Interreg
Danube Transnational Programme
JOINTISZA

www.interreg-danube.eu/jointisza

# Joint Tisza Survey Manual (Joint Tisza Survey 2)

## Deliverable 3.4.1 Guidance Manual of Joint Tisza Survey

Final Version
Date Dec 2018

Project co-funded by the European Union (ERDF, IPA funds)

DANUBE REGION strategy

# Acknowledgements

Lead authors

**Dr. Mariann Dr. Teszárné Nagy,** Middle Tisza District Water Directorate, Hungary

Contributing authors

**Attila Lovas,** MiddleTisza District Water Directorate, Hungary
**Béla Csányi**, Senior expert, Hungary
**György Rátfai,** MiddleTisza District Water Directorate, Hungary
**Melinda Váci,** Middle Tisza District Water Directorate, Hungary

# Contents

# Annexes

# 1. Introduction

A strong intention exists among the Danube countries to intensify their water management cooperation in the field of water protection and water use within the framework of the Convention on Cooperation for the Protection and Sustainable Use of the Danube River (Danube River Protection Convention, DRPC). One of the key elements of this cooperation was the collection and distribution of reliable information on water quality. The Transnational Monitoring Network (TNMN) achieved this goal producing information about water quality on annual basis since 1996.

The EU WFD and the DRPC both suggest that surface waters should be regularly controlled by surveillance, operational and investigative monitoring. The tasks of the investigative monitoring at the Danube Basin-wide level have been started with the first Joint Danube Survey (Literáthy at al. 2002) organized by the ICPDR and accomplished through two others (JDS2 and JDS3 in 2007 and 2013, respectively, Liška et al. 2008, 2015). The first Investigation of the Tisza River (ITR) program was a follow up study of the JDS1 (Csányi 2002) in order to reveal the actual situation of the Tisza one and a half year after the serious cyanide and heavy metal spills. The methodologies of this longitudinal survey and the JDS1 were the same. Therefore this first Investigation of the Tisza River (ITR)could better be nominated as Joint Tisza Survey 1 (JTS1).

Similar WFD compliant survey was organized and executed on the Sava River carried out by the experts of the Sava countries when the second largest sub-basin of the Danube was investigated in a cooperation.

The Tisza River with its tributaries represent the largest sub-basin of the total Danube Basin. For the better understanding of the present ecological status it is important to plan and execute an international longitudinal survey program along the whole Tisza with the participation of all five Tisza countries. Based on the results and experiences of the previous three JDS and one Tisza missions it is possible to organize the second longitudinal sampling program on the whole Tisza River. The experiences of these missions concerning the planning, execution and dissemination of result provide a solid base to the second international JTS. The results of this proposed survey could be compared to previous data sets detecting changes of the situation during the last decades.

One of the work packages of the framework of INTERREG JOINTISZA project (WP3 Basin characterization-SW) started to deal with the necessity of the preparation of the new Joint Tisza Survey Manual(Activity 3.4). This Manual is the thematic document of the second Joint Tisza Survey (JTS2).

This proposal for the longitudinal Tisza Survey is based on the former experiences of the three Joint Danube Surveys (JDS1-3) and the first Joint Tisza Survey (JTS1). The planning of sampled matrices, biological and chemical quality elements and other components such as microbiology, hydromorphology are following the methodologies of previous programs. However, the JDS4 to be executed in 2019 has a very different set up in many points: national teams will work on their own sections simultaneously, no ship will be used for sampling and travel downstream the Danube, certain instrumentation and matrices will not be included in the sampling program at all (SPM, sediment, etc.). Therefore a special expert group should be nominated and involved later, in the planning phase of the next Tisza Survey for the finalization procedure in order to take into consideration of all special characters and needs of the Tisza Basin that are not foreseen at this moment.

# 1 Objectives of the Joint Tisza Survey

## 1.1 General objectives of the JTS2

The Tisza River Basin, with its total extent of 157,186 km² is the largest sub-basin in the Danube River Basin. The Tisza River is the longest tributary of the Danube (966 km), and the second largest by flow, after the Sava River. There are some specific characters in the Tisza Basin that should be taken in consideration as follows:

- The metal mining was a permanent activity on the upper-middle Tisza stretch since some hundred years resulting in higher values of several heavy metal compounds in the river water and sediment. Therefore certain heavy metals could be river basin specific pollutants here;
- Due to anthropogenic effects the Tisza and some of its tributaries (deforestation, water regulation, etc.) are characterized by sudden peak flood events. The Tisza River is an excellent example of the rivers that should be managed in a harmonized manner taking into consideration some EU Directives focusing to - in certain cases antagonistic - goals (WFD, Flood Directive).

The five states (Hungary, Slovakia, Serbia, Romania, Ukraine) in the Tisza Basin agreed on a close transboundary co-operation aiming to achieve integrated management of the Tisza River Basin. The Tisza countries are ready for joint action according to the relevant EU legislations to protect the quality and improve the status of surface waters and safeguarding the sustainable use of water resources with the help of the Integrated Tisza River Basin Management Plan. The Joint Tisza Survey (JTS) is planned in order to better understanding the present ecological status and pressures of waters.

The key objective of JTS2 is to produce comparable and reliable information on selected water quality elements for the whole length of the Tisza River including the major tributaries. The outcomes of the JTS2 could decrease the information gaps as necessary for the next update of the Tisza River Basin Management Plan. Such a joint action provides an outstanding opportunity for harmonization and training in WFD related monitoring for the experts of the Tisza countries.

The general objectives and added values of the JTS2 are as follows:

- Establishing the further development of the international cooperation in water monitoring activity during the third phase of the River Basin Management Plan;
- Obtaining appropriate information to identify ecological and chemical status of surface water bodies along the Tisza Basin using WFD compliant water monitoring system in the framework of an international study that is highly similar to the previous three Joint Danube and one Joint Tisza Surveys;
- Contributing to the Tisza River Basin Management Plan (TRBMP) updates.

## 1.2 Specific objectives and added values

Several additional specific objectives should be mentioned as follows:

- Producing homogenous data set for the Tisza River and its tributaries based on a longitudinal downstream directed nautical sampling program following the water current;

- Screening of WFD Priority Substances and some other hazardous pollutants and new pollutants like micro-plastics;
- General overview of habitat morphology of the Tisza River (that is sharply different from the Danubian situation);
- Providing interactive platform of Tisza countries for sampling and assessment of different WFD compliant quality elements;
- Generating an independent sub-basin-wide platform for improving national surface water monitoring practices;
- To gather knowledge for the Participating Experts about how to organize and carry out field work on a longitudinal survey on the whole extent of the almost 1000 km long Tisza River and its tributaries;
- Practical joint testing and comparison of national methodologies for chemical, biological and hydromorphological quality elements leading to their future harmonization;
- The only source of data for a number of quality elements (especially for emerging substances) for the Tisza Basin;
- Knowledge transfer to non-member states.

## 1.3 Long term benefit

Nowadays such kind of extended longitudinal sampling campaigns are very rare around the World. JTS2 - similarly to all of the previous three JDS missions will focus the attention of several experienced leading European Laboratories (JRC, etc.) to the Tisza Basin in order to participate in the analytical work on voluntary base.

- Independent Europe-wide platform for improving chemical surface water monitoring practices;
- Practical joint testing and comparison of national methodologies for biological and hydromorphological quality elements leading to their future harmonization;
- Interactive platform for hands-on training in sampling and assessment of biological quality elements;
- The only source of data for a number of chemical quality elements (especially for emerging substances) for the whole Danube;
- Knowledge transfer to non-member states.

# 2 Survey program and preparation

The methodology of the JTS should follow the approach of the previous JDS methodologies organized by the ICPDR during the last two decades since 2001. One possible discrepancy will arise due to the fact that ships will not be used during the next JDS: some equipments will be missing that formerly were installed on board of the Laboratory Ship Argus during the previous time. These equipments are the centrifuge used for collecting SPM and the wet sieving machine for sediment samples that will not be available during the JDS4 program. A possible solution would be to use the Argus ship for the sampling program. This ship is a Serbian property since 2002 and it was used for several longitudinal sampling programs beside of the JDS missions (AquaTerra FP6 program, sediment collecting programs, etc., Slobodník et al. 2005, Stahlschmidt-Alner P. et al. 2005).

To establish a relevant longitudinal sampling program several aspects should be taken into consideration. These points are as follows:

- *Sampling network*: What particular cross-sections and tributaries are to be investigated? The answer is highly depending on the characteristics of the river basin, de environmental situation including the hydrological conditions, and, the pollution situation, as well. An appropriate sampling network have to reflect all of the main anthropogenic impacts and pressures for the reliable status assessment;
- *Time schedule of the sampling action*: it should be highly dependent on the hydrological situation (water level, water discharge conditions) ), and, in case of biological elements, on the optimal timing according to their characteristics;
- *Investigated matrices and relevant Determinands, variables*: Lists of previous expeditions have to be completed according to the present 'status of the art' concerning the different amendments of the WFD, recent findings about river basin-specific chemicals and ecotoxicological results, etc.;
- *Applied methodology during the Survey:* Relevant sampling methods and sample treatment procedures have to be given in the Manual that exactly defines what to do during the mission;
- *International Core Team:* The nomination of the International Core Team for the longitudinal ship-cruise is the responsibility of all Tisza countries based on common understanding;
- *National Expert Teams:* The individual nomination of the experts is the responsibility of each given country. The selected experts are able to join to the navigational crew on each given river section in order to carry out the common sampling;
- *Cost estimations:* The total costs will be basically influenced by the number of sampling sites and the type and number of investigated Determinands.
- *The planning of logistics during the survey:* The traffic (moving by ship and cars/motorboats), the treatment, storage and transport of different samples together with the nomination and negotiation with participating laboratories should be clarified in details;
- *Reporting:* The preparation of the Final Report of the JTS2 has to be planned in advance determining all of the responsible editors of the whole report and the different chapters;
- *Public awareness involvement:* The regular communication of the different steps of the sampling program should be provided by officials.

The financial background is the principal point because the costs will basically determine the whole action. Two general items will mainly influence the overall costs of the survey:

- The analysis of chemical compounds;
- The costs concerning the ship.

Later on, alternative scenarios should be given for the realistic and reliable selection of the optimal version sampling program: what kind of compounds are relevant in which sampling sites? The optimal version should be based on rational way determined by the WFD-/ RBMP-requirements. This Manual contains one proposal in terms of sampling sites and investigated components after analysing three versions of sampling network. The final plan of the sampling program, the investigated compounds, the methodology, the personnel background and all other aspects should be discussed in details among nominated experts of relevant topics.

## 2.1 Survey program: proposed sampling sites

The list of proposed sampling sites in this Manual follows the methodology of previous longitudinal sampling programs organized by the ICPDR. One of them was a follow up survey of the first Joint Danube Survey (Investigation of Tisza River, ITR 2001). According to the practice of the previous Joint Danube Survey this first ITR could be nominated as the First Joint Tisza Survey (JTS1) and the next international program could follow this action as the Second one (JTS2).

At that time the Tisza River just recovered the serious cyanide spill and heavy metal pollution events causing significant effects on the Biota of the river one and a half year earlier (January-March 2000). Considering the river length and the number of sampling sites several earlier Danubian experiences could help in determining the necessary number of selected sites on the Tisza River (Table 1).

*Table 1. Number of sampling sites on the Danube and average distances between sampling sites during previous JDS missions*

| ***JDS (year)*** | **Total no. of sampling sites on Danube & tributaries** | **No. of sampling sites on Danube** | **Average length / Danube site (river km)** | **Average length during three JDS programs (rkm)** |
|---|---|---|---|---|
| JDS1 (2001) | 98 | ***74*** | ***35*** | 40 |
| JDS2 (2007) | 96 | ***72*** | ***36*** | |
| JDS3 (2013) | 68 | ***53*** | ***49*** | |

The JDS1 mission covered 2600 river km, 98 sites in 2001 having 74 locations on the main river and 24 locations on the major tributaries and side arms. The average length between two sites was 35 km. During the JDS2 (2007) the number of sites was only a little bit reduced: out of 96 sites 72 was situated on the Danube and the same amount of tributaries/side arms (24) were included. That time the average distance between sites was almost the same (36 km). However, the JDS3 contained significantly smaller number of sites because altogether only 68 sites were included in the program: 53 Danube sites and 15 tributaries/side arms were sampled (average length of section between two sites was 49 km).

The first Joint Tisza Survey was dealing with 744 river km and 20 Tisza sites were investigated. At that time the average length of section between two sites was 37 km. Based on these comparisons the following Tisza and Tributary sites are suggested to involve in the survey:

According to the proposal there are **twenty Tisza locations** and thirteen **tributaries, altogether 33 sites** that are proposed for the survey program according to the following site list. (Figure 1, **Annex I, Table 1.** ). The exact position of some locations are nominated in this list according to the program of the First Joint Tisza Survey (JTS1 in 2001) and only larger tributaries are taken into consideration for the sampling program. This amount of tributary sites is realistic to be sampled (average length of section between two sites is approximately 45 km on the investigated 910 km long Tisza stretch. ***Comparing to the JDS1-2-3 and the JTS1 programs this proposal with the 33 investigated sites seems to be realistic.***

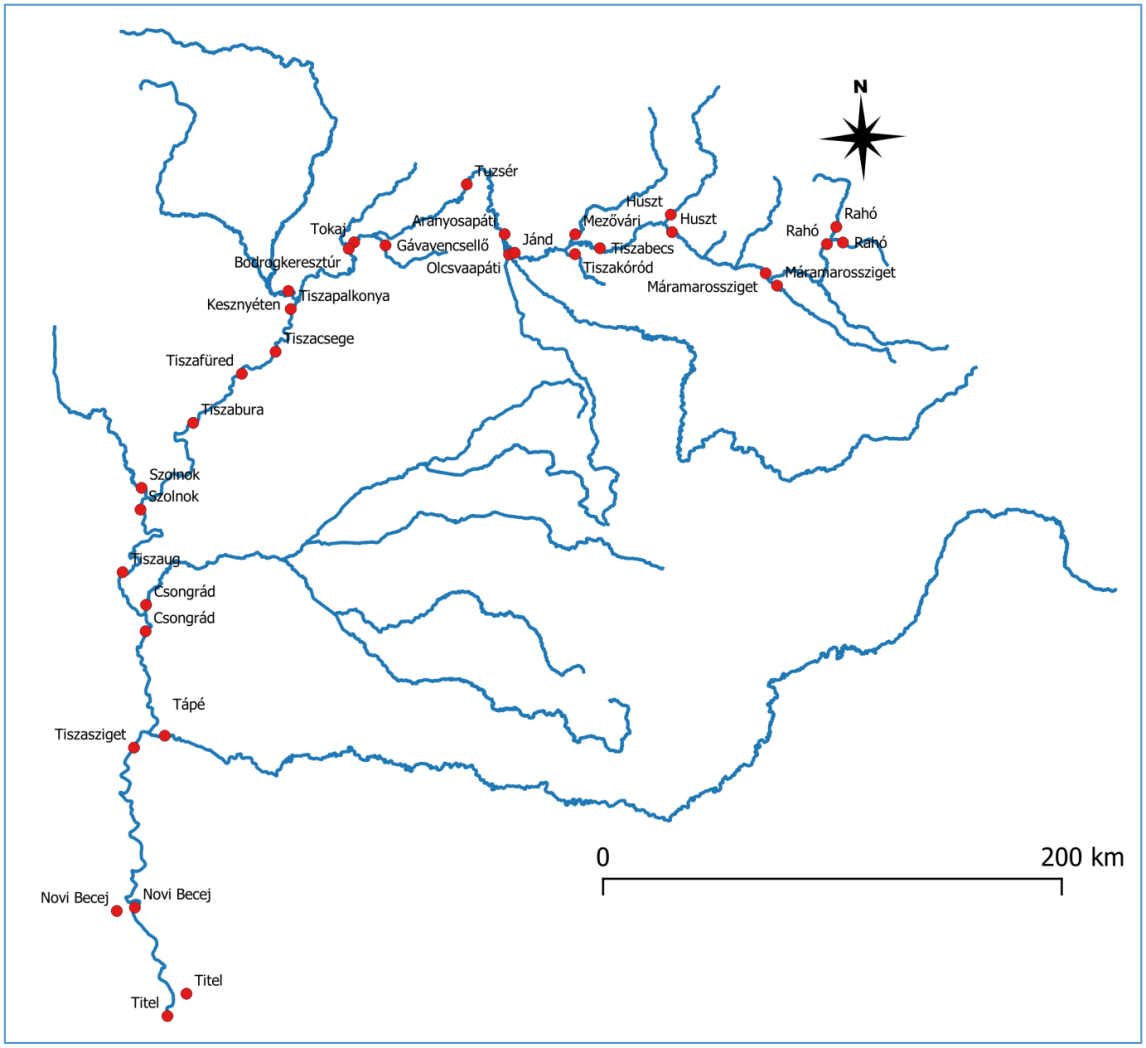



*Figure 1. Sampling sites along the Tisza and its tributaries*

## 2.2 Time schedule

The proposed starting of JTS2 is summer time when middle or low water discharge conditions exist and the season is appropriate for the Biological Elements, as well. The time schedule of the sampling program is given only, as follows:

The base of calculating the required time of the expedition is that the upper section of the Tisza could be sampled only by car with rubber boat. Although the upper end of the official navigation section of the Tisza is at Vásárosnamény (685 river km) during low water discharge the river is navigable only up to Tuzsér (616.5 river km) (this situation happened in 2001 during the JTS1 longitudinal survey). In the optimal case during navigable period the uppermost sampling site is situated at Jánd that is few km upstream Vásárosnamény and the Samos confluence. So, the different sections are sampled by various ways: car and rubber boat (Upper Tisza) and ship and motorboat (Middle and Lower Tisza) should be used, respectively. That means that approximately 350 km is sampled by car and 610 is sampled by ship and motor boat.

