lotic or lentic ecosystem – e.g., Danube or Danube Delta), and on the species concerned (autochthonous, anadromous, potamodromous or invasive), as well as on the stage of development/size of the species (e.g. the mesh sizes of the fishing gear) to be taken.
- Selection of species according to the scientific hypothesis pursued: for the evaluation of the status of the Danube-Black Sea integrated aquatic ecosystem based on bioindicators, the use of anadromous species (e.g. *Alosa immaculata*) is recommended, while for the evaluation of the Danube-Danube Delta aquatic ecosystem it is recommended to use potamodromous species (e.g. *Cyprinus carpio*). For the assessment of the status of a freshwater aquatic ecosystem, such as the Danube River, based on bioindicators, in a

specific and restricted area, it is recommended to use species with limited mobility (macrozoobentos e.g. *Corbicula fluminea*).

- The conservation of the sampled species will be specific depending on the type of subsequent analyses that will be subject to the study/monitoring (e.g. for the determination of pollutants such as heavy metals, the samples will be preserved in polymeric containers/bags while for the determination of emerging pollutants such as microplastics, the sampled species will be preserved in aluminum/paper bags to exclude the possibility of self-contamination of the samples).
- From the sampled species, it is recommended to select the most relevant tissues according to the affinities of accumulation of the targeted pollutants (e.g. for the determination of heavy metals, tissues such as muscle and liver will be selected, while for the determination of microplastics, the digestive tract will be selected).
- Before preservation, the selected tissues will be extracted and separated using instruments specific to each analysis so as to reduce the risk of self-contamination (e.g. for heavy metal analysis, only plastic instruments will be used for tissue extraction, while only metal instruments will be used for microplastic analysis).

Monitoring of invasive species

- The Danube is increasingly confronted with the problem of invasive species, which put at risk the ecological balance of the river basin.
- Monitoring these invasive species is important to assess their impact on the ecosystem, to prevent their spread, and to implement effective measures for conserving native species and maintaining ecological balance.

Section 4 Choice of sampling sites

TABLE 3 CORRESPONDING TO THE CHAPTERS OF THE STANDARDS RELATING TO THE CHOICE OF SAMPLING SITES

Clarifications ⁵	Practical implementation details required	Upgrade proposals
<p>Choice of sampling site</p>	<p>Given the complexity of an aquatic ecosystem such as the Danube, the choice of sampling areas is one of the most important phases of the preparation of activities. The following must be nuanced:</p> <ul style="list-style-type: none"> - Areas of interference with Danube tributaries. - Terrestrial maps with areas of important anthropogenic activities are needed. - The areas where municipal wastewater is discharged must be studied. - Existing monitoring stations are considered regardless of their complexity and measurement capacity. 	<p>As a result of the experience gained overtime and the consultation of the international literature, the following emerged:</p> <ul style="list-style-type: none"> - It is necessary to investigate in advance the depth of measurement and sampling as well as the structure of the bottom of the water by bathymetric determinations or physical measurements where the particularities of the water do not allow bathymetric determinations. - It is necessary to study and determine the flow velocity over larger areas and according to the flow/season to determine the possible changes in the accumulation of sediments depending on these characteristics. - Special attention is paid to areas of historical accumulation: new islands, clogged areas, etc. to study the specific parameters and dynamics of these areas. - Continuous monitoring areas should also include sediment sampling to include these features in subsequent modelling. - Confluence areas, municipal wastewater discharge areas after treatment and Danube water pumping areas for drinking water have a distinct monitoring and sampling program. - Given that the pre-delta and deltaic areas are also studied, the monitoring and sampling methodologies are customized.

⁵ According to ISO 5667–12:2017; ISO 5667–17:2008; ISO 5667–15:2010; ISO 5667–6:2017; ISO 5667–1:2023; SIMONA project - Sediment quality sampling protocol for hazardous substances in surface waters

depth, structure of the bottom of the water and the presence of vegetation.

- The areas of island formation must be mapped.
 - It is necessary to identify areas at risk of erosion (banks, meanders and sinuosities.).
 - Flow currents and turbulence areas will be measured in advance.
 - Mapping adjacent areas such as bifurcations, lakes, ponds or irrigation canals.
 - Seasonal monitoring should be carried out, or at times of rapid variation of hydromorphological parameters.
- Monitoring and sampling activities are planned on dredging areas according to a schedule that includes the periods: before dredging, during dredging and after dredging, especially when they are done on the fairway.
 - Fine sediments or pollutants can accumulate in bathymetric pits or depressions on the bed of the steeply sloping riverbed due to low current speeds.
 - Near the mouths (e.g. the confluence with the Olt River – Romania, the Vit River – Bulgaria) rapid mixtures of waters with different physicochemical characteristics can occur, which requires multiple vertical samplings.
 - In narrow areas or with submerged thresholds, turbulence and irregular redistribution of sediments occur. In such places, the samples must be taken both upstream and downstream of the obstacle.
 - During the period of low waters, the distribution of sediments and the concentration of pollutants can become uneven, requiring an increase in the density of sampling points.
 - In the more sinuous sectors of the river, erosion and deposits manifest themselves asymmetrically: erosion on the outer bank, deposition on the inner bank, which requires an adapted distribution of sampling points.

As a result of the experience gained overtime and the consultation of international literature presented in Table 3, the following emerged:

- The diagram in Annex 2 will be considered, which indicates the weight, with graduation from 1 to 5 (the strongest), of the criteria for selecting the sampling areas according to the hydro-morphological parameters of a river.
- A general predetermination of the longitudinal bathymetric profile in the sampling areas to which physical measurements should be added where conditions do not allow bathymetry.
- The bathymetric profile is important to determine because it shows us where depth variations occur. Thus, it is much easier to identify in advance the sectors where there are sediment accumulations, thresholds with sediment deposits, or steep thresholds due to deep erosion phenomena. All these elements directly influence the physico-chemical distribution of water and sediments on the riverbed.
- Knowing and mapping in advance the morphology of the riverbed through bathymetric determinations increases the degree of confidence when we want to choose the representative sampling points, at the same time it helps us to avoid an unnecessary waste of time at the time of sampling by avoiding areas where sampling is not relevant, or impossible to achieve – rocky river bottom, extremely high depths (exceeds 50 meters), or very shallow depths - less than 1 m.
- By pre-planning based on the bathymetric profile, the time of activity in the field is reduced and the risks associated with moving and positioning equipment in inaccessible areas are minimized.
- In situations where multi-beam or single-beam bathymetric measurements cannot be performed (due to too shallow depths, or excessive grassing) it is necessary to complete the data with classic topo-bathymetric measurements: using classic GPS, total topographic levels or stations, milestones or graduated targets, or correlation with available historical data.
- Extensive studies on flow velocities in different areas and under different flow conditions (as is the case for Danube flow variations).
- In the case of rivers, especially those that have similar characteristics to the Danube River, they usually have natural fluctuations of flow especially between seasons (high water level – spring, low level – summer and autumn). These major variations due to fluctuations in flow velocities influence sediment dynamics and water quality distribution both longitudinally and transversely.
- It is useful and necessary to identify flow areas with high water velocities. Usually, when high flow velocities are encountered, the fine sediments remain in suspension and move from one area to another, and the water samples taken may have a lot of suspended matter. **Thus, fast-flowing areas are therefore selected for the investigation of solid material transport.**
- **In areas where the water flow velocity drops below 0.5m/s**, the suspended material begins to settle on the bed of the riverbed, creating pits or banks of bottom sand as well as the internal bottom banks of the rivers from which **the sediment sampling points are chosen.**
- In sectors with very low speeds (below 0.2m/s) or areas with high turbulence, water stagnation and local concentration of pollutants or fine materials occur. **The sampling must include but also differentiate these areas from the main flow.**
- Gradual determinations starting from river-river/canal-lake (e.g. Danube river-Şontea canal-Fortuna lake).