According to the initial planning the sampling action is taken downstream direction in order to ensure the realisation of the quasi-synchronous 'water-line sampling' idea. This way the program of 33 sites

(20 Tisza and 13 tributary sites) could require **12 days** altogether. The 20 sites on the Tisza requires 9 days and the 13 sites on tributaries needs 3 days. This way the **average site** sampled per day value is **3 sites/day (Annex I: Table 1)**.

There are certain elasticity for compromises in the program, especially concerning the Middle and Lower Tisza sections. During the downstream travel and sampling by ship and motorboat the sampling of tributaries by car and rubber boat could be organised parallel, at the same time. The conditions of the number of experts for the sampling programs will be discussed later in details. For comparison the JTS1 program required altogether 10 days for 27 samples (**roughly 3 sites/day**) but 9 days were required for ship traffic and all of the 5 sites on the Upper Tisza stretch was taken during one day. However, it should be noticed that the WFD compliant sampling program obviously requires more time than before.

## 2.3 Matrices and Determinands

### 2.3.1 Matrices

The following matrices were investigated during the longitudinal sampling program of JDS1-3:

- Surface water (W);
- Sediment (S);
- Suspended particulate matter (SPM);
- Tissue of Biota (mussel and/or fish).

It should be decided whether or not **Suspended particulate matter (SPM)** will be included in the next Tisza sampling program. This matrix was investigated during all of the three previous JDS missions and the first JTS, as well. SPM has to be an essential matrix in case of the Tisza and its tributaries because these rivers contain much larger amount of suspended particulate matter (even during low water discharge) than the River Danube. The involvement of SPM in the JDS sampling programs was achievable because one ship (ARGUS) was equipped with a sampling equipment: the 'on board' installed centrifuge during all previous expeditions worked very well. However, other mobile sampling methods/devices could be available for car expeditions, as well. The Tisza Expert group should decide upon the SPM involvement during the JTS2 sampling program. It should be noticed that JDS4 will not deal with SPM and sediment sample collection, due to different reasons (see **ANNEX II:** Proposal for chemical analysis (JDS4 Survey Plan ANNEX D), page 34).

In case of involvement of SPM sampling and analysis in the JTS2 program the costs are similarly shaping as the costs of the sediment samples.

### 2.3.2 The types of samples in matrices

The investigated matrices along the Tisza River and its tributaries are the following: surface water, sediment, suspended particulate matter (SPM) and biota (fish and mussels). The survey program includes general *physico-chemical* and *chemical analyses* (each of them with different determinand list) and *biological samples*. These matrices including Biota samples will be investigated applying special chemical analysis, as follows:

**Water**

*Chemical analysis*

- General physico-chemical analysis

- Priority Substances analysis
- River Basin Specific Pollutant analysis: target, non-target and suspect screening
- Microplastics

*Biological samples*

- Phytoplankton
- Zooplankton
- Phytobenthos
- Macrophytes
- Benthic invertebrates
- Fish
- Microbiological samples
- Zooplankton

**Sediment**

*Chemical analyses*

- Priority Substances analysis
- River Basin Specific Pollutant analysis: target, non-target and suspect screening
- Microplastics

**SPM**

*Chemical analyses*

- Priority Substances analysis
- River Basin Specific Pollutant analysis: target, non-target and suspect screening
- Microplastics

**Biota**

*Chemical analyses*

- Priority Substances analysis
- River Basin Specific Pollutant analysis: target, non-target and suspect screening
- Microplastics

Water samples have to be collected in the middle of the river with the help of a rubber boat (Upper Tisza) or motorboat (Middle and Lower Tisza). Sediment samples should be taken from left and right bank and then should be mixed and wet sieved on-board to obtain 63 µm fraction. It has to be decided whether or not *SPM sample collection* should be included in the program. The sampling procedure was completed by an on-board centrifuge of the Argus ship during all of the three JDS missions. However, in some cases a stretch of the Danube River had been sampled between two sampling sites during the travelling downstream, due to time constraints. SPM samples from the Upper Tisza sampling sites could be collected by a centrifuge installed in a car.

During the longitudinal Tisza survey seven different types of biological samples will be collected.

*Phytoplankton* and *Zooplankton* has to be taken in the middle of the river and tributaries. M*icrobiological* determinations could be taken at the middle of the river due to the fact that - oppositely to the Danube - the total mixing occurs within relatively short distances along the Tisza after the different major confluences.

Sampling of *Benthic invertebrates* will be carried out at the left and right bank of the Tisza. Additionally, a dredging of the bottom of river bed for collection of benthic invertebrates will take place in the middle of the river from the habitats of deep water region. In case of the tributaries only one benthic invertebrate sample will be collected from the best available habitat type of the location. *Phytobenthos* samples should be taken from left and right bank both on the Tisza and tributaries. A major problem could be to find stony (or other solid) surfaces along the Tisza River. *Macrophytes* are not relevant biological elements of the Middle and Lower Tisza due to the depth and the frequently changing water level together with the high turbidity/SPM content. Macrophyte survey should be considered only on the Upper Tisza stretch if it is relevant according to local experts.

The *Fish survey* will be performed along selected sampling sites. Littoral electrofishing by night and deep-water electro-trawling at day time will be used similarly to the JDS3 program (2013). It should be mentioned that prior to the JDS3 the Danube Research Institute (DRI) crew performed successfully a Preliminary Tisza Survey in order to try out those sampling methods in the deep water region of the Tisza that would have followed in the JDS3 later on the year of 2013. Our conclusion was that the deep water methodology for both macroinvertebrate and fish sampling was very successful on the Tisza River.

The design of the *Microbiological survey* requires special expert discussion and appropriate planning similarly to the previous JDS missions. There was a clear and obvious development in the applied on-board methodology from the JDS1 until the JDS3 (and it is an ongoing process for the methodological planning of the JDS4 performed in 2019, as well) indicating the need for new and wider approach for the microbiological characterization of the environmental situation of rivers. The JDS3 approach was dealing with the following individual **research topics** on the longitudinal survey:

- Bacterial Faecal Indicators
- Microbial Faecal Source Tracking
- Spread of non-wild type antibiotic resistant phenotypes in the Danube River
- Microbial Ecology
- Microbial Metagenomics

Such a wide scope approach is described in the following ICPDR Preparatory Paper JDS4 Microbiology Program (Third draft version April 26, 2018, enclosed as **Annex VIII**): JDS4 **Microbiology Program** (JDS4 Survey Plan Annex F). It has to be noticed that this document- similarly to all other Survey Plans - is not a final version.

*Hydromorphology*

The first hydromorphological standard (CEN EN 14614:2004) has been revised by CEN/TC230/VG 25. As a result, the new hydromorphological methodology is under preparation. The hydromorphological survey on the Tisza River should include collection of background hydromorphological data for each station. A detailed hydromorphological characterisation of each JTS2 site is important to support the interpretation of biological results.

The description and evaluation of hydromorphological characteristics (i.e. physical characteristics of a water body's shape, boundaries and content) for large rivers is strongly dependent on various background data such as historical, topographical and navigation maps; satellite images; and hydrologic, morphometric (i.e. quantitative analysis of form) and land use data. The JDS3 approach recently had included a number of different studies such as:

- Continuous longitudinal survey of stretches 10 river km-long; an inventory of dams and continuum interruptions; bathymetrical (i.e. measurement of the depth of bodies of water) data to understand width and depth variability and channel incision; degree of degradation of channels and banks; gravel and sand bar occurrence and shape; data on harbours and daily traffic density (e.g. wave surge impacts); and possibly some ornithological work such as the occurrence of bird colonies adapted to open gravel and sand bars.
- Detailed hydromorphological characterizations of each JDS3 site.
- Sediment characterization, by collecting river bed material at each sampling site.
- Flow velocity and discharge measurements at selected sites.
- Suspended sediment measurements.
- Water level slope and fluctuation data. Water level slope helps understand channel forming processes (e.g. erosion, deposition) essential for habitat diversity, and can show changes in flow on rivers that have been modified. Water level fluctuation shows change in discharge and flow and can help document the effects of hydropeaking (where hydropower stations store as much water as possible before releasing it to create peak energy surges).

## 2.3.3 Determinands

### 2.3.3.1 General physico-chemical variables

Nutrient pollution, particularly by nitrogen (N) and phosphorus (P), can cause eutrophication-- an enrichment of water causing an accelerated growth of algae and higher forms of plant life that produce an undesirable disturbance to the balance of organisms present in the water and to the quality of the water concerned. N and P emissions cause eutrophication in many surface waters of the Danube River Basin and contribute to eutrophication in the Black Sea north western shelf.
As with organic pollution, nutrient pollution is mainly caused by emissions from agglomerations (cities and towns), industry and agriculture. Atmospheric deposition is also significant. Many industrial facilities, especially in the chemical sector, are significant sources. Nutrient pollution results from point sources and diffuse sources.
On-board analyses will include in-situ temperature, pH, conductivity, dissolved oxygen. External laboratory analyses will include: *Nitrogen forms, Phosphorus forms, Hardness (Calcium, Magnesium), Suspended solids in water, Dissolved organic carbon (DOC) and Total organic carbon (TOC) in water, BOD5, COD.*

### 2.3.3.2 Other chemical variables:

The document 'JDS4 Preparatory paper ANNEX D' presented in DANUBIS (the ICPDR Information System) summarises the following main issues (**Annex II** in this Manual):

The assessment of the “chemical status” of water bodies is based on compliance with environmental quality standards (EQS) defined for 45 priority substances (in some cases groups of substances) and for 5 additional substances/groups of substances originally selected according to Directive 74/464 (see directive 2013/39/EU).

As a result of the prioritisation process for the continuous update of the list of priority substances 17 substances are listed in a “Watch list” published by Commission decision 2015/495 of 20 March 2015 (notified under document C(2015) 1756).

River specific substances and their EQS may be defined by EU member states on the national level. The results of these substances contribute to the assessment of the “ecological status”.

The selection of chemical parameters for JDS4 should take into regard

- the present list of priority pollutants according to directive 2013/39/EU
- watch-list substances
- the draft list of Danube River Basin Specific Pollutants elaborated within the EU-project SOLUTIONS
- national lists of river basin specific pollutants
- LC-HRMS-screening and GC-HRMS-screening as new tools of water monitoring
- emerging pollutants based on previous results, national lists/activities and results, Europe-wide investigations run by JRC/Ispra, literature etc.
- requirements of the WFD concerning ecological status (determination of physico-chemical parameters)

The parameter selection for the longitudinal Tisza Survey has to be based on the results of previous surveys (JDS1 - JDS3) but the preparatory actions of the future JDS4 should be taken into consideration, as well (See **Annex II**: Proposal for chemical analysis (JDS4 Survey Plan ANNEX D).

Therefore a very detailed analysis is needed to evaluate which organic chemical components should really taken into consideration during the longitudinal Tisza Survey. The number of compounds is very large, the cost consequences are very serious. The available lessons learned from the previous JDS missions - particularly of JDS3 - about the whole Danube Basin can provide a solid base for a comprehensive evaluation concerning the most relevant organic compounds to be studied in the Tisza Basin. The preparation of the Joint Tisza Survey Manual requires a particularly careful planning carried out by chemical experts, on one hand to avoid the collection of "useless" data and, on the other hand to find the optimal way of the information collection.

The following organic and inorganic chemical compounds are listed in the WFD compliant analysis:

*Organic micropollutants*
PolyAromatic Hydrocarbons (PAHs); Pesticides; Short Chained Chlorinated Paraffins (SCCP); Volatile Compounds (VOCs); Brominated diphenylethers (BDEs); Industrial pollutants; Tributyltin compounds; Newcomers; Organochlorine compounds
*Inorganic micropollutants*
Metals (primarily heavy metals)

Additional compound is the group of Microplastics that will be investigated in the water phase, similarly to the JDS4 program in 2019 (see two enclosed documents: **Annex VI**: Concept-Paper for a JDS 4 - Plastic monitoring action in the Danube River (JDS4 Survey Plan Annex E and **Annex VII** Guideline for Sampling and preparation of Suspended Particulate Matter - Standard Operation Procedure (SOP), *Draft version 1.0 (2018-07-19)*).

These investigated groups of polluting materials are all presented in the lists of Determinands (together with their costs of analyses) in the **Annex I Tables:** referring to *Water PS* (**Table 3**), *Biota PS* (**Table 4**), *Sediment PS* (**Table 5**) and the *River Basin Specific Substances* (**Table 6**). All of these information are taken into account when summarising *unit costs* and *total cost* of the expertise and JTS2 samples/types given in **Table 8**.The price list of the Environmental Institute (Slovakia) - who participated in all of the previous Joint Danube Surveys - was used for calculating the costs of the analytical items.

Altogether 10 different groups of pollutants for *Water* (PAHs, Metals, Pesticides, SCCPs, VOCs, Industrial pollutants, TBTs and Newcomers), 8 groups for *BIOTA* (PAHs, Metals, BDEs, Industrial pollutants, VOCs, Newcomers and Dioxins), and 9 groups for *Sediment* (PAHs, Metals, Pesticides, BDEs, SCCPs, Industrial pollutants, TBTs, Newcomers and Organochlorine

compounds) are sorted for the analysis as the **WFD Priority Substances** regulated by the Directive 2008/105/EC and Directive 2013/39/EU (**Table 3, 4, 5,** and **ANNEX III**).

The **Annex I Table 8** summarises that the total cost of the biological samples and the HYMO work is 80 000 €. The cost of the analytical work is 84 550 **€**. The total cost of the work is 164 750 €. The number of biological and chemical samples in different sites and Matrices is shown also in **Table 8**. It should be noted that the real cost value will be finalised in case of the discussion and decision of the Tisza Expert Group (involvement of the SPM sampling and analysis, use of ship, accommodation, etc.).

## 2.4 Cruise Manual/Sampling methods

### 2.4.1 Phytoplankton

Quantitative samples (200 ml) of Phytoplankton has to be taken from the middle of the river from the surface water. Secchi-depth has to be measured at each point. Chlorophyll-a concentration is measured in laboratory with spectrometric determination according to standard ISO 10260:1992. For extraction we used 90% ethanol, and UNICAM UV4 for spectrometry. The phytoplankton sample is fixed with acetic acid Lugol solution in labelled brown screw cap glass bottles and stored in a cool dry place until examination (Utermöhl, 1958, Hillebrand et al., 1999). The samples were counted on Leica DMIL inverted microscope applying the Utermöhl's technique (EN 15204:2006, Borics et al., 2015). 400 individuals (cell, coenobium or filament) should be counted in 3 or more random transects. The individuals have to be identified mostly on species or genus taxonomical level, and finally the number of individuals (in ind/ml) is calculated.

#### 2.4.1.1 References

BORICS, G., KISS, K.T., 2015. Módszertani útmutató a Fitoplankton élőlénycsoport VKI szerinti gyűjtéséhez és feldolgozásához, MTA Ökológiai Kutatóközpont

HILLEBRAND, H., DÜRSELEN, C.-D., KIRSCHTEL, D., POLLINGHER, U. AND ZOHARY, T., 1999.Biovolume calculation for pelagic and benthic microalgae. Journal Phycology 35, 403-424.

UTERMÖHL, H., 1958. Zur Vervollkommnung der quantitativen Phytoplankton Methodik. Mitteilungen Internationale Vereinigung Limnologie 9, 1-38.

VANNOTE R.L., MINSHALL G.W., CUMMINS K. W., SEDELL J.R. & GUSHING (1980).The river continuum concept. Can. J. Fish. Aquat. Sci. 37: 130–137.

ISO 10260:1992 Water quality. Measurement of biochemical parameters. Spectrometric determination of the chlorophyll-aconcentration.

EN 15204:2006 Water quality. Guidance standard on the enumeration of phytoplankton using invertedmicroscopy (Utermöhl-technique).

### 2.4.2 Phytobenthos

For phytobenthos sampling, a river segment (usually up to 50 m long) with a suitable substrate (preferably stones or other solid substrates) has to be chosen at each sampling site. Diatom sampling followed instruction of the EN 14407:2014 and Ács et al, 2015. In principle, at least five stones occurring in the current (if possible) and euphotic zone down to 1 m of depth (preferably cobbles with a diameter between 64 to 256 mm) are used for sampling. Where hard substrata are absent, epiphyton was sampled following the EN 14407:2014 and Ács et al, 2015. After the sampling a minimum area of 10 $cm^2$is brushed thoroughly from each stone (as much concentrated as possible) into two containers (for diatoms and non-diatoms analyses) and labelled. Samples for non-diatoms analyses are

refrigerated and analysed alive on-board. If any macroscopic algae are available at site (e.g. Cladophora, Hydrodiction), a separate subsample has to be taken for determination.

Diatom samples are further treated by hot hydrogen peroxide method to obtain clean frustule suspensions. Finally, the oxidised samples are rinsed with deionised water by decantation of the suspension several times, and permanent slides are mounted with Naphrax. The microscopic analysis has to be performed using light microscopy with a Zeiss scope A1 (Axio) microscope with 100x oil immersion objective (1000x magnification). On average, 400 valves are counted on each slide in random transects. All taxa have to be identified to the lowest taxonomical level possible. A list of taxa data is made from each slide and the counts are used to calculate species relative abundance (in %). The microscopic analysis of non-diatom community is performed using light microscopy at 400 x – 1000 x magnification. The taxa identified are quantified on the scale 1 – 5 (1: rare, 5: dominant).

#### 2.4.2.1 References

ÁCS, É., BORICS, G., KISS, K.T., VÁRBÍRÓ, G. (2015): Módszertani útmutató a fitobentosz élőlénycsoport VKI szerinti gyűjtéséhez, feldolgozásához és kiértékeléséhez, MTA Ökológiai Kutatóközpont

LAMBERTI G. A. (1996). The role of periphyton in benthic food webs. In: Algal Ecology: Freshwater Benthic

Ecosystems (Eds R.J. Stevenson, M.L. Bothwell & R.L. Lowe), Academic Press, San Diego, CA. pp. 533–573.

MINSHALL G.W. (1978). Autotrophy in stream ecosystems .BioScience, 28: 767–771.

EN 14407:2014 Water quality. Guidance for the identification and enumeration of benthic diatom samples from rivers and lakes.

EN 15708:2009 Water quality. Guidance standard for the surveying, sampling and laboratory analysis of phytobenthos in shallow running water.

### 2.4.3 Macroinvertebrates

The group of benthic macroinvertebrates is one biological quality element used within the framework of the European Water Framework Directive (2000/60/EC; WFD) to assess the ecological water quality and were therefore monitored in all previously conducted Joint Danube Surveys (JDS). The JDS experiences are taken for the JTS2 longitudinal survey to be followed. The methods applied were differing due to availability of devices, financial issues and the scientific focus. While in JDS 1 grabs were used to investigate hard rocky substrates (LITERÁTHY et al., 2002), in JDS 2 air-lift samples were taken to study the faunal composition of deep water habitats (LIŠKA et al., 2008). During JDS 3 a modified Multi-Habitat-Sampling (MHS) approach has been performed to highlight the importance of specific micro-habitats in terms of biodiversity and additionally as a sound basis for river restoration efforts and water management issues in general. The data gained from JDS 3 can be seen as an important documentation of the current distribution of specific taxa and a completion regarding faunistics of earlier studies, (RUSSEV, 1998; SLOBODNIK et al., 2005; CSÁNYI & PAUNOVIC,2006) and of all previous JDS excursions. The results will significantly contribute to the currently ongoing discussions regarding the WFD compliant assessment methods of large rivers either for field work as well as the analysing aspects.

Sampling of benthic macroinvertebrates for JDS3 had three approaches carried out by three separate sampling groups:

- Sampling of different habitats (Habitat Sampling, HS) in the actual littoral zone with a Multi-Habitat-Sampling (MHS) net (BOKU);
- Cross-sectional survey by dredging in the deep water area - Deep Water Sampling, DWS (Laboratory of MTA (Hung. Acad. Sci.), Centre for Ecological Research, Danube Research Institute); and
- Kick and Sweep sampling of shore region (Siniša Stanković, University of Belgrade (IBISS)).

The sampling areas were surveyed by motorboat. Depending on the number of different habitats, the numbers of sampling units per site varied. Tributaries and side-arms were not sampled.

#### 2.4.3.1 Habitat specific sampling

The habitat specific macroinvertebrate sampling at the littoral zone was done with a Multi-Habitat-Sampling (MHS) net with a frame of 25 x 25 cm following the AQEM-STAR methodology (2002). This semi-quantitative instrument provides a sampling area of 0.0625 m² per sampling unit and is positioned upstream in the riverbed whereas the sediment in front of the frame is stirred up so that the animals are drifting into the collecting net with a mesh size of 500 µm and minimum lengths of 1 m. This method can be applied for wadable zones up to a maximum water depth of 1.5 m.

#### 2.4.3.2 Kick and sweep

The Kick & Sweep (K&S) sampling technique (EN 27828:1994) was used in the shore region up to 2.0 m water depth. Sampling was done using a hand net with 500µm mesh size, following multihabitat procedure. Free diving material collection up to 4 m water depth was additionally employed primarily in order to collect supplementary data on freshwater mussels.

#### 2.4.3.3 Dredging

Dredging was carried out with the help of the motor boat of ARGUS. The iron-forked mouth of the triangle shaped dredge had a collecting net with 500 µm mesh size (Figure 2). Pulling the dredge was carried out with the help of a rope. The upstream located boat was used backwards; so that the dredging was done from the frontal part of the boat. The dredging speed of the sampler on the bottom had to exceed the actual current velocity in order to avoid the washing out of the material from the net. The first 2 m of the pulling device was a heavy iron chain in order to keep the dredge horizontal on the bottom during the dredging. We tried to keep the degree of the rope less than 25 ° during the procedure because this orientation made the dredge capable to dig in the bottom material efficiently.

Dredging locality was marked with GPS device, water depth was measured by hydro-acoustic equipment. The dredged material was put into buckets marked with serial numbers I-V (Number I is near to right bank, II is far from right, III is in the middle, IV is far from left, V is near to left) Photos were taken to illustrate grain size distributions of the sample.

*Figure 2: Bottom dredge with chain and rope for macroinvertebrate sampling*

#### 2.4.3.4 Sorting and Identification

The macroinvertebrate samples collected from a defined habitat should be stored separately for further determination in the laboratory. The material of each sample has to be sorted completely. The animals are counted, separated into their specific orders and determined by taxonomic experts to the best level possible.

The samples collected by dredging (cross sectional sampling) and K&S could be partially processed on field. Reducing of sample volume is done by washing and removing large particles from sample and rising through hand net (mesh size 500 µm). Material is preserved with 4% formaldehyde.

### 2.4.4 Fish

#### 2.4.4.1 Littoral electric fishing

The sampling acts on the basis of the EU Water Framework Directive and the European Standard "Water Analysis – Fishing with Electricity (EN 14011; CEN, 2003) for wadable and non-wadable rivers.

The method that will be used to investigate the composition of the fish fauna on the different sites along the Danube is electric fishing, which is the most used method worldwide to sample fish in smaller rivers or shallow waters. A generator (in a boat) establishes an electric field in the water between the fixed cathode and the mobile anode. This electric field attracts and stuns the fish, so they can be collected with a net. Under normal conditions this method does not harm or damage the fish that recover very fast. However, especially with juvenile fish casualties can happen, but in general electric fishing is a non-lethal sampling method. The method is also CEN-standardized and is recommended for use as the basic sampling methods for WFD purposes. The standardised sampling procedure for each site follows the habitat specific approach (strip fishing method, Wiesner et al. 2007).

#### 2.4.4.2 Electrified benthic frame trawl

Deep water regions could be fished by the electrified benthic trawl worked out by the VITUKI Hydrobiological Department with the cooperation of DINPI and ÖK experts The electrified benthic frame trawl consists of a stainless steel frame (2 m long × 1 m high, 3,4 cm tube diameter) with a drift net attached. The drift net was 5 m long and consisted of an inner mesh bag with 5 mm mesh size and an outer mesh bag with 8 mm mesh size. Weighted metal wheels were attached to the frame to keep

the device close to the bottom and to keep the frame 6 cm above the bottom thus preventing the net from filling with substrate material. The frame was electrified with a 40 m long electrode cable which was connected to a Hans-Grassl EL65 II GI electrofishing device operated by a VANGUARD HP21 14.9 kW generator. A 6 m long copper cathode cable was attached to the tow rope and was hanging freely approximately 2 m before the electrified frame (**Hiba! A hivatkozási forrás nem található.**). Fishing (hereafter called trawling) was conducted along the flow direction with a 6,3 m long boat powered by a 50 hp outboard Mercury four stroke engine. The main sampling team consisted of three people, two handled the trawl net and the electrofishing device and one operated the boat. Occasionally an additional person, usually from a national fishing team, assisted the crew during sampling. The sampled stretches were measured by a GPS right after the trawl reached the bottom and the electroshocking started. The direct current (approx. 350 V, 33 A) was given for 5-8 sec. with 3-5 sec. breaks to minimize fright bias and injury of fish. Each time the electroshocking restarted, water depth was measured by a LOWRANCE X-50DS fishfinder. The applied trawling speed was slightly higher than the current velocity of the river (approx. 60 cm $sec.^{-1}$). The length of trawling stretches was usually 500 m. Sometimes the trawl got stuck due to large rocks or logs on the bottom thus shorter trawling stretches also occurred. Trawling was carried out only during daytime.

#### 2.4.4.3 Data assessment

Using JTS2 fish data the national fish indices should be applied for the calculation of ecological status. The Fish Index of Slovakia (FIS), the Hungarian Fish Index could be used for this purpose as WFD compliant methods. The European Fish Index (EFI) does not work well for the Lower Danube. The harmonization of different methods is challenging in case of the Tisza - particularly in the Danube Basin - because there are serious needs for a general Danubian assessment method that does not exist until now.

#### 2.4.4.4 References

Bammer V (2018) JDS4 BQE Fish – sampling method and assessment. JDS4 Prep Paper ANNEX B5 fish, ICPDR https://danubis.icpdr.org/document/18587

EN 14011:2003 Water quality — Sampling of fish with electricity.

Szalóky Z, György ÁI, Tóth B, Sevcsik A, Specziár A, Csányi B, Szekeres J, Erős T (2014)Application of an electrified benthic frame trawl for sampling fish in a very large European river (the Danube River) – Is offshore monitoring necessary? FISHERIES RESEARCH 151: pp. 12-19.

Wiesner, C., Schotzko, N., Cerny, J., Guti, G., Davideanu, G., Jepsen, N., (2007): Technical Report with Results from the Fish Sampling and Analyses from the Joint Danube Survey 2007. International Commission for the Protection of the Danube River, Vienna, 73 pp.

### 2.4.5 Invasive Alien Species (IAS)

Invasive Alien Species (IAS) have become global policy agenda in the last ten years around Europe. In the case of the DRB, the International Commission for the Protection of the Danube River (ICPDR) acts as the coordinating body for multilateral and basin-wide actions related to water management, that involve the resolving issue of the IAS. During the JTS2 it is very important to follow the same methodology concerning data collection and assessment of the IAS topic within the Tisza Basin. **Annex X** contains the details of the following ICPDR document that is providing the methodology for the JDS4 program:

(See: https://danubis.icpdr.org/document/17990)

#### 2.4.5.1 References

AQEM & STAR Site Protocol (2002): www.eu-star.at. Protocols.

EN 16150:2012(E), Water quality — Guidance on pro-rata Multi-Habitat sampling of benthic macro-invertebrates from wadable rivers.

https://danubis.icpdr.org/document/17990

ISO 10870:2012(E) Water quality — Guidelines for the selection of sampling methods and devices for benthic macroinvertebrates in fresh water.

Graf W., B. Csányi, B. Leitner, M. Paunovic, G. Chiriac, I.Stubauer, T. Ofenböck and F. Wagner 2008.Macroinvertebrates.In: Liška I., F. Wagner, J. Slobodník (Eds.):Joint Danube Survey. Final Scientific Report.41-53;ICPDR – International Commission for the Protection of the Danube River, Wien.

Hellawell, J. M. (1978): Biological surveillance of rivers. Water Research Centre, 344 p.

Liška, I., Wagner, F. & J. Slobodník (2008): Joint Danube Survey 2. Final Scientific Report. ICPDR – International Commission for the Protection of the Danube River. Wien.

LITERÁTHY, P., KOLLER-KREIMEL, V. & I. LISKA (2002): Joint Danube Survey.- Technical Report of the International Commission for the Protection of the Danube River, 261 pp.

Várbíró, G., Boda, P., Csányi B., Szekeres J. (2015): Makroszkopikus vízi gerinctelenek élőlénycsoport VKI szerinti gyűjtéséhez és feldolgozásához [Methodological guide for the collection and processing of macroscopic aquatic invertebrates according to the WFD], MTA Ökológiai Kutatóközpont.
http://www.kornyezetvedok.hu/vgt/vgt2/orszagos/6_1_hatteranyag_Makrozoobentosz_Modszertani_utmutatoVGT2.pdf

### 2.4.6 General physico-chemical parameters

Water samples will be collected directly from the river with the help of motor-boat. In-situ measurements (temperature, dissolved oxygen, pH and conductivity) will be carried out by portable instrument with dedicated probes, in three profiles of the river (left, middle and right), based on international standardised methods.

Nutrients forms and basic ions will be analysed in water samples by selected laboratories according to EN ISO standardised methods based on molecular spectrophotometry (total forms of N and P).

Quantitative characterization of organic compounds found in natural waters is used to determine the chemical oxygen demand (COD). Potassium permanganate or potassium dichromate is used as the oxidizing agent for the measurement. Another important parameter is the biochemical oxygen demand (BOD), which determines the amount of oxygen consumed during the bacterial breakdown of biodegradable substances. The essence of the applied method is to measure the amount of required dissolved oxygen under specified conditions (temperature, broth composition), usually 5 days of oxygen consumption.

### 2.4.7 Metals

Water samples will be collected directly from the river with the help of motor-boat. Samples are stored in PE-bottles after acidification with nitric acid to a pH <2. Mercury samples are stabilized with

potassium dichromate and stored in borosilicate glass bottles. In practice, the dissolved fraction of a substance in water is defined as the fraction that passes a 0.45 µm filter.

Cadmium (Cd), Lead (Pb), Nickel (Ni), Aluminium (Al), Iron (Fe), Copper (Cu), Zinc (Zn), Chromium (Cr) and Arsenic (As) are determined by use of AAS (FAAS, AAS-ETA; required air-acetylene flame or argon gas, metal free water, standard metal solutions) according to ISO 1484-3:2006 or by use of ICP-MS according to ISO 17294-2 for. Mercury (Hg) is analysed by AFS following EN ISO 17852:2008.

The investigated elements are categorised into two groups as follows:

- Group 1: Heavy metals included in the Priority List of the Water Framework Directive (WFD): Cadmium (Cd), Mercury (Hg) Nickel (Ni) and Lead (Pb);
- Group 2: Other heavy metals and metalloids: Arsenic (As), Chromium (Cr), Copper (Cu), Aluminium (Al) and Zinc (Zn).

### 2.4.8 Microplastics

Plastics are an emerging environmental concern. Studies from the marine environments dramatically show the increase of plastic pollution in the seas. Many scientific studies quote freshwater systems (rivers) as major pathway of plastics into the marine environment. Studies from fresh waters are existing but the used methods are not harmonised. So far it is not possible to compare such data. Plastics barely degrades in the environment, but alters and brakes into small pieces of micro-plastics.

Until present there is no exact information about plastic loads in the Danube River Basin. However, results from studies on other European rivers show that plastics are ubiquitous in freshwater systems. It is therefore important to evaluate the load of rivers to characterize riverine inputs into the marine environments and to close the knowledge gap of pathways, sinks and fragmentation and impact of plastics in freshwater environments. The scientific program of the JDS4 will contain a detailed survey on this topic. Therefore the Concept paper of the microplastics are enclosed to the Manual in **Annex VII**: **Guideline for Sampling and preparation of Suspended Particulate Matter - Standard Operation Procedure (SOP) - ICPDR Document for the JDS4.** In this initial phase of the research only the contribution of the suspended particulate phase is investigated, the microplastics embedded in the sediment phase and entered in the digastrics system of aquatic organisms will be an issue for future studies after having enough experiences of the status of the art.

#### 2.4.8.1 References

https://danubis.icpdr.org/document/18886

### 2.4.9 Priority Substances

**Annex III** contains the list of the WFD Priority substances taken into consideration for the JDS4 sampling program according to the present scenario. The document is not a final version at the moment. (See: **JDS4 - Proposal for the analysis of chemical parameters (draft 6, 20.04.2018) Annex III – Priority substances**

https://danubis.icpdr.org/document/18592

### 2.4.10Watch list substances

See: JDS4 - Proposal for the analysis of chemical parameters
**Annex IV – Watch list substances**
https://danubis.icpdr.org/document/18593

### 2.4.11River Basin Specific Pollutants

See: JDS4 - Proposal for the analysis of chemical parameters
**Annex V – Danube River Basin Specific Pollutants (draft)**

https://danubis.icpdr.org/document/18594

### 2.4.12BIOTA samples

Fish and/or mussel samples should been taken during JTS2 at 10 sites for the analysis of specific biomarkers, heavy metals and isotopes. Omnivorous benthic/pelagic (*Abramis brama, Alburnus alburnus*)and carnivorous benthic species (*Neogobius melanostomus, Neogobius kessleri*) would be ideal sort of fish whereas the filtering large Unionidae together with either the *Dreissena polymorpha* or *Corbicula fluminea* mussels are appropriate Biota species for analysis, as well.

3-5 adults have to be collected at 10 selected sites, the specimens have to be stored in deep freezer until the chemical analysis.

## 2.5 JTS2 Core Team

The number of Core Team should be kept in moderate size due to the fact that probably the size of the ship for sampling will be moderate, as well. The most detailed fieldwork is connected to the sampling of benthic macroinvertebrates. Therefore it is not necessary to represent all biological and chemical elements on board. The ideal solution would be to deal with more than one BQE sampling by one expert, similarly to the JDS missions where benthic invertebrate experts were able to deal with the following sampling subjects (including driving the motorboat):

1/. Benthic invertebrates; 2/. all kinds of chemical samples; 3/. sediment sampling; 4/. microbiological sampling; 5/. zooplankton sampling.

Biologists could help the work of chemists in many terms (sample preparation, labelling, preparation for transport) and *vica versa*. This way the Core Team should consists of the following experts:

*Coordinating Experts*:

1. Project Manager - not present on board
2. Technical coordinator - Responsible for organizing the Survey - not present on board

*On-board experts (minimum one expert/subject, maximum two experts/subject)*:

- Core Team Leader- preferably having wide expert scope concerning more than one BQE
- Deputy Core Team Leader - preferably having wide expert scope concerning more than one BQE
- Chemical expert 1 - responsible for sample labelling, treatment, preparation of sample transport

- Chemical expert 2 - responsible for sample labelling, treatment, preparation of sample transport
- Biology expert 1 - responsible for sample labelling, treatment, preparation of sample transport
- Biology expert 2 - responsible for sample labelling, treatment, preparation of sample transport
- Microbiologis 1 - responsible for sampling, sample treatment and processing
- Microbiologist 2 - responsible for sampling, sample treatment and processing
- Hydromorphologist 1 - on-site measurements at each cross section
- Hydromorphologist 2 - on-site measurements at each cross section

Dealing with the hydromorphological sampling and the field measurement there were three experts on the JDS3 to carry out the field work. Depending on the exact preparation of the plan for the Hydromorphology subject it could be decided to follow the same way or not. In this document **two** persons are added to the Core Team.