Depending on the specifics of the area, within the monitoring and research activities, the correct choice of water and sediment sampling areas such as the Danube Delta, as well as on the canals connected to the Danube River is strongly influenced by the local hydraulic characteristics. These local conditions are due to the types of existing canals: main canals, secondary canals, or hydraulic connections to lakes, ponds or small canals. Each of these conditions must be considered when choosing the sampling area:

In river areas (e.g. the Danube River):

- The flow rate and flow velocity are more constant and usually with higher values, however, having seasonal variations.
- The sampling areas must capture both the fairway and the secondary area of the river width (the area outside the fairway).
- To choose the sampling area, points are chosen at the entrances/exits in areas of multiple bifurcations or branches or in the vicinity of bathymetric thresholds or lake mouths.

On connecting canals/rivers (main tributaries) and small canals (irrigation canals):

- The speeds of the currents are reduced, and the hydrological regime is much more dependent on the weather conditions (precipitation, evaporation, etc.).
- These channels and rivers represent areas with a high potential for accumulation of fine sediments and organic matter.
- The sampling must include sampling points both in the flow area and in the vicinity of the banks, where clogging is more pronounced.

In interconnected lakes and ponds:

- The currents are weak or almost non-existent, and the water mixture is influenced by wind or temperature variations.
- It is recommended to take samples from several layers (superficial, intermediate, deep) to capture chemical and sedimentological stratification.

If the sampling area has peculiarities similar to a delta, such as the Danube Delta, the following aspects must be considered:

- The canal-lake connection areas (e.g. Gorgova, Fortuna, Roşu lakes) may have reverse flow regimes depending on the water level on the Danube River, which complicates the establishment of sampling points, multiplying their number.
- Small artificial canals (e.g. Crişan–Caraorman) can show rapid clogging and large variations in current speed depending on the season.
- Temporarily isolated lakes may require rapid sampling after reconnection to the main system to capture rapid changes in water quality.
- During periods with high flows (in spring, after the melting of the snow or following intense rains), the speed of the current increases, favoring the erosion of the banks and the bottom of the riverbed and, implicitly, the increase in the concentration of suspended sediments.
- During the summer and early autumn, when the flow decreases and the water temperature rises, the flow velocities are reduced, leading to the deposition of sediment on the bottom of the riverbed and a general decrease in the concentration of suspended sediments. Intense evaporation and lack of precipitation accentuate this process. Also, high temperatures can favor the development of algae, which additionally contributes to the modification of sedimentary characteristics by trapping fine particles in biofilms. This is very common in related areas, such as lakes, puddles, cascading islands, or canals with low water circulation.
- Mapping of areas with different dynamics such as those with rapid clogging, newly formed islands, dynamics of the area of newly formed islands.
- Mapping erosion areas and identifying the main causes of erosion, as well as their dynamics depending on the season, flow, temperature, rainfall regime, etc.
- Observation of the confluence areas with the tributaries, the areas of influence according to the flows as well as the sediment load of the tributaries.
- The mapping of areas with changing morphological dynamics, areas of rapid clogging, as well as the formation of new islands or the variation of the area of existing islands, has an important role in the strategic choice of sediment sampling points.

- These geomorphological processes directly affect the physical and chemical characteristics of the aquatic environment and require a constant adaptation of sampling campaigns. **In areas where new islands appear, sedimentation and erosion processes are intense and rapid. Here, the sampling points must be chosen in such a way as to capture the spatial variations between the active part (continuous deposition) and the periphery exposed to erosion.**
- **Geospatial change around islands — their seasonal or annual expansion or reduction — is an indirect indicator of variation in hydrological and sedimentological regimes. The sampling must include both the proximity of the new sediment formations and the unstable edges, where erosion processes can bring the sediments back into motion.** Thus, the detailed mapping of the islands helps to choose representative sampling areas for the processes of: very rapid sediment deposition or changing from one season to another; active erosion or secondary sediment transport caused by intensive navigation.
- Particular attention will be paid to areas with increased anthropogenic impact such as: municipal wastewater discharge areas after treatment, areas of intense agricultural activity, pumping areas for irrigation, etc.
- In the process of selecting sediment sampling areas, special attention should be paid to areas affected by intense anthropogenic impact, as these areas can introduce disturbing factors into the natural aquatic environment.
- Treated discharged water can contain organic and inorganic compounds, pathogenic microorganisms, and therefore it is important to take samples downstream and upstream of the discharge points. The input of nutrients (chemical fertilizers), pesticides and sediments from agricultural land can lead to water eutrophication, chemical contamination and changes in sediment characteristics. Sampling must be carried out both in surface drainage areas and near irrigation or drainage channels.
- **The activities of pumping water for irrigation from natural water courses modify the local flow regime, favoring water stagnation, recirculation or even reversal of the current in some cases. They can also contribute to the redistribution of fine sedimentary particles or pollutants. It is important to take samples at immediate confluence points and in areas of hydraulic water mixing.**
- To correctly and efficiently inform the Danube communities, the pumping areas for drinking water will be mapped and studied, both from the point of view of the dynamics of clogging by sediment accumulation and by interaction with large pumping stations.
- Water and sediment quality monitoring will also be conducted at drinking water pumping stations, including the assessment of biological indicators. This will provide decision-makers with essential data to optimize treatment processes, ensuring the delivery of high-quality drinking water that meets health and safety standards efficiently.
- In collaboration with the responsible structures, activities will be carried out to monitor the quality of water and sediments from a chemical, physical and biological point of view, both during dredging operations and after dredging. The sediments taken over by the dredging equipment will be analyzed.

Identification and marking of deballasting zones for river-sea navigation vessels will be considered.

Section 5 Choice of sampling methods and sampling equipment

TABLE 4 CORRESPONDING TO THE CHAPTERS OF THE STANDARDS RELATING TO THE CHOICE OF SAMPLING METHODS AND EQUIPMENT

Clarifications ⁶	Practical implementation details required	Upgrade proposals
Choice of sampling method	<ul style="list-style-type: none"> - The size of the dredge must be adapted not only according to the depth of the water, the type of sediment, and the depth of penetration into the sediment layer according to the standards in force, but also considering factors such as the speed of water flow and the intensity of the currents. These parameters significantly influence the performance of the equipment used and the efficiency of the bottom sediment sampling process. 	<ul style="list-style-type: none"> - It is essential that the weight and stability of the sampler are adjusted to cope with local current conditions, thus ensuring that sediment is collected from the desired location or as close to it as possible, without being influenced by current forces. For example, in fairway areas, where the depths and currents are greater, it is recommended to use high-capacity, mechanically operated dredges. They allow the equipment to be submerged at the bottom of the river, without being significantly diverted by strong currents and without disturbing sediments. On the other hand, near the banks, where the currents are weaker and the depths are shallower, it is recommended to use small dredges, which are easier to handle, even manually, and more efficient in shallow areas, but which are still able to capture the sediment layers effectively.
Sampling equipment	<ul style="list-style-type: none"> - In the context of large aquatic ecosystems and the complexity of the Danube, these factors become particularly important due to the variable characteristics of the river, such as seasonal fluctuations in water level, the wide width of the watercourse and local changes in currents. In particular cases, where different depths between sampling points or different types of sediment 	<ul style="list-style-type: none"> - As for borehole-type dredges, they are not recommended in high-flow areas, as the strong currents at the level of the river floor can seriously affect the use of the equipment. Thus, currents can deflect the dredge or require the use of long cables, which can complicate the sampling process. Moreover, the

⁶ According to ISO 5667–12:2017; ISO 5667–17:2008; ISO 5667–15:2010; ISO 5667–6:2017; ISO 5667–1:2023; SIMONA project - Sediment quality sampling protocol for hazardous substances in surface waters

are found in the same sampling site, the choice of dredge type must take these into account (e.g. Sulina canal).

choice of the type of dredge must be correlated with the objectives and purpose of the study carried out.