Beside of these persons it would be beneficial to have another small team of two experts plus one driver who could deal with the mutual sampling carried out by car on a terrestrial mission, parallel to the shipping expedition. This would result in serious time saving during the survey. These experts should carry out the BQE sampling and the collection the Chemical samples using rubber boat on the Upper Tisza section, if necessary (this kind of additional sample team was used during the first Joint Tisza Survey, too).

The independently working Fish Team would have at least **three experts** who are able to deal with both littoral and deep water electrofishing.

That means that the JTS2 Core Team could consist either 2 + 10 (6+4) and 3 experts working separately as fish experts - **altogether 11 or 15 persons.** An additional team could be organized for saving time on the upper stretch: plus **three experts** are needed for the execution of the separate car expedition. In ideal case **altogether 14 or 18 persons could perform well in the JTS2**.

## 2.6 JTS2 National Teams

The National Teams could join to the JTS2 Core Team at any Tisza sections. Their number should not be limited because one purpose of the basin wide longitudinal survey is training and scientific cooperation.

The cost consequences are serious, of course. In case of the previous surveys organized by the ICPDR the working time of the Core Team members were paid by their own countries as their "in kind" contribution. Only daily allowance was paid for them and their total accommodation and food were covered by the central budget.

The financial support needed for the realization of the JTS2 should be discussed by the Tisza Group countries and the ICPDR.

## 2.7 Logistics

The necessary logistical arrangement for the cruising and sampling action, the sample treatment, storage and transport, accommodation of the Core Team and National Team members together with

the involvement of Laboratories should be taken into consideration. This subject concerning the previous JDS missions was primarily completed by the Project Manager and the Technical coordinator, together with the assistance of the Monitoring and Assessment Working Group (MA WG) of the ICPDR. The preparatory work lasted for several years. The Cruise Manual can be finalised only after expert discussions and agreement together with the starting date of the survey. This chapter cannot deal with these specific organizations in details, only some direct problems related to the practical execution of the survey are discussed here.

Several refrigerators and deep freezers have to be placed on the board of the ship. To provide the appropriate cooling and freezing capacity was always very crucial during the previous programs. Therefore special care should be taken to this question during the planning.

Another crucial point is the perfect labelling of samples. The appropriate labelling could be organized prior to the survey, similarly to the preparation of the JDS4 for 2019.

Concerning logistics the regular sample transport is also important. To provide transport after each three days seems to be sufficient to handle the samples correctly. It should be noted that prior to the transport there are many preparation work necessary in order to provide sufficient care and safe treatment of samples. Vessels, samples should be collected from several refrigerators and freezers that was always very complicated work needing special care. Bad weather conditions often can made the whole action to be very difficult (rain, darkness, strong wind, etc.) as it was frequently experienced during the former JDS missions.

Several logistic problems will came up during the survey simultaneously that cannot be foreseen, primarily concerning the actual field work. Usually these problems could be handled well if the sampling team has special experiences.

### 2.7.1 Travel on ship (river-cruise) and land (car)

It can be shown that the sampling program by car will happen approximately along a 400 km Upper Tisza stretch and the navigation program covers the 600 km Middle and Lower Tisza. According to the original idea the sampling action happens to downstream direction in order to follow the water flow. It should be mentioned that the three JDS and the AquaTerra mission followed the downstream direction sampling but the reason was not to follow the water flow. During the first Joint Tisza Survey (2001) the direction of navigation and sampling was upstream. Generally it can be summarized that either upstream or downstream direction of sample collection is equally accepted in case if no serious water discharge happens during the program. The longitudinal survey have to be planned for a hydrologically "quiet" period, anyway.

In this Time Schedule chapter a summary is given about the duration and time requirement of the suggested program. Based on this calculation the following information is the most essential :

*Table 2. Number samples covered and days in case of the **suggested program** (33 sampling sites)*

| **Method of moving during sampling** | **Number of sites** | **Days required** |
|---|---|---|
| Travelling by car, sampling by rubber boat | 10 | 3 |
| Travelling by ship, sampling by motorboat | 23 | 9 |
| **Total** | 33 | **12** |

This way the average site sampled per day value is less than **3 site/day (Annex Table 2)**. It has to be noted that the required time could be even less if simultaneous sampling is organized, i.e. another sampler team is formed that could work independently the shipping team. **The application of the Scenario B is suggested in order to optimize the financial resources and human effort during the longitudinal Tisza survey.**

### 2.7.2 Sample storage and transport

Samples are stored generally in refrigerators, Biota samples should be kept in deep freezers.

The first transport of samples could be organized after completing the third day program at Tiszakóród. If the central laboratory is nominated in Szolnok, the necessary distance to be taken is approximately 600 km to Tiszakóród and back (the necessary kms are calculated roughly). The second transport is taken from Tiszapalkonya and the 400 km is needed for that. The third round of sample transport is taken from Csongrád (200 km) and the fourth one from Titel (700).

Two persons are necessary for the transport execution in order to help each other not only in driving the car but handling the samples - if necessary - and particularly during border crossing.

### 2.7.3 Laboratories, services

The following Hungarian accredited Laboratories could participate in the sample collection, storage and analysis*:*

Hajdú-Bihar Megyei Kormányhivatal Népegészségügyi Főosztály Laboratóriumi Osztály Környezetvédelmi Mérőközpont (Debrecen)
KÖTIVIZIG Regional Laboratory (Szolnok)
Csongrád Megyei Kormányhivatal Népegészségügyi és Élelmiszerlánc-biztonsági Főosztály Laboratóriumi Osztály (Szeged)
Bálint Analitika Kft. (Budapest)

## 2.8 Database

The ICPDR has an Information System called DANUBIS that contains all of those data that were collected during the JDS1, JDS2, JDS3 and the Investigation of the Tisza River and its tributaries (ITR or JTS1). It is evident that the results of the longitudinal Joint Tisza Survey (JTS2) should be centrally stored in the DANUBIS and in the updated Danube GIS.

## 2.9 Assessment of results

### 2.9.1 Technical (Scientific) Report

A detailed technical reference will be compiled using the standard ICPDR JDS reporting format for all variables and determinands related to JTS2. The detailed time schedule will be included as well. Furthermore, all methods for sampling, pre-treatment, preservation, storage, analysing, etc. shall be referenced/documented.

### 2.9.2 Public Report

A summary report for public will be compiled based on the technical report and will provide key highlights of JIS2 findings together with an explanation of the topics and results. Public report should give emphasis on the visual information provided to the reader and should be written in an easy-to-read and attractive manner.

### 2.9.3 Public awareness

Tisza has special concern for the public in many countries. The sad story of the cyanide spill and the subsequent heavy metal containing sludge pollution happened in 2000 January-March was widely known. After the pollution event the United Nations sent here an International Expert Group to try helping the Hungarian Authorities in the follow up activities. There was a very active communication about the subsequent events, remedial actions and follow up research on the Tisza to inform the society about the results. Tisza River became a well known river even in the USA where several experts came to Hungary for studying on-site the consequences of the pollution. Therefore such an international Joint Survey on the Tisza River most probably will create a big interest around Europe similarly to the case of the Joint Danube Surveys. People are interested in the environmental situation of the Tisza and its water shed. The appropriate way of spreading the information concerning the actual status of the Survey is crucial. The active involvement of stakeholders and civil society will be important at the beginning of the execution of the JTS2.

# 3 References

Csányi, B., 2002: Joint Danube Survey: Investigation of the Tisza River and its tributaries. Final Report of the ICPDR/VITUKI, Budapest, 1-135.

EU, 2000. Directive 2000/60/EC of the European parliament and of the council of 23 October 2000 establishing a framework for community action in the field of water policy. Off. J. Eur. Union L327, 1–72.

EU, 2013. Directive 2013/39/EU of the European Parlament and of the Council of 12 August 2013 amending directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. Off. J. Eur. Union L226, 1–17.

https://danubis.icpdr.org/tag/1835

JDS4_Survey_Plan_ANNEX_B6_-_Zooplankton.doc

181119_JDS4_proposal_for_chemical_analysis_draft7.doc

https://danubis.icpdr.org/document/18886: JDS4 - Proposal for the analysis of chemical parameters (draft 7, 14.11.2018) - Annex D1 – List of WFD Priority substances
JDS4_Survey_Plan_ANNEX_D1.docx
https://danubis.icpdr.org/document/18593: JDS4 - Proposal for the analysis of chemical parameters – Watch list substances

JDS4_Survey_Plan_ANNEX_E_-_Concept_paper_microplastics.doc

JDS4_Survey_Plan_ANNEX_E1_-_SOP-Danube_Microplastic_Sampling_JDS_04_v1.docx

JDS4_Survey_Plan_ANNEX_F_Microbiology_Program-Draft_20180510.docx

Graf, W., Csányi, B., Leitner, B., Paunovic, M., Chiriac, G., Stubauer, I., Ofenböck, T. & F. Wagner (2008). Macroinvertebrates. In: Liška,I.,Wagner, F., Slobodník, J. (Eds.): 2nd Joint Danube Survey. Final Scientific Report, ICPDR – International Commission for the Protection of the Danube River, Wien, pp. 41-53.

Liška, I., Wagner, F., Slobodník, J. (2008): 2nd Joint Danube Survey. Final Scientific Report, ICPDR – International Commission for the Protection of the Danube River, Wien, p. 242.

Liška ,I., Wagner, F., Sengl M., Deutsch K., Slobodník, J. (2015): Joint Danube Survey 3. A Comprehensive analysis of Danube Water Quality. Final Scientific Report, ICPDR – International Commission for the Protection of the Danube River, Wien, p. 369.

Literáthy P., Koller-Kreimel V., Liška I. (2002) Final Report of the Joint Danube Survey. ICPDR – International Commission for the Protection of the Danube River, Wien, p. 261.

Slobodník, J., Hamchevichi, C., Liška, I., Shearman, A., Csányi, B., Makovinská, J., Paunović, M., Tóthová, L., Stahlschmidt-Allner, P., Allner, B. (2005). Final report on sampling, chemical analysis and ecotoxicological studies - . Project no. 505428 (GOCE), AquaTerra - Integrated Modelling of the river-

sediment-soil-groundwater system; advanced tools for the management of catchment areas and river basins in the context of global change, Integrated Project,Thematic Priority: Sustainable development, global change and ecosystems, Deliverable No.: BASIN 5.11, May 2005, pp. 148.

Stahlschmidt-Allner P., Slobodník J., Makovinska J., Paunovic M., Csányi B., Hamchevici C., Maringer F.J., Hrachowitz M., Fekete J., Nagy P., Ignjatovic J., Liska I., Rijnaarts H. (2005). AquaTerra Danube Survey: Towards understanding of inter-relations among chemical pollution of sediments and suspended matters, its ecotoxicological effects and occurrence of biota in the Danube River. CONSOIL 2005, Proceedings of the 9th International FZK/TNO Conference on Soil-Water Systems 3-7 October 2005, Bordeaux. Special Session.

# Annex I

## A.1. Table 1. Sampling locations on the Tisza and its tributaries with sample delivery

| Serial no. | River/tributary name | Site name | Rkm | Type | Daysd | |
|---|---|---|---|---|---|---|
| 1 | Fehér-Tisza | Rahó | 1.0 | Tributary | | |
| 2 | Fekete-Tisza | Rahó | 1.0 | Tributary | | |
| 3 | Tisza | Rahó | 910.0 | Main river | 1. day | |
| 4 | Iza | Máramarossziget | 1.0 | Tributary | | |
| 5 | Tisza | Máramarossziget | 853.5 | Main river | | |
| 6 | Tisza | Huszt | 792.1 | Main river | | |
| 7 | Rika (Nagy-Ág) | Huszt | 1.0 | Tributary | 2. day | |
| 8 | Tisza | Tiszabecs | 744.2 | Main river | | |
| 9 | Borzsa | Mezővári | 1.0 | Tributary | | |
| 10 | Túr | Tiszakóród | 1.0 | Tributary | 3. day | First sample transport |
| 11 | Tisza | Jánd | 687.0 | Main river | | |
| 12 | Szamos | Olcsvaapáti | 1.0 | Tributary | | |
| 13 | Tisza | Aranyosapáti | 683.0 | Main river | 4. day | |
| 14 | Tisza | Tuzsér | 616.5 | Main river | | |
| 15 | Lónyai-főcsatorna | Gávavencsellő | 1.0 | Tributary | | |
| 16 | Tisza | Tokaj | 544.7 | Main river | 5. day | |
| 17 | Bodrog | Bodrogkeresztúr | 1.0 | Tributary | | |
| 18 | Sajó | Kesznyéten | 1.0 | Tributary | | |
| 19 | Tisza | Tiszapalkonya | 485.9 | Main river | 6. day | Second sample transport |
| 20 | Tisza | Tiszacsege | 453.0 | Main river | | |
| 21 | Tisza | Tiszafüred | 431.0 | Main river | 7. day | |
| 22 | Tisza | Tiszabura | 395.0 | Main river | | |
| 23 | Zagyva | Szolnok | 1.0 | Tributary | | |
| 24 | Tisza | Szolnok | 330.0 | Main river | 8. day | |
| 25 | Tisza | Tiszaug | 266.4 | Main river | | |
| 26 | Hármas-Körös | Csongrád | 1.0 | Tributary | | |
| 27 | Tisza | Csongrád | 237.3 | Main river | 9. day | Third sample transport |
| 28 | Maros | Tápé | 1.0 | Tributary | | |
| 29 | Tisza | Tiszasziget | 167.0 | Main river | 10.day | |
| 30 | DTD | Novi Becej | 1.0 | Tributary | | |
| 31 | Tisza | Novi Becej | 68.0 | Main river | 11.day | |
| 32 | Béga | Titel | 1.0 | Tributary | | |
| 33 | Tisza | Titel | 1.0 | Main river | 12.day | Fourth transport |

| | **Legend** |
|---|---|
| | Main river |
| | Tributary |
| | Transport by car |
| | Transport by ship |

## A.1. Table 2. Scenario B: Sample types (biological & chemical samples)

| No. | River/ tributary name | Site name | Rkm | MZB | Phytobenthos | Phytoplankton | Zooplankton | Macrophytes | Fish | Microbiology | HYMO | Water sample* | Biota sample | Sediment sample | SPM | Target screening | Suspect screening | Non-target screening | RBSPs - 4 metals |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Fehér-Tisza | Rahó | 1.0 | 1 | 1 | | | | | | 1 | 1 | | | | | | | |
| 2 | Fekete-Tisza | Rahó | 1.0 | 1 | 1 | | | | | | 1 | 1 | | | | | | | |
| 3 | Tisza | Rahó | 910.0 | 1 | 1 | | | | 1 | 1 | 1 | 1 | | | | | | | |
| 4 | Iza | Máramarossziget | 1.0 | 1 | 1 | | | | | | 1 | 1 | | | | | | | |
| 5 | Tisza | Máramarossziget | 853.5 | 1 | 1 | | | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 6 | Tisza | Huszt | 792.1 | 1 | 1 | | | | 1 | | 1 | 1 | | | | | | | |
| 7 | Rika (Nagy-Ág) | Huszt | 1.0 | 1 | 1 | | | | | | 1 | 1 | | | | | | | |
| 8 | Tisza | Tiszabecs | 744.2 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 9 | Borzsa | Mezővári | 1.0 | 1 | 1 | | | | | | 1 | 1 | | | | | | | |
| 10 | Túr | Tiszakóród | 1.0 | 1 | 1 | | | | | | 1 | 1 | | | | | | | |
| 11 | Tisza | Jánd | 687.0 | 1 | 1 | 1 | 1 | | 1 | | 1 | 1 | | | | | | | |
| 12 | Szamos | Olcsvaapáti | 1.0 | 1 | 1 | 1 | 1 | | | | 1 | 1 | | | | | | | |
| 13 | Tisza | Aranyosapáti | 683.0 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 14 | Tisza | Tuzsér | 616.5 | 1 | 1 | 1 | 1 | | | | 1 | 1 | | | | | | | |
| 15 | Lónyai-főcsatorna | Gávavencsellő | 1.0 | 1 | 1 | 1 | 1 | 1 | | | 1 | 1 | | | | | | | |
| 16 | Tisza | Tokaj | 544.7 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 17 | Bodrog | Bodrogkeresztúr | 1.0 | 1 | 1 | 1 | 1 | | | | 1 | 1 | | | | | | | |
| 18 | Sajó | Kesznyéten | 1.0 | 1 | 1 | 1 | 1 | 1 | | | 1 | 1 | | | | | | | |
| 19 | Tisza | Tiszapalkonya | 485.9 | 1 | 1 | 1 | 1 | | | | 1 | 1 | | | | | | | |
| 20 | Tisza | Tiszacsege | 453.0 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 21 | Tisza | Tiszafüred | 431.0 | 1 | 1 | 1 | 1 | | | | 1 | 1 | | | | | | | |
| 22 | Tisza | Tiszabura | 395.0 | 1 | 1 | 1 | 1 | | | | 1 | 1 | | | | | | | |
| 23 | Zagyva | Szolnok | 1.0 | 1 | 1 | 1 | 1 | 1 | | | 1 | 1 | | | | | | | |
| 24 | Tisza | Szolnok | 330.0 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 25 | Tisza | Tiszaug | 266.4 | 1 | 1 | 1 | 1 | | | | 1 | 1 | | | | | | | |
| 26 | Hármas-Körös | Csongrád | 1.0 | 1 | 1 | 1 | 1 | | | | 1 | 1 | | | | | | | |
| 27 | Tisza | Csongrád | 237.3 | 1 | 1 | 1 | 1 | | 1 | | 1 | 1 | | | | | | | |
| 28 | Maros | Tápé | 1.0 | 1 | 1 | 1 | 1 | | | | 1 | 1 | | | | | | | |
| 29 | Tisza | Tiszasziget | 167.0 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 30 | DTD | Novi Becej | 1.0 | 1 | 1 | 1 | 1 | | | | 1 | 1 | | | | | | | |
| 31 | Tisza | Novi Becej | 68.0 | 1 | 1 | 1 | 1 | | 1 | | 1 | 1 | | | | | | | |
| 32 | Béga | Titel | 1.0 | 1 | 1 | 1 | 1 | 1 | | | 1 | 1 | | | | | | | |
| 33 | Tisza | Titel | 1.0 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| **Number of samples** | | | | **33** | **33** | **24** | **24** | **4** | **13** | **9** | **33** | **33** | **8** | **8** | **8** | **8** | **8** | **8** | **8** |