Features of microplastic sampling techniques:

- This issue was discussed in the Iemelianov's publication⁷ and concerns the need to standardize methods for sampling bottom sediments for research on MP content in their surface layer. The methodological guidelines of European authorities on microplastic sampling in environmental objects provide for the use of various sampling methods for bottom sediments, in particular scoops and corers of various models (Hanke & Ruiz-Orejón, 2023; JRC SCIENTIFIC AND POLICY REPORTS. Guidance on Monitoring of Marine Litter in European Seas, 2013);
- Experimental studies have shown that the quality of samples and the effectiveness of subsequent laboratory studies depend significantly on the use of different types of samplers. It is known that microplastics have similar dispersion behavior to low-density suspended solids, and the quantitative distribution of its fragments in bottom sediments significantly correlates with the percentage of pelitic components in samples (Cunningham et al, 2020). The main part of polymer fragments accumulated in the "silt," a significant amount of which consists of fibers and their fragments, is extremely unstable to external influences and depends on the preservation of the semi-liquid surface layer of bottom sediments. Visual observations during the initial description of bottom sediment samples indicate a high probability of loss of part of their upper, semi-liquid layer when sampling with a scoop compared to a multicorer. This is indicated by changes in the composition of the sampled material, in particular, different degrees of water content and the amount of dispersed components. The loss of the liquid layer of the sample present on its surface during sampling with a scoop, unlike samples collected with a multicorer, significantly reduces the number of MP particles in the sample, thereby slightly distorting the research results. The quantitative indicators of the distribution of artificial polymer particles when sampling bottom sediments by different means can be reconciled by obtaining a reliable series of corresponding results of comparison of sampling methods based on special experimental studies.

⁷ [V.O. Iemelianov, MAIN FACTORS OF INFLUENCE ON GEOECOSYSTEMS OF WATER BASINS AND RISKS OF THEIR DESTRUCTION | Geology and Mineral Resources of World Ocean](#)

Section 6 Frequency of sampling

- There are situations in which it is necessary to unclog aquatic areas where naval transport is difficult and which require the dredging of large volumes of sediment that can significantly disturb the habitats of aquatic communities. For this reason, it is important to develop a rigorous sediment sampling plan that involves a constant frequency of sample collection at different stages of unclogging (Table 5).
- Given the fact that emerging pollutants are not yet regulated at European level, it is important to continuously monitor them in large aquatic ecosystems and the complexity of the Danube in order to contribute to the development of strategies to reduce pollution with them. All this information can contribute to their regulation and the establishment of the maximum permissible concentrations for each type of ecosystem and quality classes.

TABLE 5 CORRESPONDING TO THE CHAPTERS OF THE STANDARDS RELATING TO THE FREQUENCY OF SAMPLING

Clarifications ⁸	Practical implementation details required	Upgrade proposals
Frequency and time of sampling	<ul style="list-style-type: none"> - In the case of projects that involve dredging sediments from the bottom of the water, it is very important to take sediment samples at different times of the execution of the works. This is necessary to capture the influence that dredging activities have on both sediment quality and benthic biodiversity. - In the case of monitoring emerging pollutants for which there are still many gaps in the databases, a higher frequency of sampling is recommended so that certain regulations can be established at decision-making level for each type of compound. 	<ul style="list-style-type: none"> - When unclogging activities are carried out on canals, lakes, fairways, etc. It is recommended to establish a rigorous sampling program at different stages of dredging activities. - It is necessary to take sediment samples at least once before, during and after dredging activities. - Higher sampling frequency when monitoring emerging pollutants. - Pulsed inputs e.g. from rain events are often difficult to monitor even at „higher“ frequency of sampling. Thought, different strategies may be used e.g. passive sampling. However, these may also come with challenges.

⁸ According to ISO 5667–12:2017; ISO 5667–17:2008; ISO 5667–15:2010; ISO 5667–6:2017; ISO 5667–1:2023; SIMONA project - Sediment quality sampling protocol for hazardous substances in surface waters

Section 7 Transport and stabilization of the collected samples

Ensuring the **integrity and representativeness** of sediment samples during the post-collection phase is essential for the accuracy of subsequent chemical, physical, and biological analyses. Improper transport or inadequate preservation can result in **oxidation, microbial degradation, loss of volatile compounds**, or alteration of granulometric and geochemical properties.

TABLE 6 CORRESPONDING TO THE CHAPTERS OF THE STANDARDS RELATING TO THE STORAGE, TRANSPORT AND STABILIZATION OF THE COLLECTED SAMPLES

Clarifications ⁹	Practical implementation details required	Upgrade proposals
<p>Storage, transport and stabilization of samples</p>	<ul style="list-style-type: none"> - The standard only mentions the temperature range that must be maintained during transport, but there is no information on the maximum duration in which it is recommended to carry out the transport of samples from the field to the laboratory. Even though the ISO 5667-15 standard contains information regarding the maximum shelf life of samples, it is important to mention in ISO 5667-12 both the duration of transport and the retention period, because the information is useful from the moment of taking and transporting the samples to the laboratory. - This chapter does not provide clear information on the preservation methods suitable for each compound analyzed, although they must be known and included right from the transport and stabilization stage. - Preservation methods should be listed and developed separately, depending on subsequent analyses. - The standard does not specify information about environmental conditions other than temperature during transport. - The standard does not provide a monitoring sheet for the recording of temperatures during transport and storage to ensure traceability. - There is no clear delineation related to the storage of samples, although there are separate requirements for biological and chemical analyses. - There is no recommendation for a sufficient sample volume for the planned cumulative simultaneous analyses. 	<ul style="list-style-type: none"> - Given the diversity of target compounds, it is also necessary to highlight a maximum duration for the transport of samples. - The maximum duration of transport + preservation must be clearly specified so that the chemical composition of the sample is not affected. - Also, the transport and/or preservation time must be correlated with the type of preservation practiced (chemical, physical, etc.). - Due to the sensitivity of some compounds, samples must sometimes be isolated from light during transport. - Monitoring and recording temperature during transport in a document that can be accessed and used later. - Delimitation of transport and storage conditions according to the type of analysis (chemical, biological). - Minimum quantities of sediments can be collected depending on the number of analyzes and their type so as to minimize both the amount of sample and the consumables used, specific containers, logistics and transport and storage space.

⁹ According to ISO 5667–12:2017; ISO 5667–17:2008; ISO 5667–15:2010; ISO 5667–6:2017; ISO 5667–1:2023; SIMONA project - Sediment quality sampling protocol for hazardous substances in surface waters

- The standard does not specify instructions on a possible correction in case of non-compliance with the conditions of carriage.
- It is proposed to take a minimum recommended mass of approximately 500 g for chemical analysis and 200 g for physical analysis.

In the Table 6 are presented practical implementation details required and upgrade proposal corresponding to the chapters of the standards (ISO 5667–12:2017; ISO 5667–17:2008; ISO 5667–15:2010; ISO 5667–6:2017; ISO 5667–1:2023) and SIMONA project - Sediment quality sampling protocol for hazardous substances in surface waters.