**In water samples general physico-chemical compounds and WFD Priority Substances (PS) have to be analyzed at all sampling locations*

## A.1. Table 3. Summary on the price estimation for WFD Priority Substances (Environmental Institute, Slovakia) measured in **WATER** (Directive 2013/39/EU)

| No. | Name of group | Substances in group | EI_price per group (EUR) |
|---|---|---|---|
| **1** | PAHs | **Anthracene**; Benzo(a)pyrene; Benzo(b)fluoranthene; Benzo(g,h,i)perylene; Benzo(k)fluoranthene; **Fluoranthene**; Indeno(1,2,3-cd)pyrene; **Naphthalene** | **70** |
| **2** | Metals | **Cadmium and its compounds; Lead and its compounds;** Mercury and its compounds**, Nickel and its compounds** | **50** |
| **3** | Pesticides | **Trifluralin; Simazine; Atrazine; Hexachlorocyclohexane; Alachlor; Chlorpyrifos ethyl; Endosulfan-alpha,beta; p,p'-DDT; DDT-total; Chlorfenvinphos; Aldrin; Dieldrin; Endrin; Isodrin** | **100** |
| **4** | | **Isoproturon; Diuron** | **40** |
| **5** | SCCP | **C10-13 Chloroalkanes** | **120** |
| **6** | VOCs | **1,2-Dichloroethane; Dichloromethane; Trichloroethene; Trichloromethane;** Hexachlorobutadiene**; Carbon-tetrachloride; Trichlorobenzenes; Benzene; Tetrachlorethylene** | **80** |
| **7** | Industrial poll. | **DEHP; Hexachlorobenzene; Nonylphenol (4-Nonylphenol); Octylphenol (p-tert-Octylphenol); Pentachlorobenzene; Pentachlorophenol** | **80** |
| **8** | TBT | **Tributyltin compounds** | **110** |
| **9** | Newcomers | **Cypermethrin; Heptachlor and heptachloro epoxide; Aclonifen; Dicofol; Quinoxyfen; Bifenox; Terbutryn; Cybutryne; Dichlorvos; Hexabromocyclododecane** | **150** |
| **10** | | **PFOS and its derivatives** | **110** |
| **Total price in € per one sample** | | | **910** |

**All compounds to be determined according to the Directive 2013/39/EU are in bold**
Compounds commonly checked along with the WFD PS are in regular

## A.1. Table 4. Summary on the price estimation for WFD Priority Substances (Environmental Institute, Slovakia) measured in **BIOTA** (Directive 2013/39/EU)

| No. | Name of group | Substances in group | EI_price per group (EUR) |
|---|---|---|---|

| | | | |
|---|---|---|---|
| 1 | PAHs | Anthracene; **Benzo(a)pyrene;** Benzo(b)fluoranthene; Benzo(g,h,i)perylene; Benzo(k)fluoranthene; **Fluoranthene**; Indeno(1,2,3-cd)pyrene; | **90** |
| 2 | Metals | Cadmium and its compounds; Lead and its compounds; **Mercury and its compounds**, Nickel and its compounds | **70** |
| 3 | BDEs | **BDE 28, 47, 99, 100, 153, 154** | **150** |
| 4 | Industrial poll. | DEHP; **Hexachlorobenzene;** Nonylphenol (4-Nonylphenol); Octylphenol (p-tert-Octylphenol); Pentachlorobenzene; Pentachlorophenol | **80** |
| 5 | VOCs | **Hexachlorobutadiene** | **40** |
| 6 | Newcomers | **Cypermethrin; Heptachlor and heptachloro epoxide; Dicofol; Hexabromocyclododecane** | **150** |
| 7 | | **PFOS and its derivatives** | **120** |
| 8 | Dioxins | **Dioxins and dioxin-like compounds** | **300 - 500** |
| **Total price in € per one sample (minimum range)** | | | **1000 - 1200** |

**All compounds to be determined according to the Directive 2013/39/EU are in bold**
Compounds commonly checked along with the WFD PS are in regular

### A.1. Table 5. Summary on the price estimation for WFD Priority Substances (Environmental Institute, Slovakia) measured in **SEDIMENT** (Directive 2013/39/EU)

| No. | Name of group | Substances in group | EI_price per group (EUR) |
|---|---|---|---|
| 1 | PAHs | Anthracene; Benzo(a)pyrene; Benzo(b)fluoranthene; Benzo(g,h,i)perylene; Benzo(k)fluoranthene; Fluoranthene; Indeno(1,2,3-cd)pyrene; Naphthalene | **90** |
| 2 | Metals | Aluminium, Arsenic, Cadmium, Chromium, Copper, Lead, Mercury, Nickel, Zinc | **80** |
| 3 | Pesticides | Trifluralin;Hexachlorocyclohexane; Chlorpyrifos ethyl; Endosulfan-alpha,beta; p,p'-DDT; DDT-total; Chlorfenvinphos; Aldrin; Dieldrin; Endrin; Isodrin | **120** |
| 4 | BDEs | BDE 28, 47, 99, 100, 153, 154 | **100** |
| 5 | SCCP | C10-13 Chloroalkanes | **120** |
| 6 | Industrial poll. | DEHP; Hexachlorobenzene; Nonylphenol (4-Nonylphenol); Octylphenol (p-tert-Octylphenol); Pentachlorobenzene | **100** |
| 7 | TBT | Tributyltin compounds | **120** |
| 8 | Newcomers | PFOS and its derivatives | **120** |

| | | | |
|---|---|---|---|
| 9 | Organochlorine compounds | Heptachlor and heptachloro epoxide; Dicofol; Hexabromocyclododecane; Quinoxyfen | **150** |
| Total price in € per one sample | | | **1000** |

## A.1. Table 6. Costs of chemical compounds investigated in the framework of identifying **River Basin Specific Pollutants**

| Matrix | Non-target screening | Price per group/€ |
|---|---|---|
| W,S | GC-MS (EI, PCI, NCI) | **0-100 identified substances =350 Euro 100-500 identified substances = 550 Euro >500 identified substances = 25 Euro/ 100 additional substances** |
| | **Target screening** | |
| W,B,S | LC-HR-MS; LC-MS/MS | **Database of >2100 compounds = 480 Euro** |
| | **Suspect screening (DSFP)** | |
| W,B,S | LC-HR-MS | **Database of >40.000 compounds = 500 Euro** |

## A.1. Table 7. General physico-chemical parameters measured in water samples

| Parameter | Unit price [EUR] | Comment |
|---|---|---|
| Temperature | | Field measurement |
| pH | | Field measurement |
| Dissolved oxygen | | Field measurement |
| PO4 | | Laboratory |
| NO3 | | Laboratory |
| NO2 | | Laboratory |
| NH4 | | Laboratory |
| TN | | Laboratory |
| TP | | Laboratory |
| BOD5 | | Laboratory |
| COD | | Laboratory |
| Alkalinity | | Laboratory |
| Conductivity | | Laboratory |
| Hardness | | Laboratory |
| **Costs** | **100** | |

## A.1. Table 8. Unit and total costs of EXPERTS AND ANALYSIS - Substances measured in different **matrices** (Price of analysis is based on the Environmental Institute, Slovakia)

| **BIOLOGICAL SAMPLES** | **Unit price [EUR]** | **No. of samples** | **Total price [EUR]** |
|---|---|---|---|
| MZB | 600 | 33 | 19800 |
| Phytobenthos | 400 | 33 | 13200 |
| Phytoplankton | 400 | 24 | 9600 |
| Zooplankton | 400 | 24 | 9600 |
| Macrophytes | 300 | 4 | 1200 |
| Fish | 700 | 13 | 9100 |
| Microbiology | 500 | 9 | 4500 |
| **HYMO** | 400 | 33 | 13200 |
| ***TOTAL BIOL.+HYMO*** | *3700* | *173* | ***80200*** |

| **CHEMICAL SAMPLES** | **Unit price [EUR]** | | | **No. of samples** | | | **Total price [EUR]** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Water** | **Biota** | **Sediment** | **Water** | **Biota** | **Sediment** | **Water** | **Biota** | **Sediment** |
| WFD priority substances | 910 | 1200 | 1000 | 33 | 8 | 8 | 30030 | 9600 | 8000 |
| River Basin Specific Substances | | | | | | | | | |
| Target screening LC-MS | 480 | 480 | 480 | 8 | 8 | 8 | 3840 | 3840 | 3840 |
| Suspect screening LC-HR-MS | 500 | 500 | 500 | 8 | 8 | 8 | 4000 | 4000 | 4000 |
| Non-target screening GC-MS | 350 | 0 | 350 | 8 | 8 | 8 | 2800 | 0 | 2800 |
| RBSPs - 4 additional metals | 50 | 0 | 50 | 8 | 10 | 8 | 400 | 0 | 400 |
| Physico-chemical parameters | 100 | 0 | 0 | 37 | 0 | 0 | 3700 | 0 | 0 |
| Microplastics | 100 | | | 33 | | | 3300 | | |
| *SUBTOTAL* | *2490* | *2180* | *2380* | *135* | *42* | *40* | *48070* | *17440* | *19040* |
| ***TOTAL W/B/S*** | | | | | | | | | ***84550*** |
| **JTS2 TOTAL EXPERT & ANALYSIS COSTS** | | | | | | | | | **164750** |

## A.1. Table 9. Calculated cost of sample transport during JTS2

| **Item** | **Travel** | **Σ Km** | **Cost (0.22 Euro/km)** |
|---|---|---|---|
| 1. Round | Szolnok-Tiszakóród-Szolnok | 600 | 132 |
| 2. Round | Szolnok-Tiszapalkonya-Szolnok | 400 | 88 |
| 3. Round | Szolnok-Csongrád-Szolnok | 200 | 44 |
| 4. Round | Szolnok-Titel-Szolnok | 700 | 154 |
| Salary | Driver + Assistant | 4 days | 240 |
| **Total cost of sample transport** | | | **658** |

# Annex II

## Proposal for chemical analysis (JDS4 Survey Plan ANNEX D)

**Draft 7**

**Karin Deutsch, Carmen Hamchevici, Hana Hudcova, Elena Rajczykova, Manfred Sengl, Draženka Stipaničev, Zoran Stojanović, Peter Tarabek, Istvan György Toth, Natalia Zgircu**

**19.11.2018**

### 1. Introduction

Chemical parameters play an important role in the assessment of water quality according to the European Water Framework Directive (WFD).
The assessment of the "chemical status" of water bodies is based on compliance with environmental quality standards (EQS) defined for 45 priority substances (in some cases groups of substances) and for 5 additional substances/groups of substances originally selected according to Directive 74/464 (see directive 2013/39/EU).

As a result of the prioritisation process for the continuous update of the list of priority substances 17 substances are listed in a "Watch list" published by Commission decision 2015/495 of 20 March 2015 (notified under document C(2015) 1756).

River specific substances and their EQS may be defined by EU member states on the national level. The results of these substances contribute to the assessment of the "ecological status".

The selection of chemical parameters for JDS4 should take into regard

- the present list of priority pollutants according to directive 2013/39/EU
- watch-list substances
- the draft list of Danube River Basin Specific Pollutants elaborated within the EU-project SOLUTIONS
- national lists of river basin specific pollutants
- LC-HRMS-screening and GC-HRMS-screening as new tools of water monitoring
- emerging pollutants based on previous results, national lists/activities and results, Europe-wide investigations run by JRC/Ispra, literature etc.
- requirements of the WFD concerning ecological status (determination of physico-chemical parameters)

The main objective is producing a homogeneous data set for the entire Danube River course for selected determinants and quality elements and **not** a chemical status assessment.

Another starting point of parameter selection are the results from the previous surveys JDS1 - JDS3 available through the JDS database in DANUBIS.

**2. General aspects of the new sampling approach of JDS4**

JDS4 will have a new survey design, i.e. there will be no ships going down the Danube from sampling point to sampling point.

In JDS4 sampling will be done by national teams from the banks and from small boats all along the Danube more or less in parallel within a time frame of ca. 2 weeks.

For this reason the following sampling and sample preparation devices will not be available:

- centrifuge for collecting SPM
- sediment grabber (as was mounted on the Argus)
- wet sieving machine for sediments

SPM-sampling with centrifuge

Centrifuges are available only in single countries.

**JDS4: No sampling of SPM**

Sampling of sediments

Harmonization of sediment sampling by national teams is difficult as the availability of sediment along the Danube shows huge variations.

Sediment data from JDS1-JDS3 are available. Directive 2013/39/EU again is not setting EQS for sediments.

Sediment analysis for trend monitoring requires more than one sampling per year and should take place every three years, thus results from JDS1 – JDS4 would not be a reliable base for trend monitoring. On the other hand there are national data available e.g. for metals and PAH.

**JDS4: No sampling of sediments**

Early sampling for pesticides

JDS 1-3 were done in August/September and thus only low concentrations of pesticides could be detected as pesticides are mostly used during March-July and end of September-beginning of November.

The new survey design (sampling by national teams) gives the chance to have an early sampling campaign for pesticides e.g. in June 2019 in order to get a more realistic picture. The main application month varies between the different countries (climate zones) along the Danube and is also dependent on the weather.

**JDS4: Recommendation for an early sampling campaign in June for pesticides in water and an additional flexible second sampling in May or July. Sampling should be done in the Danube and the tributaries.**

***MAEG-28 agreed to do only one sampling for pesticides (together with general JDS4 sampling)***

**3. Priority substances according to directive 2013/39/EU**

**3.1 Analysis in water**

**MAEG-28 agreed that water samples should be taken from the middle of the river, otherwise from left or right. For transboundary sites a bilateral agreement is necessary.**

The necessity of the analysis of priority substances must be seen in the light of existing EQSs which are defined for whole water samples (except for heavy metals which are analysed from filtered samples).

**Requirements of the QA/QC Directive should be fulfilled**

### 3.1.1 Polar pesticides

(alachlor, atrazine, chlorfenvinpos, chlorpyrifos, diuron, isoproturon, simazine, trifluralin, dicofol, quinoxyfen, aclonifen, bifenox, cybutryne, cypermethrine, dichlorvos, terbutryn)

As the main period of pesticide application is March-July the sampling should be done in these months. With Directive 2013/39/EU new pesticides were added and should be covered for the first time in JDS4.

**JDS 4: Analysis of all polar pesticides in June and an additional flexible sampling in May or July**
***MAEG-28 agreed not to analyse cypermethrine and dichlorvos in water.***
***MAEG-28 agreed to do only one sampling for pesticides (together with general JDS4 sampling)***

### 3.1.2 Volatile organic compounds (VOCs)

Only few VOCs were found in a small number of JDS 1 and JDS 2 samples in low concentrations close to the limit of quantification and well below EQS. No measurement in JDS3, no hints for new sources for these substances.

**JDS4: 1,2-dichloroethane, benzene, dichloromethane, hexachlorobutadiene, trichloromethane, tetrachloromethane and trichloroethylene – no need for analysis**

### 3.1.3 Organotin compounds

In JDS3 at 7 out of 68 sampling sites the concentration of tributyltin exceeded the extremely low EQS of 0,0002 µg/l and therefore analysis should be repeated.

**JDS 4: Tributyltin should be analysed in water (together with other tinorganic compounds)**
***MAEG-28 agreed not to analyse tributyltin in water***

### 3.1.4 Alkylphenoles, pentachlorphenole and di-(2-ethylhexyl)phthalate (DEHP)

4-iso-Nonylphenol (NP) and p-octylphenol (OP) can be found in water samples along the Danube in concentrations below the EQS, thus they were not analysed in JDS3. As no new emission sources are known water concentrations are expected to be constantly low. Pentachlorophenol (PCP) can be found only in the low ng/l-range and should not be analysed in JDS4.
DEHP was exceeding the high EQS of 1,3 µg/l during JDS2 in 44% of all water samples. In JDS3 these high concentrations could not be found again, 0,5 EQS was exceeded only in one water sample. National data confirm the JDS3 results.

**JDS4: NP, OP, PCP– no need for analysis**
**DEHP** ***Conditionally***

### 3.1.5 Heavy metals and arsenic

Cadmium, lead, nickel and mercury are monitored in water in TNMN.

JDS4 should be an opportunity to get a homogenous data set for mercury in fish.

**JDS 4: No analysis in water, for mercury in biota - see chapter 3.2**

### 3.1.6 C10-C13-Chloroalkanes

C10-C13-Chloroalkanes were not detected in water in JDS3. The concentration identified in SPM were relatively low (a theoretical calculation from SPM to water concentration via KOC-value from literature gives results well below the water EQS).

**JDS4: No need for analysis**

### 3.1.7 PAH

Naphthalin and anthracene were well below the water EQS in JDS3.
The EQS for fluoranthene was lowered significantly, so exceedance is expected at most sampling sites. Benzo(a)pyrene is now the leading parameter for 5 "larger" PAH (including benzo(b)- and benzo(k)fluoranthen as well as benzo(g,h,i)perylene and indeno(1,2,3)pyrene). The new EQS for benzo(a)pyrene was lowered significantly, so exceedance is expected at almost all sampling sites.

**JDS 4: No analysis in water, for fluoranthen and benzo(a)pyrene analysis in mussels - see chapter 3.2**
**Possible use of passive samplers – see chapter 11**

### 3.1.8 Brominated diphenyl ethers (BDE)

In JDS2 samples the AA-EQS of 0,0005 µg/l for each single BDE was never reached in water. Directive 2013/39/EU skipped the AA-EQS and defined a relatively high MAC-EQS of 0,14 µg/l which is not expected to be exceeded.
Therefore BDE should be monitored in biota, as a new EQS was defined.

**JDS4: BDE should be analysed in biota – see chapter 3.2**

### 3.1.9 Organochlorine compounds

(aldrin, dieldrin, endrin, isodrin, DDT, endosulfane, hexachlorobenzene, hexachlorocyclohexane, pentachlorobenzene, trichlorobenzene, heptachlor/heptachlorexpoxide)

No or very few results at low concentrations were gathered for the organochlorine compounds (including trifluralin) in water samples. The results from JDS1 and JDS2 in sediments and SPM do not indicate that these "old" substances are relevant pollutants.

**JDS4: Organochlorine compounds: no need for analysis in water, heptachlor/heptachlorepoxide analysis in biota – see chapter 3.2**

### 3.1.10 PFOS

**JDS4: No analysis in water, should be analysed in biota – see chapter 3.2**

3.2 Analysis in fish and mussels

Since 2013 EQS for biota are now defined for more substances.
While EQS of HCB and HCBD were never exceeded in muscle and liver samples during JDS 2, mercury is known to exceed the EQS for biota in the majority of the samples of European rivers. Mercury in fish is a new parameter in TNMN.

It must be decided if whole fish or fish muscle tissue should be analysed, as both approaches are used by the Danube countries in their national monitoring.
Whole fish is a clearly defined sample. 4 out of 12 biota EQS are derived from the top predator approach (accumulation in the food chain, "secondary poisoning"). For most substances higher concentrations are found in whole fish in comparison to muscle fillets (exemption: mercury).

**JDS4 – analysis of whole fish**

**JDS4:**
**- no need for analysis: HCB and HCBD**
**- analysis in whole fish:**
**mercury**
**brominated diphenylethers**
**HBCDD *Conditionally (as the EQS is very high)***
**PFOS**
**dicofol**
**heptachlor/heptachlorepoxid**
**dioxins and dioxin-like substances**
***MAEG-28 agreed that tendering of biota analyses of parameters without inkind-contributions should be prioritised as follows: a) PFOS, dicofol b) dioxins c) HBCDD (analysis of heptachlor/heptachlorepoxid can only be done by very few labs and will be too expensive)***
**- analysis in mussels**
- **fluoranthen and benzo(a)pyrene**
***MAEG-28 agreed that also Gammaridae can be analysed if mussels are not available***

The fish species used for analysis must be specified in order to get comparable results: 3-5 years-old chub (Leuciscus cephalus) or breams (Abramis brama) or barbel (Barbus barbus).
It is recommended to freeze whole fish as fast as possible. All fish should be prepared (grinding at deep temperature) by one experienced institution.

Mussels can be frozen immediately after sampling to process them in the laboratory. It is also possible to prepare the mussel bodies and freeze this material. It is possible, that (not protected) mussels cannot be found at every sampling site. In this case the sampling of Gammaridae is possible.

**Essential additional parameters for comparison of the fish data:**
**age, length, weight, dry weight, lipid content**

### 3.3 Analysis of priority substances for trend monitoring

According to directive 2013/39/EU member states shall arrange for the long-term trend analysis of those priority substances listed that tend to accumulate in sediment and/or biota. Particular

consideration should be given to substances number 2, 5, 6, 7, 12, 15, 16, 17, 18, 20, 21, 26, 28, 30, 34, 35, 36, 37, 43 and 44 listed in Part A of Annex 1.
Many of these substances were already analysed during JDS1 to JDS3. With this data a simplified trend can be determined as according to the rules, trend analysis is based on average values from single years.

| **No sampling for trend analysis – national data available for metals and PAH, trend analysis needs more comparable data than JDS can supply** |
|---|

An overview of the proposal for the analysis of priority substances is given in Annex 1.

## 4. General physico-chemical quality elements in JDS3

### 4.1 Water

Analysis of general physico-chemical parameters is done in all water samples by national laboratories

**JDS 4:**

- **temperature**
- **dissolved oxygen concentration and saturation**
- **pH, alkalinity**
- **conductivity**
- **nutrients: N-$NH_4$, N-$NO_2$, N-$NO_3$, Total N, P-$PO_4$, Total P**
- **TOC**

**Additional for microbiology: total suspended solids, chlorophyll a**

## 5. "Watch list" published by Commission decision 2015/495 of 20 March 2015 and proposal for next Watch list substances

The Commission decision lists 10 substances or groups of substances (in total 17 single substances). The watch list substances had to be analysed in 2016 in all member states at selected sampling sites and results (average value of at least 4 measurements) had to be reported to the Commission by the end of 2016.
Until May 2017 data from 25 countries were reported and an assessment was done by JRC. The PNEC values given in 2015 used for the assessment were updated (lowered) in the meantime for 9 single substances based on additional information from Switzerland.
For 9 out of 17 substances the quantification frequency was below 10%, for acetamipride and methiocarb below 1%. For diclofenac sufficient data were collected. Thus it is forseen to remove diclofenac, 2-ethylhexyl-4-methoxycinnamate, oxadiazon, triallate and 2,6-di-tert-butyl-4-methylphenol from the Watch list.
Exceedances of PNEC values were primarily observed for EE2, E2, E1, imidacloprid, diclofenac, azithromycin and clarithromycin. Substances that were not found in concentrations above the PNECs (except single outliers) should not be analysed in JDS4.

JRC also proposed to select new watch list substances with low monitoring data quality and quantity and a high ranking from the prioritisation monitoring and modelling exercise 2016. The proposed substances are, amoxicillin, ciprofloxacin and metaflumizone. Analytical methods for "old" and "new"

watch list substances were discussed during a JRC-workshop in March 2018. As a conclusion analytical methods were described that meet the requirements of the proposed EQS concentrations.

**JDS4:**
**Analysis of watch list substances with results above the PNEC if a suitable method meeting the PNEC values is available: 17-alpha-ethinylestradiol (EE2), 17-beta-estradiol (E2), estrone (E1), imidacloprid, diclofenac, azithromycin and clarithromycin.**

**Analysis of amoxicillin, ciprofloxacin and metaflumizone according to the conclusions of the JRC-workshop.**

**The substances are listed in Annex 2**

**6. - Draft list of Danube River Basin Specific Pollutants (elaborated within the EU-project SOLUTIONS)**

In 2013 a list of 20 substances relevant for the Danube river has been compiled on the results of the JDS3 target screening of 654 substances. This first preliminary list of Danube River Basin Specific Pollutants was further elaborated in the SOLUTIONS project using more data and new ecotoxicological information. At the end of September a new version was presented listing substances in six categories according to the NORMAN prioritisation scheme.

**JDS4: Based on list of Danube River Basin Specific Pollutants (SOLUTIONS, version March 2018) analysis of substances in categories 1 and 2**
**→except those substances which are analysed in countries (metals), or planned to analyse as watch list parameters, priority substances or pesticides.**
**The substances are listed in Annex 3**

**7. Emerging pollutants based on previous results, national lists and national results or results from European-wide surveys**

During JDS1-3 the analysis of emerging pollutants was mostly done as in-kind contributions of laboratories or (EU-)projects running specialised analytical methods.

**7.1. Pharmaceuticals**

Pharmaceuticals were found in JDS1-3 mainly in small concentrations <50 ng/L. Pharmaceuticals were analysed in many studies of European rivers so a broad screening approach is not recommended for JDS4.

Regarding exotoxicity antibiotics are the most relevant group of pharmaceuticals (see macrolides in the watch list) and should be followed first. As data for metabolites of pharmaceuticals are often not available, they should be targeted if analytical methods are available.

If WWTP effluents will be sampled during JDS4 pharmaceuticals should be analysed to characterise these point sources.

**Some pharmaceuticals are already covered by watch list and DRBSP.**
**Further compounds: to be selected, when the results of the ongoing WTTP effluent screening are available**

**7.2 Illicit drugs**

Illicit drugs were in the focus of the public and should be covered in JDS4.

| **JDS4: Analysis in water – essential compounds from the group of illicit drugs based on COST score Action should be analysed** |
|---|

**7.3 Organophosphorous compounds (OPC)**

In JDS3 OPC were found far below the effect levels for aquatic biota. Concerning ecotoxicity the substances tris(methylphenyl)phosphate (TMPP) and triphenylphoshate (TPhP) were the most relevant.

| **JDS4: Analysis of TMPP and TPhP in water -– conditionally** |
|---|

**7.4 Glyphosate and AMPA**

Glyphosate and its metabolite AMPA are widely found in surface waters. These substances are very polar and require a separate analytical method using derivatisation. Quantitative results are far below the proposed PNEC values.

| **JDS4: No analysis in water** |
|---|

**7.5 …**

**Additional emerging substances can be added (e.g. in-kind contributions)**

- 1,4-Dioxan in water samples (option for in-kind contribution Germany)
- …

**8. Non-target screening of organic pollutants using GC- and LC-HRMS techniques**

In JDS3 LC-HRMS-systems using high-resolution mass spectrometry (Time of flight-MS, Orbitrap) were applied by three different laboratories. The data were used for target and non-target screening.
The stored raw data can also be used for further (retrospective) analysis and data comparisons.

As HRMS-systems show a continuous improvement in sensitivity and useful software tools for data comparison and analysis, non-target screening should be repeated in all water samples preferably by different laboratories. As there are many efforts for harmonisation of non-target-workflows, the laboratories involved should plan their analytical approach, data evaluation and data storage together.

LC-HRMS-systems will probably be available in Croatia, Serbia, Germany and Slovakia (end of 2017). Measurements should be done according to the routine workflow of each laboratory. This is usually direct injection of water samples.
Possibly also online-SPE can be applied (Germany?).

The raw data can also be uploaded to the NORMAN Digital Sample Freezing Platform to enable additional data analysis. In this context the use of a retention time index mixture is essential.

*Additional idea: If possible LC-HRMS-measurements of single selected sampling sites could be done in 2019 on a monthly basis for comparing consistency or differences over time.*

GC-MS screening was done during JDS1-3 and a large number of substances could be provisionally identified. These measurements could be repeated using a GC-HRMS system if available.

| **JDS4: LC-HRMS screening of all water samples; GC-HRMS if possible** |
|---|

**9. Effect based tools (EBT)**

Main goal is the comparison of chemical analyses and the results of EBT. Tools selection based on SOLUTION results – Toolbox!

List of target substances corresponding to toolbox have to be defined.

**Discussion between ICPDR, UBA Berlin and UFZ Leipzig**

**10. Required chemical analysis supporting the microbiology program (proposal Alexander Kirschner, TU Vienna, July 2017)**

List of ancillary variables (**bold: essential**; light: nice to have):

- Chemophysical variables: **pH, electrical conductivity, water temperature, oxygen, total suspended solids**
- Nutrients: **TOC, Ptot, Ntot**, NO3, NH4
- Biological variables: **chlorophyll a, (macro-zoobenthos)**
- Chemical parameters: **fluoroquinolones, sulfonamides, tetracyclines; heavy metals: at least once,** more would be nice

| **JDS 4: -for physico-chemical parameters see chapter 4**<br>**-antibiotics – the relevance of the proposed substances will be checked by the results of the WTTP effluent screening –** ***a NORMAN offer of University of Athens covers antibiotics***<br>**-heavy metals – national results should be used** |
|---|

**11. Passive sampling**

The use of passive samplers could be interesting e.g. for the analysis of PAH (flouranthen, benzo(a)pyrene) and pesticides, but input by experts in this field is necessary as only few countries have practical experiences.

| **Passive samplers – further discussion needed**<br>***MAEG-28: There is an offer from NORMAN lab from CZ, exposure of passive samplers should start in May to "catch" pesticides during the main application period*** |
|---|

# Annex III

## JDS4 - Proposal for the analysis of chemical parameters (draft 7, 14.11.2018) - Annex D1 – **List of WFD Priority Substances**

| Substance | CAS No. | Water | Fish/Mussel |
|---|---|---|---|
| Alachlor | 15972-60-8 | | |
| Anthracene | 120-12-7 | | |
| Atrazine | 1912-24-9 | | |
| Benzene | 71-43-2 | | |
| Brominateddiphenylethers | not applicable | | fish |
| Cadmium anditscompounds | 7440-43-9 | | |
| Chloroalkanes, C 10-13 | 85535-84-8 | | |
| Chlorfenvinphos | 470-90-6 | | |
| Chlorpyrifos (Chlorpyrifos-ethyl) | 2921-88-2 | | |
| 1,2-dichloroethane | 107-06-2 | | |
| Dichloromethane | 1975.09.02 | | |
| Di(2-ethylhexyl)phthalate (DEHP) | 117-81-7 | *conditional* | |
| Diuron | 330-54-1 | | |
| Endosulfan | 115-29-7 | | |
| Fluoranthene | 206-44-0 | | mussel (passive sampler?) |
| Hexachlorobenzene | 118-74-1 | | |
| Hexachlorobutadiene | 87-68-3 | | |
| Hexachlorocyclohexane | 608-73-1 | | |
| Isoproturon | 34123-59-6 | | |
| Lead and its compounds | 7439-92-1 | | |
| Mercury and its compounds | 7439-97-6 | | fish |
| Naphthalene | 91-20-3 | | |
| Nickel and its compounds | 7440-02-0 | | |
| Nonylphenols | not applicable | | |
| Octylphenols | not applicable | | |
| Pentachlorobenzene | 608-93-5 | | |
| Pentachlorophenol | 87-86-5 | | |
| Polyaromatic hydrocarbons (PAH) | not applicable | | |
| Benzo(a)pyrene | | | mussel (passive sampler?) |
| Simazine | 122-34-9 | | |
| Trichlorobenzenes | 12002-48-1 | | |
| Trichloromethane (chloroform) | 67-66-3 | | |
| Trifluralin | 1582-09-8 | | |
| Dicofol | 115-32-2 | | fish |

| | | | |
|---|---|---|---|
| Perfluorooctane sulfonic acid and its derivatives (PFOS) | 1763-23-1 | | fish |
| Quinoxyfen | 124495-18-7 | | |
| Dioxins and dioxin-like compounds | not applicable | | fish |
| Aclonifen | 74070-46-5 | | |
| Bifenox | 42576-02-3 | | |
| Cybutryne | 28159-98-0 | | |
| Hexabromocyclododecanes (HBCDD) | not applicable | | Fish *conditional* |
| Heptachlor and heptachlor epoxide | 76-44-8/ 1024-57-3 | | fish |
| Terbutryn | 886-50-0 | | |

# Annex IV

## JDS4 - Proposal for the analysis of chemical parameters - Annex D2 – **List of Watch list substances**

**Draft 6, 20.04.2018**

| Substance | CAS No. | PNEC value updated (µg/l) | Water | Sediment |
|---|---|---|---|---|
| Diclofenac | 15307-79-6 | 0,05 | X | |
| 17-Beta-estradiol (E2) | 50-28-2 | 0,0004 | X | |
| Estrone (E1) | 53-16-7 | 0,0036 | X | |
| 17-Alpha-ethinylestradiol (EE2) | 57-63-3 | 0,000035 | X | |
| Oxadiazon | 19666-30-9 | 0,088 | | |
| Methiocarb | 2032-65-7 | 0,002 | | |
| 2,6-Di-tert-butyl-4-methylphenol | 128-37-0 | 3,16 | | |
| Triallate | 2303-17-5 | 0,41 | | |
| Imidacloprid | 105827-78-9/ 138261-41-3 | 0,0083 | X | |
| Thiacloprid | 111988-49-9 | 0,01 | | |
| Thiamethoxam | 153719-23-4 | 0,042 | | |
| Clothianidin | 210880-92-5 | 0,13 | | |
| Acetamiprid | 135410-20-7/ 160430-64-8 | 0,5 | | |
| Erythromycin | 114-07-8 | 0,2 | | |
| Clarithromycin | 81103-11-9 | 0,12 | X | |
| Azithromycin | 83905-01-5 | 0,019 | X | |
| 2-Ethylhexyl 4-methoxycinnamate | 5466-77-3 | 6 (200 µg/kg forsediment) | | |
| ***New Watch listsubstances*** | | | | |
| Amoxicillin | 26787-78-0 | 0,078 | X | |

| | | | | |
|---|---|---|---|---|
| Ciprofloxazin | 85721-33-1 | 0,089 | X | |
| Metaflumizone | 139968-49-3 | 0,0654 | X | |

# Annex V

## List of the first twenty most important River Basin Specific Pollutants (RBSPs) in the Danube River

| No. | Substance | CAS No. | No. of countries with measurements | Position prioritisation 2014 |
|---|---|---|---|---|
| 1 | Arsenic - dissolved | 7440-38-2 | 7 | DRBSP |
| 2 | PFOS | 1763-23-1 | 10 | 2 |
| 3 | Chloroxuron | 1982-47-4 | 8 | 3 |
| 4 | Caffeine | 58-08-2 | 10 | - |
| 5 | Bromacil | 314-40-9 | 7 | 6 |
| 6 | Copper - dissolved | 7440-50-8 | 7 | - |
| 7 | Diazinon | 333-41-5 | 10 | 10 |
| 8 | Carbamazepine | 298-46-4 | 10 | - |
| 9 | Metolachlor | 51218-45-2 | 9 | - |
| 10 | Zinc - dissolved | 7440-66-6 | 7 | DRBSP |
| 11 | Metazachlor | 67129-08-2 | 9 | 14 |
| 12 | Nickel - dissolved | 7440-02-0 | 7 | PS |
| 13 | Lead - dissolved | 7439-92-1 | 7 | PS |
| 14 | Desethylterbutylazine | 30125-63-4 | 11 | 4 |
| 15 | Linuron | 330-55-2 | 8 | 12 |
| 16 | Diclofenac | 15307-86-5 | 11 | 17 |
| 17 | Tebuconazole | 107534-96-3 | 9 | - |
| 18 | Isoproturon | 34123-59-6 | 9 | PS |
| 19 | Bisphenol A | 80-05-7 | 10 | 8 |
| 20 | Amoxicillin | 26787-78-0 | 7 | 13 |

# Annex VI

## Concept-Paper for a JDS 4 - **Plastic monitoring** action in the Danube River (JDS4 Survey Plan Annex E)

Authors: Bannick (UBA DE), Braun (BAM), Gödecke (BAM), Heidemeier (UBA DE), Hohenblum UBA AT), Liebmann (UBA AT), Liska (ICPDR), Ricking, (UBA DE)

### 1 Introduction

Plastics are an emerging environmental concern. Studies from the marine environments dramatically show the increase of plastic pollution in the seas. Many scientific studies quote freshwater systems (rivers) as major pathway of plastics into the marine environment. Studies from fresh waters are existing but the used methods are not harmonised. So far it is not possible to compare such data. Plastics barely degrades in the environment, but alters and brakes into small pieces of micro-plastics.