- During the transport of samples from the field to the laboratory, there is a possibility of fluctuations in environmental parameters (temperature, light, humidity, etc.). Therefore, it is recommended to systematically monitor and record these parameters to ensure traceability.
- In case of exceptional situations (failure of the air conditioning system, accidental cross-contamination, accidental degradation of the transport container, etc.) that lead to non-compliance with the specific conditions of transport and preservation, it is recommended to adopt measures such as: specific labeling, prioritization of samples for analysis (first in, first out/last in, first out), consideration of the sample as non-compliant and resumption of the sampling procedure.
- In order to streamline the sampling time, storage space, but also to reduce the consumption of consumable materials, it is recommended to collect a sample volume/mass for compounds that have the same conditions of sampling, handling, and preservation (for example, in the case of volatile organic compounds and microplastics).

Section 8 Good practice examples

In line with the Grant Agreement, the project focuses on building upon the key results of previous initiatives, while also creating strong synergies with ongoing projects. In the following paragraphs a list of such projects is given.

Implemented projects

- POIM type project "Improvement of hydrological conditions in natural aquatic habitats in the Danube Delta Biosphere Reserve for the conservation of biodiversity and fish resources - Gorgova-Uzlina, Roșu-Puiu lake complexes", POIM 120890;
- POIM type project "Improvement of hydrological conditions in natural aquatic habitats in the DDBR for the conservation of biodiversity and fish resources - Dunăvăț-Dranov, Razim-Sinoie, Sinoie-Istria-Nunțași lake complexes";
- POIM type project, "Improvement of hydrological conditions in natural aquatic habitats in the BDDR for the conservation of biodiversity and fish resources - Lake complexes Șontea-Furtună, Matiața-Merhei, Somova Parcheș" Code SMIS 2014+ 120889.

Within the projects: POIM 120889 (Șontea-Fortuna, Matiața-Merhei, Somova-Parcheș) POIM 120890 (Dunăvăț-Dranov), POIM 120892 (Gorgova-Uzlina, Roșu-Puiu), monitoring campaigns were carried out before and after unclogging, involving measurements of flow rates, flow velocities, sediments and physico-chemical parameters of the water. To access narrow or clogged channels, special boats with reduced draft and modern sampling equipment were used. The projects aimed to restore hydrological connectivity between lakes and canals, with the aim of reducing siltation and supporting aquatic biodiversity. Physico-chemical monitoring was essential for assessing the efficiency of ecological reconstruction works. Within the above-mentioned projects, dredging works aimed at restoring the natural circulation of water between lakes, combating eutrophication processes and supporting fish and ornithological habitats of high conservation value. All these projects demonstrated the need for an integrated, scientifically substantiated approach to unclogging works in the Danube Delta, respecting the principle of protecting biodiversity and maintaining ecological balance.

Projects under implementation

- Project "HORIZON-MISS-2021-OCEAN-02, DANUBE REGION WATER LIGHTHOUSE ACTION", Project: 101094070 — DALIA.
- Project „HORIZON-CL5-2021-D6-01-09, Resilience-centric Smart, Green, Networked EU Inland Waterways, - ReNEW.

The DALIA project is focused on the Danube River basin and fresh water, it is an innovation event, and it is supporting the new EU Mission Restore our Ocean and Waters by 2030. This project brings together 23 partners from 9 EU countries. DALIA Knowledge and Monitoring System (KMS) is the DALIA project digital innovation hub focused on implementation and demonstration of development done at pilot sites. DALIA KMS combines technical and social tools to support replication of methodologies and approaches developed within DALIA project and stimulates cooperation with other projects within EU Waters and Ocean Mission.

The ReNEW (Resilience-centric Smart, Green, Networked EU Inland Waterways) project, funded by Horizon Europe, brings together 24 partners from 11 EU countries to advance Inland Waterway Transport (IWT) towards a smarter, greener, and more climate-resilient future. Key objectives include developing a decision-support framework for IWT resilience and sustainability, implementing innovative infrastructure solutions, and creating a Green Resilient IWT Dataspace alongside digital twin technologies. These efforts are exemplified through four Living Labs across Europe, focusing on integrated inland waterway and hinterland infrastructure. This initiative aligns with the objectives of the SUNDANSE project, which aims to develop innovative and sustainable sediment management solutions for the Danube River–Black Sea system. Both projects emphasize enhancing the resilience and sustainability of inland waterways, addressing environmental challenges, and promoting coordinated approaches across European regions.

The **Joint Danube Survey (JDS)**, organized by the **International Commission for the Protection of the Danube River (ICPDR)**, represents one of the most comprehensive river monitoring initiatives in Europe. JDS campaigns (JDS1–JDS5, 2001–2025) aim to provide a unified, transnational overview of the Danube’s ecological status using **standardized sampling methodologies** for sediment, biota, and water quality.

The **Joint Danube Survey (JDS)** :

- ensures a unified assessment of the ecological status of the river and its tributaries, the International Commission for the Protection of the Danube River (ICPDR) regularly organizes joint monitoring expeditions, known as the Joint Danube Survey (JDS).
- these expeditions are the largest monitoring campaigns in Europe and aim to collect comparable scientific data along the entire length of the Danube. One of the key objectives of the JDS is to harmonize sampling and analysis techniques for aquatic bioindicator communities, which are essential for the assessment of ecological status under the Water Framework Directive (2000/60/EC).
- are an example of good practice in transnational monitoring of water bodies. By promoting the harmonization of sampling and analysis techniques for aquatic bioindicators, they contribute significantly to increasing the consistency of ecological assessments, protecting biodiversity and strengthening international cooperation for the protection of the Danube River.

The harmonization of sediment sampling protocols must build upon existing national and international experience in large river systems, especially within transboundary basins like the Danube. Several successful case studies and coordinated monitoring efforts provide a solid foundation for methodological alignment, ensuring replicability, comparability, and efficiency in data collection and interpretation.

JDS introduced harmonized methods for:

- Sediment sampling for trace metals, PAHs, PCBs;
- Biological assessment (macroinvertebrates, fish, phytobenthos);
- Hydromorphological characterizations in line with WFD standards.

Outcome: Establishment of **comparability of ecological status assessments** across the entire basin, which remains a cornerstone for WFD implementation in the region.

Section 9 Conclusions

The proposed recommendations for updating the sediment sampling protocols within the SUNDANSE project aim to improve methodological coherence, data comparability and the capacity for multidisciplinary interpretation. The harmonized protocols are designed to be applicable in a wide range of hydromorphological contexts specific to large aquatic ecosystems, with a particular focus on the Danube River and its deltaic system.

Building on previous experience (especially from SIMONA project and ISO standards), field studies and literature review, this deliverable presents upgrades to the sampling methods, criteria for site selection, frequency and equipment, and introduces complementary analyses for emerging pollutants and microplastics. It also emphasizes the importance of sediment sampling in understanding the environmental impact of dredging and the strategic role of sediments in ecosystem dynamics and contaminant transport.

Implementing these harmonized protocols will support the achievement of the Water Framework Directive goals and facilitate the transferability of methodologies to other European river basins. Moreover, it enhances the scientific robustness and policy relevance of sediment monitoring in complex riverine and deltaic systems.

Examples of previous initiatives are included in the last chapter and their results are summarized.

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ANNEX 1

TABLE 7 MARITIME DANUBE SECTION OF THE DANUBE RIVER WITH DIFFICULT NAVIGATION AREAS

Maritime Danube (km 0 – km 175)					
River Km	Name of the area	Navigation type	Minimum Navigation Depth [m]/Minimum Signal Width	Critical Season	Observations
km 0 – km 2	Sulina Bar	River-Maritime	7.01 / 120 The required width must allow two ships or a convoy to sail simultaneously.	summer	Massive sediment deposits; frequent dredging, sometimes monthly.
km 44	The roadstead of the port of Sulina	River-Maritime		summer	Critical point for the reception of the pilot; minimum depth 7.01 m.
km 175	Port of Brăila	River-Maritime		summer	Regular dredging to maintain the fairway.

TABLE 8 MIDDLE DANUBE SECTION OF THE DANUBE RIVER WITH DIFFICULT NAVIGATION AREAS

Middle Danube (km 175 – km 943)					
River Km	Name of the area	Navigation type	Minimum Navigation Depth [m]/Minimum Signal Width	Critical Season	Observations
km 345 – km 346	Zimnicea/Sviștov	River	2.50 / between 80 m and 150 m, depending on the sector	summer	Low water levels; necessary dredging.
km 375 – km 376	Giurgiu	River		summer	Sediment deposits; periodic dredging.
km 685 – km 686	Belene	River		summer	Formation of sandbanks; requires dredging.

TABLE 9 DANUBE UPSTREAM SECTION OF THE DANUBE RIVER WITH DIFFICULT NAVIGATION AREAS

Danube upstream (km 943 – km 1,075)					
River Km	Name of the area	Navigation type	Minimum Navigation Depth [m]/Minimum Signal Width	Critical Season	Observations
km 943 – km 948	Iron Gates I	River	2.50 / between 80 m and 150 m, depending on the sector	summer	Level fluctuations; requires constant monitoring.
km 965 – km 975	Iron Gates II	River	In some more sensitive areas (e.g. Belene, Iron Gates) decreases of up to 2.20 m are exceptionally accepted in the dry season.	summer	Sediment deposits near the locks.
km 1.070 – km 1.075	Baziaș	River		summer	Low levels; possible dredging required.

ANNEX 2

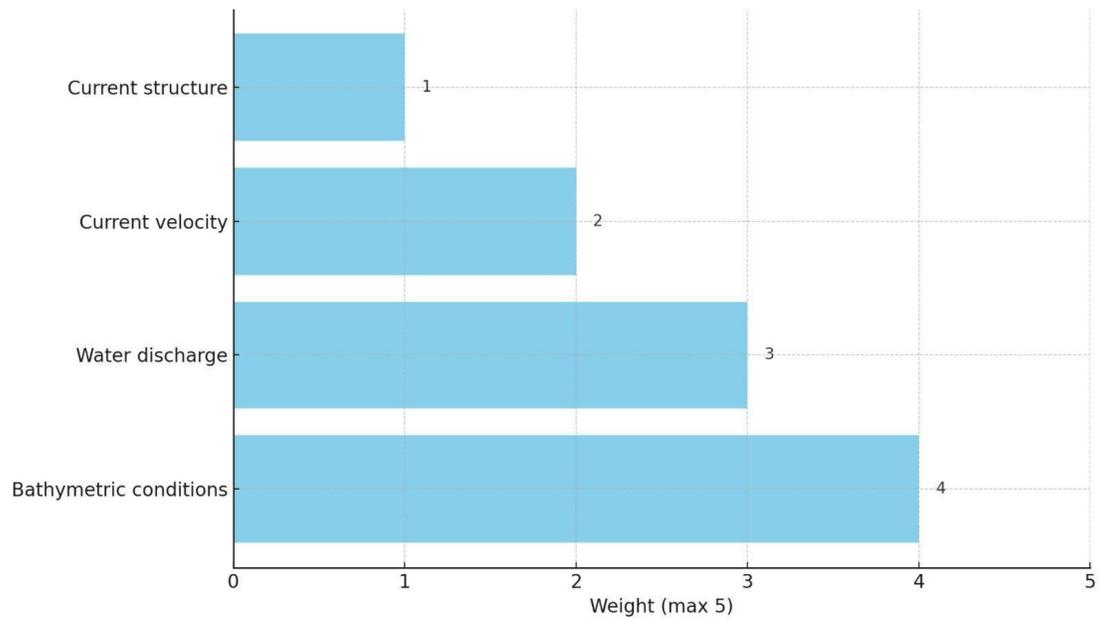


FIGURE 2 HYDROMORPHOLOGICAL CRITERIA FOR SELECTING SAMPLE COLLECTION AREAS

ANNEX 3

Criteria for Site Selection

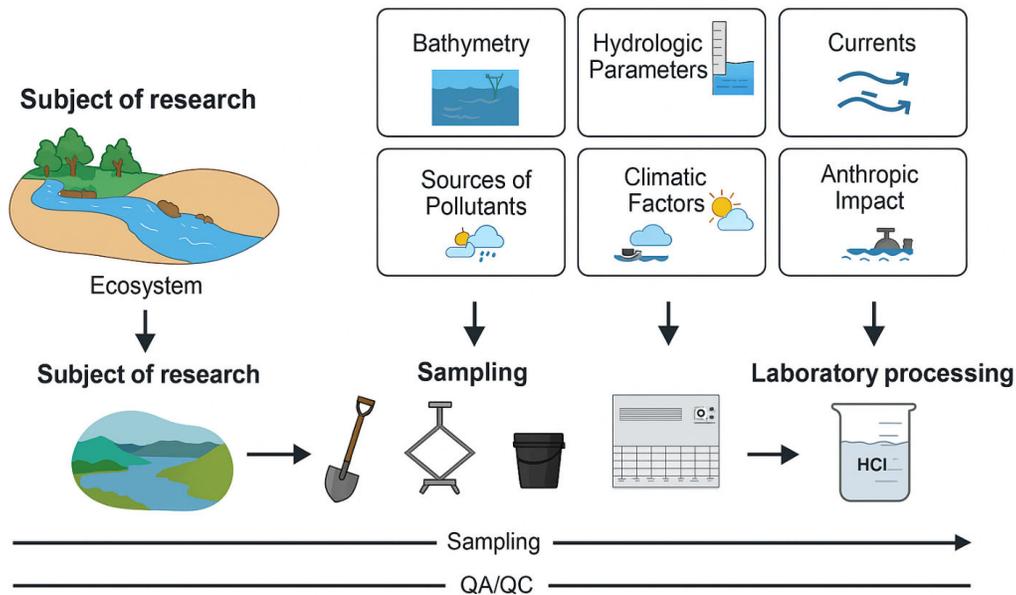


FIGURE 3 CRITERIA FOR SITE SELECTION¹⁰

Image based on information taken from:

- 1). Danube Commission – "Danube Navigation Manual", - [MANUAL ON DANUBE NAVIGATION - VIADONAU](#)
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¹⁰ QA = QUALITY ASSURANCE. QC = QUALITY CONTROL

ANNEX 4

Co-funded by the European Union

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sundanse
Sustainable Sediment solutions for
the Danube - Black Sea system

In-Situ Sampling Form

Project acronym: **Sample matrix:**

Collection date: **Collection time:** **Sample ID:**

(DD/MM/YYYY) (HH:MM) **Sampling standard:**

Monitoring site details

Monitoring site name: **Monitoring site ID:**

Location - Latitude: **Longitude:** **River km (rkm):**

Sample location description:

Type of monitoring site: **Aim of sampling:**

Intended analysis type:

Monitoring site before after at distance

Is it the same waterbody as the monitoring site? Yes No

If not, describe the connection between the waterbody and the monitoring site:

Waterbody name: **Waterbody ID:** **Type of waterbody:**

River width (m): **estimated value** **measured value**

Depth (m): **estimated value** **measured value**

Flow rate/Water velocity (m/s): **estimated value** **measured value**

Discharge data (m³/s): **estimated value** **measured value**

Geology and background value of parent material/lithology in the area:

Extreme conditions: **Weather conditions:**

Water sample information

T (°C): **pH:** **EC (µS/cm):** **DO (mg/L):**

ORP (mV): **TDS (mg/L):** **Turbidity (NTU):** **Transparency - Secchi disk (cm):**

Sediment sample information

Water depth above sample (m): **Sediment sample depth (cm):**

Collection device: **Distance between the first and last sampling site (m):**

Composite sample: Yes No *If yes, number of collected samples*

Sample replicate: Yes No **Replicate ID:**

Sample is duplicates? Yes No

Sampling volume estimated - wet weight (L): **Sample temperature on field (°C):**

Sediment pH (undisturbed): **Sediment pH (post-homogenization):**

Colour (Munsell soil colour chart number): **Odour:**

Texture (particle size):

Information on sediment components (seashells, animals, peat, wood, tar, stones, waste, plastics, others):

Sample photograph identification:

Additional information:

Sampler name

Signature

FIGURE 4 SAMPLE REPORT FORM (UPDATE THE FORM PRESENTED ON SIMONA PROJECT SAMPLING PROTOCOLS DELIVERABLE¹¹)

¹¹ SEDIMENT QUALITY SAMPLING PROTOCOL FOR HSS_Output 4.1_SIMONA project.pdf

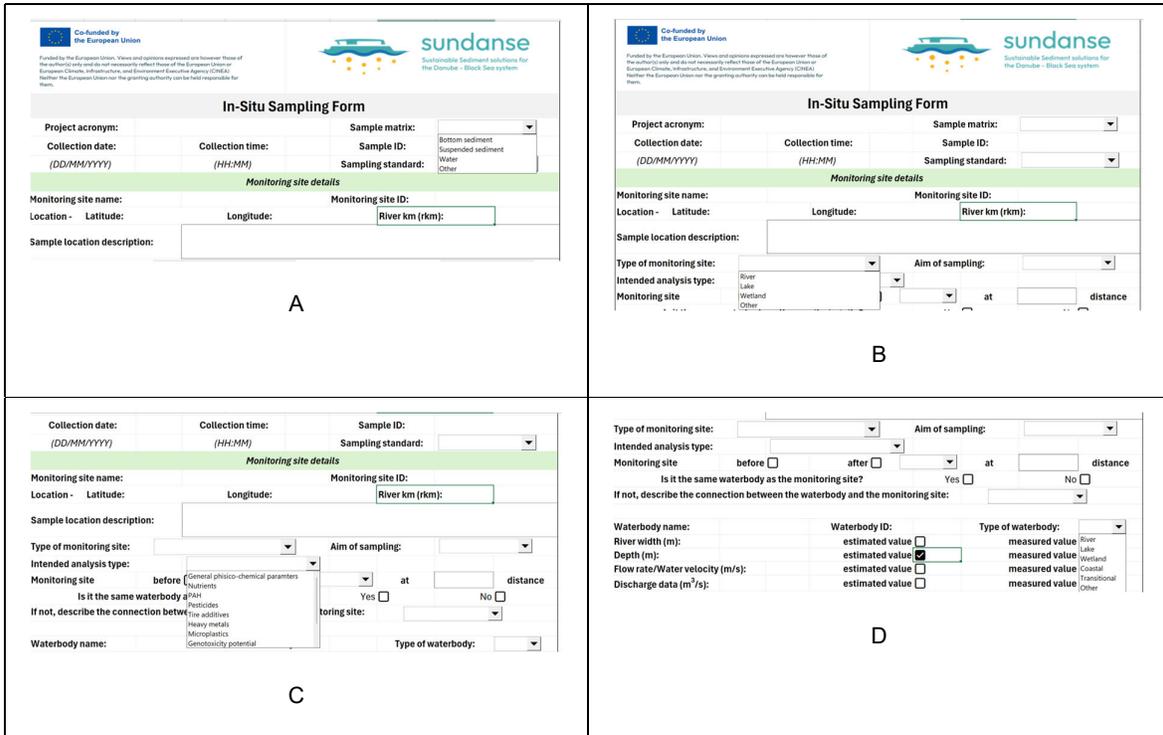


FIGURE 5 EXAMPLE OF UPDATES OF SIMONA PROJECT SAMPLING PROTOCOLS DELIVERABLE: A – POSSIBILITY OF CHOSING THE TYPE OF THE SAMPLE; B - POSSIBILITY OF CHOSING THE TYPE OF THE INTENDED ANALYSIS TYPE; C – MULTIPLE CHOISE FOR THE TYPE OF MONITORING SITES; D - POSSIBILITY OF CHOSING THE TYPE OF THE WATER BODY¹²

ANNEX 5

Crt. No.	Document title	Observations
1	SR EN ISO 5667-1:2023 Water quality — Sampling Part 1: Guidance on the design of sampling programmes and sampling techniques	<p>This document sets out the general principles for, and provides guidance on, the design of sampling programmes and sampling techniques for all aspects of sampling of water (including waste waters, sludges, effluents, suspended solids and sediments). This document does not include detailed instructions for specific sampling situations, which are covered in various other parts of the ISO 5667 series and in ISO 19458.</p> <p><u>Status:</u> in effect</p>
2	SR EN ISO 5667-15:2010 Water quality — Sampling Part 15: Guidance on the preservation and handling of sludge and sediment samples	<p>ISO 5667-15:2009 provides guidance on procedures for the preservation, handling and storage of samples of sewage and waterworks sludge, suspended matter, saltwater sediments and freshwater sediments, until chemical, physical, radiochemical and/or biological examination can be undertaken in the laboratory.</p> <p>The procedures in ISO 5667-15:2009 are only applicable to wet samples of sludge, sediment and suspended matter.</p> <p><u>Status:</u> in effect</p>
3	SR EN ISO 5667-19:2005 Water quality — Sampling Part 19: Guidance on sampling of marine sediments	<p>ISO 5667-19:2004 provides guidance for the sampling of sediments in marine areas for analyses of their physical and chemical properties for monitoring purposes and environmental assessments. It encompasses sampling strategy, requirements for sampling devices, observations made and information obtained during sampling, handling sediment samples, and packaging and storage of sediment samples.</p> <p><u>Status:</u> in effect</p>
4	ISO 5667-12:2017 Water quality. Guidance on sampling of bottomsediments from rivers, lakes and estuarine areas	<p>ISO 5667-12:2017 provides guidance on the sampling of unconsolidated sediments for the determination of their geological, physical and chemical properties, as well as the determination of biological, microbiological and chemical properties at the water and sediment interface. Guidance on achieving sediment cores is given specifically for the measurement of rates of deposition and detailed strata delineation. The main emphasis of this document is to provide methods that achieve sediment samples.</p> <p>The environments considered are</p> <ul style="list-style-type: none"> - limnic (rivers, streams and lakes, natural and man-made), and - estuarine, including harbours. <p><u>Status:</u> in effect</p>
5	SR EN ISO 22032:2006 Water quality — Determination of selected	<p>ISO 22032:2006 specifies a method for the determination of selected polybrominated diphenyl ethers (PBDE) in sediment and sludge using gas chromatography/mass</p>

Crt. No.	Document title	Observations
	polybrominated diphenyl ethers in sediment and sewage sludge — Method using extraction and gas chromatography/mass spectrometry	<p>spectrometry (GC-MS) in the electron impact (EI) or negative ion chemical ionization (NCI) mode.</p> <p>When using GC-EI-MS, the method is applicable to samples containing 0,05 to 25 micrograms per kilogram of tetra- to octabromo congeners and 0,3 to 100 micrograms per kilogram of decabromo diphenyl ether (BDE-209), respectively.</p> <p><u>Status</u> : Expected to be replaced by ISO/DIS 22032 Water quality - Determination of polybrominated diphenyl ethers (PBDE) in sediment, suspended (particulate) matter and biota - Method using gas chromatography-tandem mass spectrometry or high resolution mass spectrometry (GC-MS/MS; HRMS).</p>
6	SR EN ISO 18635:2016 Water quality — Determination of short-chain polychlorinated alkanes (SCCPs) in sediment, sewage sludge and suspended (particulate) matter — Method using gas chromatography-mass spectrometry (GC-MS) and electron capture negative ionization (ECNI)	<p>ISO 18635:2016 specifies a method for the quantitative determination of the sum of short-chain polychlorinated n-alkanes also known as short-chain polychlorinated paraffins (SCCPs) in the carbon bond range, n-C10 to n-C13, inclusive in mixtures with chlorine mass fractions ("contents") between 50 % and 67 %, including approximately 6 000 of approximately 8 000 congeners.</p> <p>This method is applicable to the determination of the sum of SCCPs in sediment and suspended (particulate) matter, sewage sludge, and soil using gas chromatography-mass spectrometry with electron capture negative ionization (GC-ECNI-MS).</p> <p>Depending on matrix and the detection capabilities of the GC-ECNI-MS, the method can be applied to samples containing 0,03 µg/g to 3 µg/g sum of SCCPs.</p> <p><u>Status</u>: in effect</p>
7	ISO 23646:2022 Soil quality — Determination of organochlorine pesticides by gas chromatography with mass selective detection (GC-MS) and gas chromatography with electron-capture detection (GC-ECD)	<p>This document specifies a method for quantitative determination of organochlorine pesticides (OCPs) and semi-volatile chlorobenzenes in soil and sediment, using GC-MS and GC-ECD.</p> <p>The limit of detection and the limit of application depends on the determinants, the sample intake, the equipment used, the quality of chemicals used for the extraction of the sample and the clean-up of the extract.</p> <p>Under the conditions specified in this document, lower limits of application from 1 µg/kg (expressed as dry matter) for soils to 10 µg/kg (expressed as dry matter) for sediments can be achieved. The necessity to achieve these lower limits of application depends on the analyses order and the current limit values.</p> <p>Soils and sediments can differ in properties as well as in the expected contamination levels of OCPs and the presence of interfering substances. These differences make it impossible to describe one general procedure. Based on the properties of the samples, this document contains decision tables</p>

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8	ISO 18475:2023 Environmental solid matrices — Determination of polychlorinated biphenyls (PCB) by gas chromatography - mass selective detection (GC-MS) or electron-capture detection (GC-ECD)	<p>regarding drying-, extraction- and clean-up procedures. This method is performance based.</p> <p><u>Status:</u> in effect</p> <p>This document specifies methods for quantitative determination of seven selected polychlorinated biphenyls (PCB28, PCB52, PCB101, PCB118, PCB138, PCB153 and PCB180) in soil, sludge, sediment, treated biowaste, and waste using GC-MS and GC-ECD (see Table 2).</p> <p>The limit of detection depends on the determinants, the equipment used, the quality of chemicals used for the extraction of the sample and the clean-up of the extract.</p> <p>Under the conditions specified in this document, lower limit of application from 1 µg/kg (expressed as dry matter) for soils, sludge and biowaste to 10 µg/kg (expressed as dry matter) for solid waste can be achieved. For some specific samples the limit of 10 µg/kg cannot be reached.</p> <p>Sludge, waste and treated biowaste may differ in properties, as well as in the expected contamination levels of PCB and presence of interfering substances. These differences make it impossible to describe one general procedure. This document contains decision tables based on the properties of the sample and the extraction and clean-up procedure to be used.</p> <p><u>Status:</u> in effect</p>
9	EPA Method 1699: Pesticides in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS	<p>EPA Method 1699 determines organochlorine, organophosphorus, triazine, and pyrethroid pesticides in environmental samples by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) using isotope dilution and internal standard quantitation techniques. This method has been developed for use with aqueous, solid, tissue and biosolids matrices.</p> <p><u>Status:</u> in effect</p>
10	EPA Method 1633 : Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS - December 2024	<p>Method 1633A is for use in the Clean Water Act (CWA) for the determination of the per- and polyfluoroalkyl substances (PFAS) in aqueous, solid (soil, biosolids, sediment) and tissue samples by liquid chromatography/mass spectrometry (LC-MS/MS).</p> <p>The method calibrates and quantifies PFAS analytes using isotopically labeled standards. Where linear and branched isomers are present in the sample and either qualitative or quantitative standards containing branched and linear isomers are commercially available, the PFAS analyte is reported as a single result calculated from the combined responses of the linear and branched isomers.</p> <p><u>Status:</u> in effect</p>
11	EPA Method 7473 (SW-846): Mercury in Solids and Solutions by Thermal	<p>This method is for the determination of the total Mercury (organic and inorganic) in solids, aqueous samples, and</p>

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	Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry	<p>digested solutions in both the laboratory and field environments.</p> <p>Total mercury (organic and inorganic) in soils, sediments, bottom deposits, and sludge-type materials as well as in aqueous wastes and ground waters can be determined without sample chemical pretreatment using this method.</p> <p>Alternatively, this method can be used for the detection of total mercury from total decomposition sample preparation methods, or for detection of extracted or leached mercury compounds or species.</p> <p><u>Status:</u> in effect</p>
12	EPA Method 8061A Phthalate esters by gas chromatography with electron capture detection (GC/ECD)	<p>Method 8061 is used to determine the identities and concentrations of various phthalate esters in aqueous and solid matrices including groundwater, leachate, soil, sludge and sediment.</p> <p>This method involves gas chromatography with electron capture detection (ECD) to determine the concentration and identification of phthalate esters. Sediment samples are first extracted, often using Soxhlet extraction or other methods, to isolate the phthalate esters. The extract is then analyzed by GC-ECD.</p> <p><u>Status:</u> in effect</p>
13	ISO 13859:2014 Soil quality — Determination of polycyclic aromatic hydrocarbons (PAH) by gas chromatography (GC) and high performance liquid chromatography (HPLC)	<p>ISO 13859:2014 specifies the quantitative determination of 16 PAH in sludge, soil, and treated biowaste using GC-MS and HPLC-UV-DAD/FLD covering a wide range of PAH contamination levels.</p> <p>When using fluorescence detection, acenaphthylene cannot be measured.</p> <p>Sludge, soil, and treated biowaste can differ in properties and also in the expected contamination levels of PAH and presence of interfering substances. These differences make it impossible to describe one general procedure. ISO 13859:2014 contains decision tables based on the properties of the sample and the extraction and clean-up procedure to be used. Two general lines are followed, an agitation procedure (shaking) or use of Soxhlet/pressurized liquid extraction.</p> <p><u>Status:</u> in effect</p>
14	ISO 24187:2023 Principles for the analysis of microplastics present in the environment	<p>This document describes the principles to be followed in the analysis of microplastics in various environmental matrices. This includes the unique particle size classification of plastics, the use of certain apparatus with regard to sampling, sample preparation, and the determination of representative sample quantities.</p> <p>The purpose of this document is to specify minimum requirements until specific standards for the different case situations are available. This is important to ensure that the development of the specific standards is done on a</p>



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		<p>consistent basis to ensure that comparison or correlation of results is possible.</p> <p><u>Status:</u> in effect</p>
15	SR EN 14757:2015 – Water Quality. Sampling fish in lakes, using benthic multi-mesh gillnets.	<p>This European Standard specifies a standardised method for sampling fish in lakes, using benthic multi-mesh gillnets. The method provides a whole-lake estimate for species occurrence, quantitative relative fish abundance and biomass expressed as Catch Per Unit Effort (CPUE), and size structure of fish assemblages in temperate lakes. It also provides estimates, which are comparable over time within a lake and between lakes. This European Standard specifies routines for sampling, data handling and reporting, and provides information on applications and further treatment of data. This European Standard also provides guidance on sampling of fish with pelagic multi-mesh gillnets and sampling of fish for age and growth analyses.</p> <p>Evaluation of population parameters, species composition, abundance and age structure, for fish populations in rivers, lakes and transitional waters requires appropriate sampling methods. The appropriate sampling methods depend on the objective, as well as on the type and category of water, the species of fish investigated and their body length. The sampling methods are selected in relation to the wide variety of inland waters and the diversity of fish species and their habitats. The adequacy of the established methods has been classified for the purpose of carrying out a valid evaluation.</p> <p><u>Status:</u> in effect</p>
16	SR EN 14011:2003 - Water quality - Sampling of fish with electricity	<p>This European Standard provides procedures to be used by trained persons in evaluating fish communities in streams, rivers and littoral areas for the purpose of classification of ecological status. These procedures allow standardisation of sampling methods for descriptions of fish communities. The use of standardised methods is a critical requirement for the comparability of results.</p> <p>This European Standard describes an electric fishing method to be used when catching fish for the purpose of characterising species richness, composition, abundance and age structure of a given fish community. Sampling-related issues include obtaining permissions, concerns about endangered species, protective measures of importance for the user of the sampling apparatus and co-ordination of activities with other sampling programmes.</p> <p>The processing of samples covers taxonomic identification, counting, measurement of biologic parameters (length, weight etc.), and examination of fish for external anomalies.</p> <p><u>Status:</u> in effect</p>
17	SR EN 14962:2006 - Water quality - Guidance on the	<p>This document defines methods for sampling fish and procedures for selection of methods in order to evaluate fish populations in rivers, lakes and transitional waters. This</p>

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18	<p>scope and selection of fish sampling methods</p> <p>EN 15910:2014 - Water quality - Guidance on the estimation of fish abundance with mobile hydroacoustic methods</p>	<p>document refers to the standards "Water quality - Sampling of fish with electricity" (EN 14011) and "Water quality - Sampling of fish with multi-mesh gillnets" (EN 14757).</p> <p><u>Status:</u> in effect</p> <p>This European Standard specifies a standardized method for data sampling and procedures for data evaluation of fish populations in large rivers, lakes and reservoirs, using hydroacoustic equipment deployed on mobile platforms (boats and vessels).</p> <p>This standard covers fish population abundance estimates of pelagic and profundal waters > 15 m mean depth with the acoustic beam oriented vertically, and the inshore and surface waters of water bodies > 2 m depth with the beam oriented horizontally. The size structure of fish populations can only be determined to a relatively low degree of precision and accuracy, particularly from horizontally-deployed echosounders. As acoustic techniques are presently unable to identify species directly, other direct fish catching methods should always be used in combination.</p> <p>This standard provides recommendations and requirements on equipment, survey design, data acquisition, post-processing of data and results and reporting.</p> <p>Acoustic monitoring is performed to investigate large volumes of water. Transect-based monitoring, therefore, is based on the assumption that the measurements that are made along the monitoring lines are representative samples of the wider distribution of target species in the monitored volume of water.</p> <p>Acoustics is used to collect information from a distance, by transmitting a beam in pulses of sound energy in a body of water. Acoustic systems are usually launched from a moving boat into large bodies of water.</p> <p><u>Status:</u> in effect</p>
19	<p>SR EN 14184:2014 - Water quality - Guidance for the surveying of aquatic macrophytes in running waters</p>	<p>This European Standard specifies a method for surveying aquatic macrophytes in running waters for the purpose of assessing ecological status, using these organisms as elements of biological quality. The information provided by this method includes the composition and abundance of the aquatic macrophyte flora.</p> <p>This European Standard is applicable to all kinds of surface running water bodies, like natural brooks, streams and rivers and their heavily modified equivalents, as well as to artificial water bodies like canals or run-of-river reservoirs.</p> <p>The general principles of the approach described in this European Standard may also be applied when monitoring water bodies in the fluvial corridor of a river, such as side channels and oxbows.</p> <p><u>Status:</u> in effect</p>

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20	SR EN 15460:2008 - Water quality - Guidance standard for the surveying of macrophytes in lakes	<p>This standard defines a method for surveying aquatic macrophytes in lakes primarily for the purpose of assessing ecological status, using these organisms as an element of biological quality.</p> <p>The information provided by this method includes the composition and abundance of the aquatic microphyte flora.</p> <p>This European Standard describes a methodological approach for determining the ecological status of lakes using aquatic macrophytes.</p> <p>The status of a lake is assessed by establishing its deviation from the natural conditions of a lake of a similar ecological type.</p> <p>The presence of aquatic macrophyte taxa in the individual lakes is recorded. Macrophyte abundance, measured in terms of the spatial extension of taxa or macrophyte beds, and/or macrophyte abundance estimates or biomass, is assessed by different methods adapted to the scales and purpose of the study.</p> <p>Numerical derivatives or metrics of macrophyte composition and abundance in a lake survey can be used to identify the divergence from type-specific, natural conditions.</p> <p><u>Status:</u> in effect</p>
21	SR EN 13946:2014 - Water quality - Guidance for the routine sampling and preparation of benthic diatoms from rivers and lakes	<p>This European Standard specifies a method for the sampling and laboratory preparation of benthic diatoms for ecological status and water quality assessments. Data produced by this method are suitable for production of water quality indices based on the relative abundance of taxa.</p> <p><u>Status:</u> in effect</p>
22	SR EN 14407:2014 - Water quality - Guidance for the identification and enumeration of benthic diatom samples from rivers and lakes	<p>This European Standard specifies methods for the identification and enumeration of relative proportions of diatom taxa on prepared slides and of data interpretation relevant to assessments of water quality in rivers and lakes. It is suitable for use with indices and assessment methods based on the relative abundance of taxa. The methods for identification and enumeration may also be applied to the study of benthic diatoms in other habitats provided that data interpretation methods appropriate to these habitats are used.</p> <p>Benthic diatoms, cleaned of cell contents and mounted in a medium with a high refractive index, are identified and counted using a high power light microscope until an appropriate sample size has been obtained. These data are then interpreted using one or more indices or other assessment methods.</p> <p><u>Status:</u> in effect</p>
23	SR EN ISO 10870:2012 - Water quality — Guidelines for the selection of sampling	<p>This International Standard specifies criteria for the selection of sampling methods and devices (operation and performance characteristics) used to evaluate benthic</p>

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24	<p>methods and devices for benthic macroinvertebrates in fresh waters</p> <p>AQEM - The Development and Testing of an Integrated Assessment System for the Ecological Quality of Streams and Rivers throughout Europe using Benthic Macroinvertebrates</p>	<p>macroinvertebrate populations in fresh waters (rivers, canals, lakes, and reservoirs). The methods and devices considered in this International Standard are suitable for sampling all major components of the benthic assemblage. They are not suitable for sampling meiofauna.</p> <p><u>Status:</u> in effect</p> <p>The AQEM assessment system is the main result of the European Union funded project AQEM, which was carried out from March 2000 to February 2002.</p> <p>It serves the implementation of the EU Water Framework Directive and provides a system for assessing ecological quality in European streams with benthic macroinvertebrates.</p> <p>Aims of the AQEM system are:</p> <ul style="list-style-type: none"> • To classify a stream stretch in a quality class from 5 (high) to 1 (bad) based on a macroinvertebrate taxa list. • To give information about the cause of a possible degradation to help direct future management practices. <p>In contrast to many other comparable projects, the development of the AQEM system has been based on a new dataset covering both the fauna and general stream characteristics of 28 common European stream types.</p>