Recent studies in Danube River demonstrate that up to 41 Tonnes of plastics (macro and micro plastics) are transported annually by the river in Austria (Hohenblum et al. 2015). To present, there is no information about plastic loads in other parts of the Danube River. However, results from studies on other European rivers show that plastics are ubiquitous in freshwater systems. It is therefore important to evaluate the load of rivers to characterize riverine inputs into the marine environments and to close the knowledge gap of pathways, sinks and fragmentation and impact of plastics in freshwater environments.

The idea of this proposal is a plastic monitoring during the next JDS in 2019. Here a joint action between the various JDS-members should be organized.

### 2 Rationale and political dimension

Plastics are indispensable to the society and much of our high living standard is owed to the use of plastic products. However, the use of plastic products and materials results in plastic contamination of the aquatic environment, especially if adequate waste management and awareness of proper handling of plastics products are lacking. Plastic particles are ingested by a wide variety of animals and the transfer of these particles to the aquatic food web is of growing concern. There is also interaction with other environmental and societal elements by the transfer of plastics to soil by irrigation.

Once in the environment, additives of plastics can leach into the surrounding matter and can negatively influence the biosphere. Plastic, therefore, is a cross-cutting issue and has interfaces to a number of legislative fields along its life cycle from production to waste management and re-integration into the

material flow. Thus, on the one hand, knowledge about the environmental processes and best practices need to be shared to raise awareness. On the other hand, prevention of loss of plastic into the environment has to be tackled by legislative frameworks which need to be fed by sound environmental data and knowledge about material flows to manage the problem efficiently at source.

Basically, there is increasing but little environmental data available which can be compared with other riverine data to assess the situation and to inform authorities and the public. This process should include as many stakeholders as possible to develop, share and distribute best practices.

The Danube River is the second largest river in Europe and uniquely, the International Commission for the Protection of the Danube River (ICPDR) organised already three Joint Danube Surveys (JDS) to monitor the river's quality from source to Sea. For the next, fourth edition of the JDS in 2019, it would be a valuable demonstration to include micro plastics in the scope of the survey to provide data on the river's pollution by micro plastic.

**3 Aim and Expected impact**

Main aim of this concept is to elaborate comparable, riverine (micro) plastic data for the entire length of the Danube River. This action is a first screening and would be a unique set of data to describe the river's burden of micro plastics. Besides that, results should reflect a first burden at regional level, which can be compared with other data along the Danube River. For this purpose, locations for sampling are proposed in Annex 1.

The described approach would contribute to increase knowledge about plastics and micro plastics along the Danube River and raise awareness of involved national experts (as multipliers) by dealing with the issue. Involving trained local experts generates transition of awareness to plastics and micro plastics to the countries and boosts international, transboundary co-operation, which is needed to tackle the problem efficiently.

There is the idea to provide specific micro plastic passive samplers personally to the local/national experts, to hand them over at the occasion of the summer school seminar and to allow them to practice and familiarize with sampling in advance of the survey. At the end, property of the sampling device can be transferred to the expert.

**4 Methodology**

There are several approaches available to sample plastics and micro plastics from riverine systems. A comprehensive compilation of approaches has been provided by the European Joint Research Centre in its "Riverine Litter Monitoring – Options and Recommendations" (González et al., 2016), which vary from visual observations to multi-spot sampling regimes. Multi spot sampling has the advantage to

take into account strong temporal and local variations caused by hydro-morphological effects. These approaches, however, are time and cost intensive, require assisting technology and manpower.

On the other hand, passive sampling allows for unattended sampling over a defined period of time. Beauty of this approach is that samplers are exposed and left alone without further need for maintenance. After exposure they are collected and samples can be extracted for analysis. Annex 2 describes the principle of passive collectors as they are to be used in the project. Various fractions are generated via sampling with the sedimentation box.

For the detection various methods are applicable. A determination of the total contents is proposed with TED GC MS (Dümichen et al 2016). The TED GC MS procedure has the advantage that it delivers reliable results in a short time. In order to obtain a first impression of the occurrence of plastic, the determination of the total contents of plastic in the abovementioned fractions of suspended matter is sufficient. Additional other methods (e.g. IR-spectroscopy) could be used during the monitoring action. With IR it possible to determine particle sizes and particle numbers.

To our knowledge, the ICPDR will change its concept for taking the samples from the Danube River from ship-based to land-based sampling. Moreover, local experts will be involved in conceptualizing the survey and taking samples. It is recommended to recruit national water experts who are affiliated with national authorities or ministries with broad experience in taking water samples. They shall be trained for exposing and handling the passive samplers properly.

Training regarding micro plastics is recommended to take place in form of centralized seminars to bring experts together, provide specific training on the job and to teach background knowledge on the subject of plastics in the environment. Training shall comprise proper use of the sampling device, selection of sites for exposure with comparable features along the entire river. Ideally, this could be organised in a summer school over a period of several days.

In order to make the survey consistent and comparable over the entire Danube River, selection of sampling sites shall be done on basis of the same principles and parameters for all sites. This strategy shall fit into the overall design of the JDS and need to consider accessibility, similar hydrological conditions, effects of dilution or concentration, possible “hot spot” issues next to effluents of emitters, transboundary and boundary effects etc. The approach shall be discussed and agreed with the ICPDR.

**5 Currently participating institutions**

Environment Agency Austria (UBA AT) – Sampling, Analytics
Environment Agency Germany (UBA DE) – Sampling, Sample Preparation
Federal Institute for Material Research and Testing (BAM DE) – Detection

ICPDR - Logistic

## 6 Literature

***Hohenblum Philipp, Liebmann Bettina, Liedermann Marcel; 2015***: Plastic and Microplastics in the Environment; UBA Reports 0551, Wien

***Dümichen E, Eisentraut P, Bannick CG, Barthel AK, Senz R, Braun U; 2017***: Fast identification of microplastics in complex environmental samples by a thermal degradation method, Chemosphere 174

***González, D., Hanke,G., Tweehuysen, G., Bellert, B., Holzhauer, M., Palatinus, A., Hohenblum, P., and Oosterbaan, L. 2016***. Riverine Litter Monitoring - Options and Recommendations. MSFD GES TG Marine Litter Thematic Report; JRC Technical Report; EUR 28307; doi:10.2788/461233

## Annex 1: Suggestions for sampling sites for (micro)plastics in the Danube River

The proposal for the sampling sites is based on the regional characteristics of the Danube. The aim is the investigation of relevant and distinctive sections of the river. The investigations are intended to provide initial ideas on the occurrence of plastics over the entire length of the river.
In addition to the overall criteria of describing relevant parts of the river stretch, the following criteria are important for the specific sampling sites selection:

- the water column should be well mixed, so higher turbulence is better than a laminar flow;
- a flow gauge should be in the vicinity of the site to allow for a load calculation;
- safe position against the influence of third parties

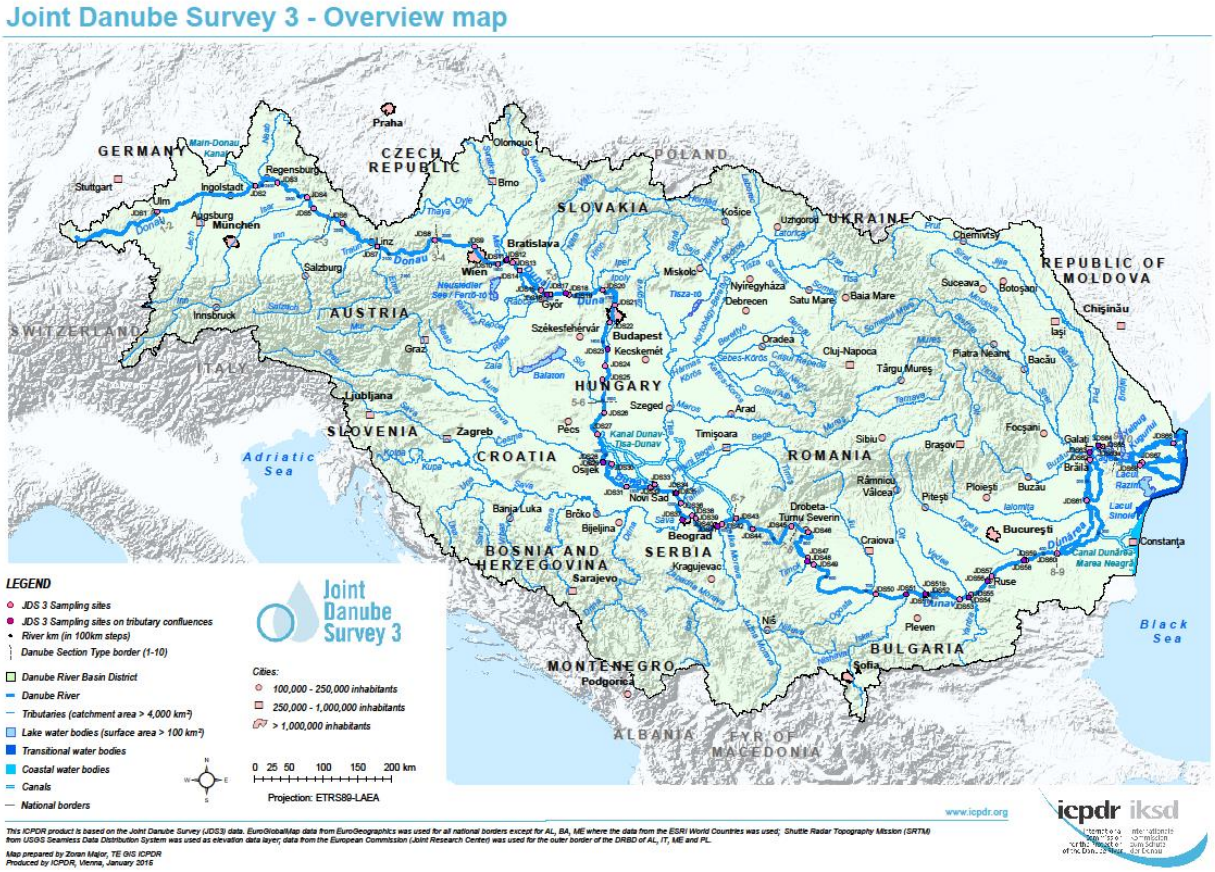



**Figure 1: Sampling Sites during JDS 3**

**Table 1: Proposal for sampling sites during JDS 4 (microplastics) – under discussion**

| Number | JDS code | Name | Remarks |
|---|---|---|---|
| **1** | JDS1 | Böfinger Halde | |
| **2** | JDS5 | Mühlau | |
| **3** | JDS6 | Jochenstein | |
| **4** | JDS9 | Klosterneuburg | |
| **5** | JDS10 | Wildungsmauer | |
| **6** | JDS11 | Upstream Morava (Hainburg) | |
| **7** | JDS13 | Bratislava | |
| **8** | JDS20 | Szob | |
| **9** | JDS27 | Hercegszanto | |
| **10** | JDS32 | Upstream Novi-Sad | |
| **11** | JDS35 | Tisa | |
| **12** | JDS39 | Downstream Pancevo | |
| **13** | JDS43 | Banatska Palanka/Bazias | Upstream Irongate |
| **14** | JDS46 | Vrbica/Simijan | Downstream Irongate |
| **15** | JDS60 | Chiciu/Silistra | |
| **16** | JDS65 | Reni | |
| **17** | JDS66 | Vilkova – Chilia arm/Kilia arm | |
| **18** | JDS68 | Sf.Gheorghe – Sf.Gheorghe arm | |

| Number | JDS code | Name | Remarks |
|---|---|---|---|
| **19** | | Black sea | |

**Annex 2: Sedimentation Box for suspended solid sampling in the aquatic environment**

The sedimentation box is the tool for the sampling of suspended solids for the *German Environmental Specimen Bank (see Fig. 1)*

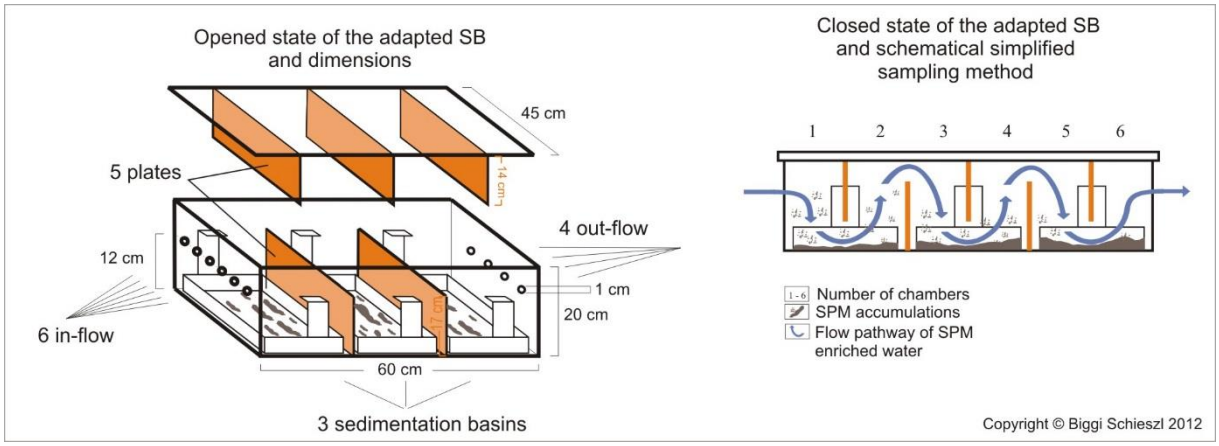



***Figure 1: Basis scheme of the sedimentation box of the German Environmental Specimen Bank***

Two operating modes are possible:

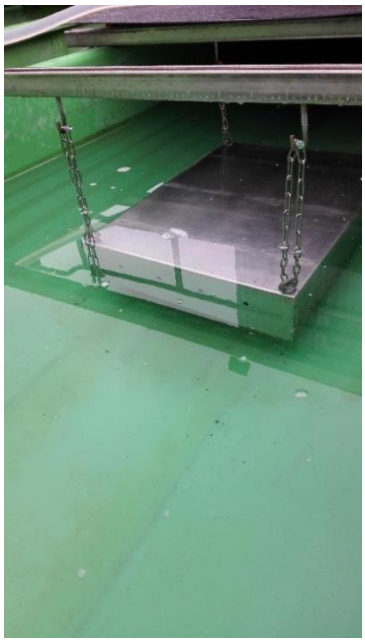


***Figure 2: Open water version (here: during a testing in the artificial stream and pond system (UBA DE -Experimental Site Marienfelde).***

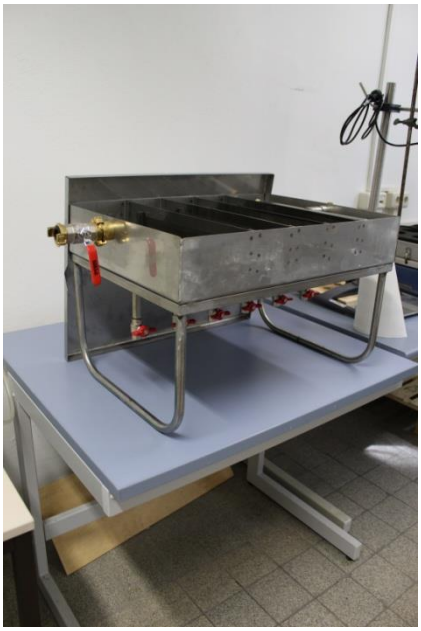


***Figure 3: Stationary version (supplied via a pump) (UBA Lab – Corrensplatz)***

Various methods of fixing are possible:

***Figure 4: Fixation under a bridge: sampling a WWTP-effluent in Ruhleben (Berlin).***

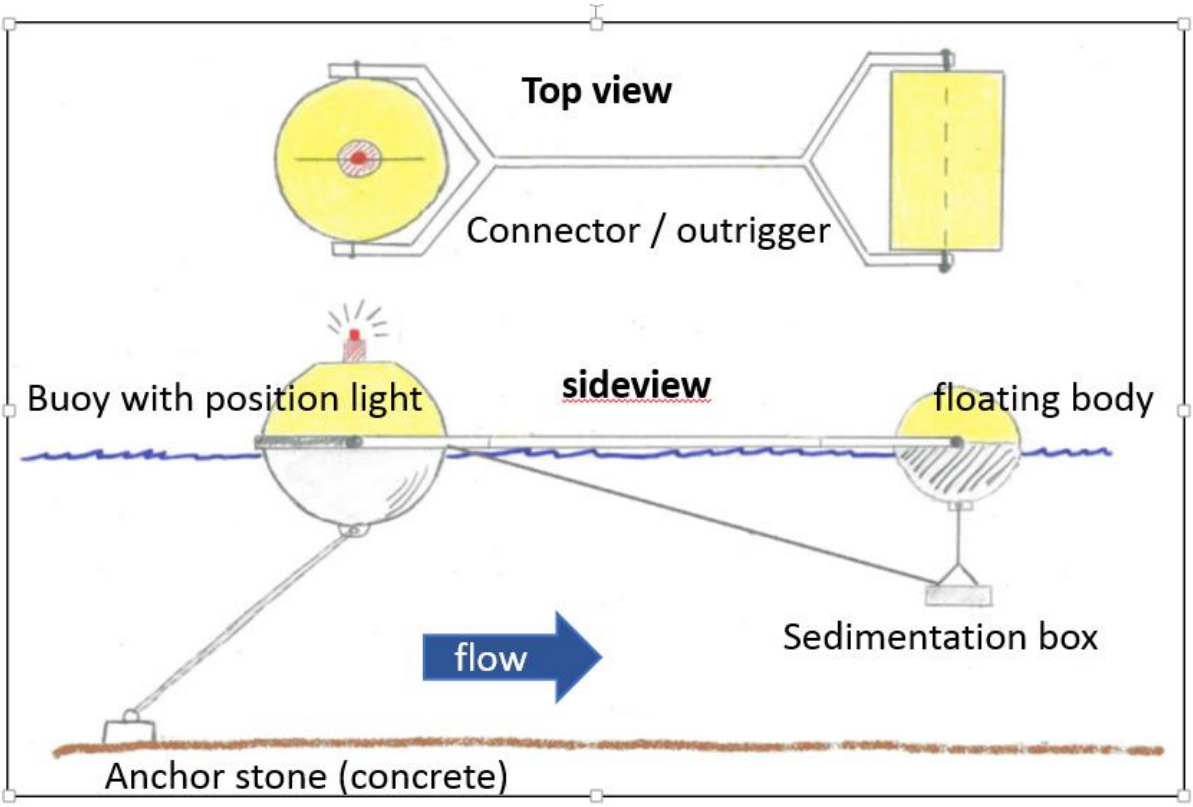



***Figure 5: Buoy construction with free-hanging sedimentation box***

*Figure 6: Open SB (stationary version) after weeks of sampling the effluent in a WWTP (Berlin).*

# Annex VII

## Guideline for Sampling and preparation of Suspended Particulate Matter - Standard Operation Procedure (SOP) *Draft version 1.0 (2018-07-19)*

**Guideline for Sampling and preparation of Suspended Particulate Matter - Standard Operation Procedure (SOP)**
*Draft version 1.0 (2018-07-19)*
**Guideline Objective**
Consistent sampling of suspended particulate matter including microplastic during the JDS 04 on the Danube River.
During the JDS 04 suspended particulate matter samples will be taken by means of sedimentation boxes. Subsequently the suspended particulate matter has to be processed through a 5 mm mesh as upper limit of the microplastic definition. The samples contain organisms like small Gammarids and worms not passing through the mesh.
The sampling takes place during one to two weeks and the composite sample has to be sent to the EA in Berlin for processing.

**Fixation of the sampling**
The sampling has to be performed by trained personal only.
The sampling of the suspended particulate matter will be realized on a two-week to monthly basis between May and September. Exceptional events (e.g. very high discharges, high precipitation, dredging activities in the water body) are to be documented.

**Realization of the sampling**
All information during sampling and the description of the sample have to be recorded in data sheets. For each sampling campaign a separated protocol has to be delivered.
Disturbances (excavation activities, activities within the river stretch, water level fluctuations close to dams and weirs) within the sampling location are to be documented in the protocol. Regular contact to water authorities have to be carried out.

**Devices for sampling and cleaning instructions**
All sampling devices need to be free of plastic material. Otherwise the application has to be noted and the certain polymer will not be analyzed for in the sample.
<u>Sampling of water samples</u>

- Water sampler (e.g. according to Niskin or Ruttner) made out of glass or stainless steel
- 1 L glass bottles for the water samples
- Thermometer
- Water-proof pen
- Cool box (for transport at 4°C)

<u>Sampling of suspended particulate matter</u>

- Sedimentation box made of stainless steel (V4A)

- Site-specific fixation for the exposition of the sedimentation box (according to the site-specific sampling plan)
- Nitril gloves
- Kleenex tissues
- Demineralized water ( 5 L) and wash bottles
- Stainless steel circular mesh (5 mm) according to ISO 3310-2: 1999
- Stainless steel container (50 L with lid and clamp) with code number engraved
- Additional glass bottles for the remains during sieving
- Photo camera (for documentation)
- Cooling box for the transport to the EA (4°C)

Pre-cleaning of devices

The pre-cleaning of the small devices and containers has to be performed in the lab by means of an automatic laboratory washing machine with chlorine-free intensive cleaner and subsequent hot washing at 90-95°C. Afterwards neutralization with 30% phosphoric acid follows and finally a hot and cold water flushing with demineralized water is applied. The devices and containers are dried at 130 °C in a drying cabinet.

The sedimentation boxes are cleaned in the field by means of native river water and brushes with only natural bristles – no plastic material is allowed. If necessary, diluted $HNO_3$ (5-10%) or bleaching lye can be applied. For organic coatings isopropanol is utilized.

Afterwards the whole box is intensively washed with river or tap water to remove remains of the cleaning process. Finally, the box is dried via white Kleenex tissues.

Sampling technique

Analysis of the content of suspended particulate matter

The sampling has to be performed at three depths (upper 50 cm of the water column, middle section of the water body, 50 cm above the sediment surface). Care has to be taken not to resuspend accumulated sediment.

The samples are transported to the lab in a cooling box at 4°C and processing should be performed within 8 hours after arrival at the lab.

The content of suspended particulate matter in 1 L of river water has to be processed according to DIN 38409-2 by vacuum filtration through 0.45 µm glass fiber filters and drying at 105°C until a constant weight is achieved. The weight of the dried filters is measured on a microbalance.

**Realization of the sampling by means of a sedimentation box within the field**

The sedimentation box is deployed directly in the water body according to the main current by means of stainless steel ropes, stainless steel chains or in necessary by a fixed stainless steel construction (see figures2-6). The 3-chamber sedimentation box has more wholes for the incoming water masses, easy to recognize (see appendix 1).

The sedimentation box should be deployed on a dynamic fixing point (e.g. a buoy, a pontoon) for a constant exposition depth of 50 cm below the water surface.

Close to a weir, lock or dam with a regulated water level the deployment can be realized by a fixed system, keeping in mind the minimal water level throughout the year.

At flow velocities above 1.5 m/s a disturbation or failure of sampling is possible. The sampling efficiency is reduced. In that case, the number of incoming wholes has to be reduced by means of silicon stoppers (see appendix 1).

**Removal of the suspended particulater matter out of the sedimentation boxes**

At the termination of the sampling campaign the whole content of the sedimentation box is gained by means of a vacuum suction (see figure 1). Afterwards the box is cleanedwith brushes within authentic river water. This subsample is added to the sample to include parts of sorbedmaterial from the walls of the sedimentation box.

*Figure 1: Vacuum suction device.*

**Documentation**

All sample information is documented in the protocol and will be handled within the net-based information system.

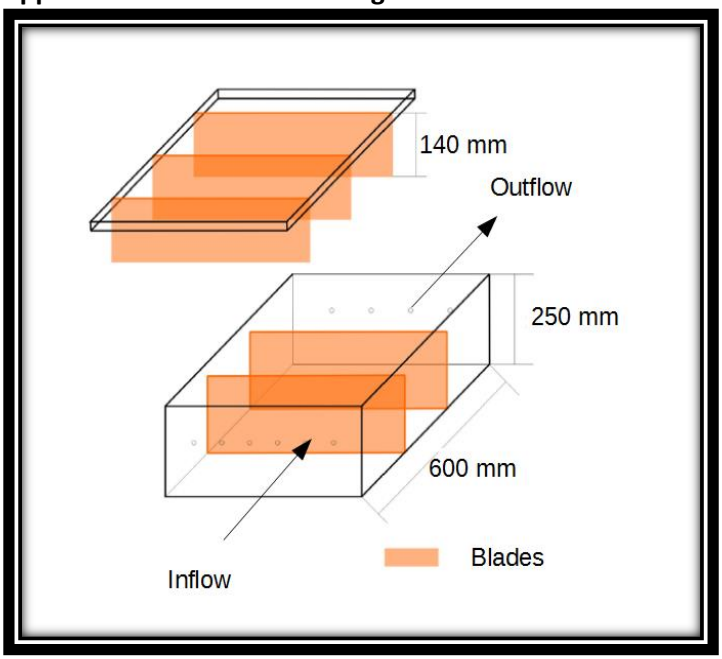
For every sampling location a master sampling plan has to be prepared, including detailed information for the definite sampling point within the river stretch. Gaussian-Krueger data of the sampling are to be givenand are to be displayed in a detailed map (1: 200 or 1: 500). Possible disturbances have to mentioned in the master sampling plan and the sampling protocol for each sampling campaign, including possible deviations from the master sampling plan.

The samples are to described according to the protocol listed below (appendix 2)

**Literature:**

Schubert, B., Heininger, P., Keller, M., Ricking, M., Claus, E. 2012: Monitoring of contaminants in suspended particulate matter and sediments – a comparison; Trends in Analytical Chemistry, 36, 58-70.

**Appendix 1: Schematic drawing of the sedimentation box**




**Appendix 2: Documentation**

| **Sampling datasheet** | |
|---|---|
| JDS 04 Microplastic screening | |
| Sampling location | Date: |
| Name and Organisation of the Sampling technician | |
| Detailed information about the sampling location | |
| Gaussian-Krueger-coordinates:<br>Latitude: | Longitude: |
| River kilometer [km]: | |
| River branch (in flow direction): | |
| Water Level: | |
| <u>Sampler deployment</u>:<br><br>stainless steel rope | stainless steel chain fixed exposition |
| Deployment depth: | |
| Sampling Time [days]: | |

| |
|---|
| Motivation of the Sampling Campaign |
| <u>Basic Parameters:</u><br>Conductivity<br>Temperature<br>Colour<br>Odour |

**Appendix 3: Photo documentation**

*Figure 2: Exposition by means of stainless steel ropes from a bridge.*

*Figure 3: Exposition by means of stainless steel chains at a weir.*

*Figure 4: Exposition by means of stainless steel chains from a bridge.*

*Figure 5: Exposition by means of stainless steel chains on a buoy directly in the water body.*

*Figure 6: Rigid fixation of the sedimentation box for high water currencies.*

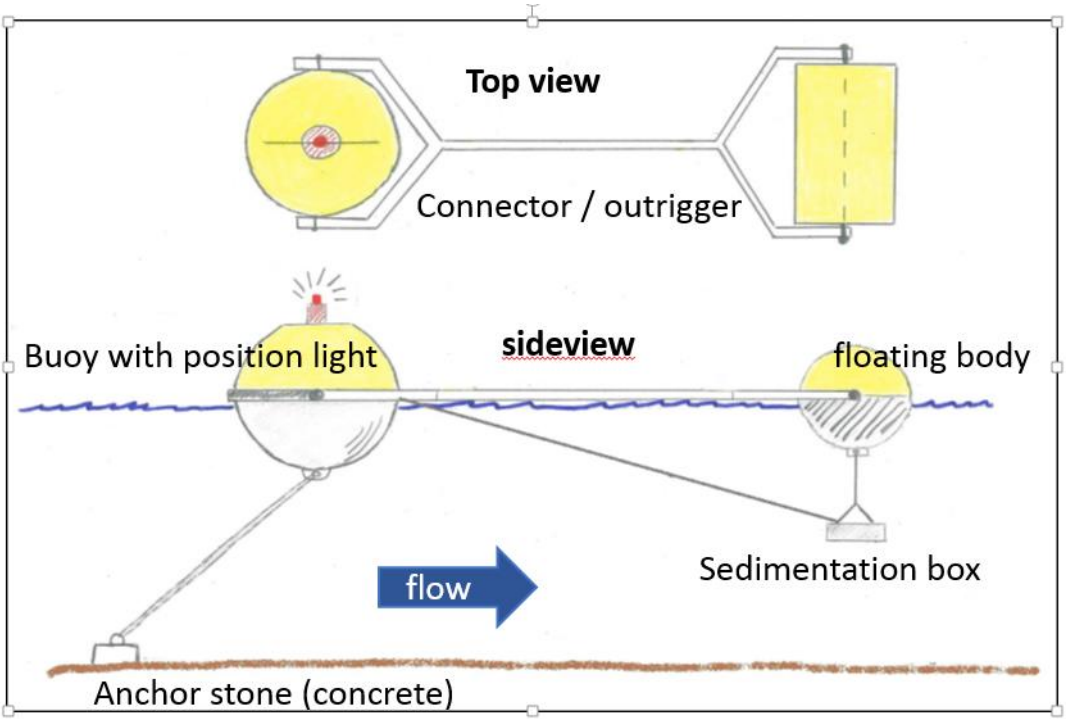



*Figure 7: Construction of a buoy for the deployment of a sedimentation box in the water body.*

# Annex VIII

## JDS4 **Microbiology Program** (JDS4 Survey Plan Annex F)

(Third draft version April 26, 2018)

*Alexander Kirschner[1,2], Andreas Farnleitner[1,3], Stoimir Kolarevic[4], Klemens Kittinger[5], Gernot Zarfel[5]*
[1]Interuniversity Cooperation Centre for Water and Health, [2] Medical University Vienna, Karl Landsteiner University of Health Sciences, Krems, [4] University of Belgrade, [5] Medical University Graz

**STATEMENT**

During JDS 4 an ambitious and scientifically innovative microbiology program shall be performed. This program shall combine standard faecal pollution monitoring, microbial faecal source tracking, bacterial microbiome analysis, antibiotic resistance analysis and microbial ecology. Several innovative methodical and conceptual approaches will be included in all mentioned topics. With this program the knowledge on the most relevant aspects of the microbiology of the Danube will be significantly advanced leading to a better understanding and management of this most important river in Europe.

**EXPERT TEAM**

The expert team developing the microbiology concept will consist of members of the Interuniversity Centre for Water and Health at the Medical University Vienna (PI: Assoc. Prof. Dr. Alexander Kirschner) and at the Karl Landsteiner University of Health Sciences (PI: Assoc. Prof. Dr. Andreas Farnleitner), the Medical University Graz (PI: Dr. Clemens Kittinger, Priv.-Doz. Dr. Gernot Zarfel) and the University of Belgrade (PI: Dr. Stoimir Kolarevic). All these experts have explicit practical and scientific experience in the field of microbiology of rivers and have already participated in earlier JDS (see literature list, 9 peer-review journals, 1 book chapter, 10 JDS report chapters).

Several partners from all participating countries along the Danube will be integrated into the research team. Collaborations already exist for Germany and Hungary, for other countries (especially Slovakia, Croatia, Romania and Bulgaria) potential partners still have to be found. These national partners shall help with sampling, perform parallel measurements for quality assurance, and enable connection to national microbiological programs.

Dr. Alexander Kirschner will be the coordinator of the whole team and will be nominated as JDS Core team member.

**FINANCING**

As for JDS3, the overwhelming part (approx. 400.000 €) of the JDS4 microbiology program will be financed by third parties (like the Austrian Science Fund FWF and others). However, we propose that a basic funding for standard faecal pollution monitoring will be covered by the ICPDR/BMST (approx. 15.000 €). As well, we need to have the opportunity to use the logistic infrastructure (sample transport, cooling, freezing, sampling permissions) of the ICPDR.

**LOGISTICS**

For being able to perform the planned microbiology program, selected microbiology team members will have to travel down the Danube for about three weeks. The survey will be conducted in cooperation with eDNA team which also expressed interest in a longitudinal survey of the Danube. It is optimally to start the campaign between July and August (range mid June - mid September) which will be decided in coordination with eDNA team. For this purpose, a laboratory bus will be hired allowing basic on-site laboratory work (filtration of samples, cultivation of bacteria, incubation at 37°C and 44°C, short-term storage of samples at 4°C and -20°C, running an on-line flow cytometer and

epifluorescence microscope) and accommodation for two people. Most probably, two 3-person teams will change in Belgrade, as during previous JDS.

- **Help of national teams/coordinators**

○ We count on the help of the national teams primarily by means of providingbasic hydrological data such as actual discharge information, whether data at the sites approached within the microbiological campaign. Yet, this kind of support has to be confirmed by the national coordinators.
○ Moreover, we would need a list of possible stations for the lab-truck (university campus, camping place, etc.) with permanent electricity supply which has to be defined for each country by the national coordinators. Preferable stations are indicated in blue in the **Table 1**.
○ Additional samples prepared/analyzed by national teams highly welcome and needed only for basic microbiology program. Therefore, national experts for microbiology are necessary to be nominated within the national teams (Germany, Slovakia, Hungary, Romania); in Austria: Alexander Kirschner, in Serbia: Stoimir Kolarevic. Training of the national experts for basic program in advance is possible and necessary only when no funding of the advanced program is provided by third parties. In this case, national teams have to perform sampling for all microbiological samples.

- **Logistic support by ICPDR**

○ Regular (2 days) transport of samples (4°C, -20°C) from the lab-bus to the main lab in Vienna
○ Regular (2 days) transport of lab material (4°C, -20°C) from the main lab in Vienna to the lab bus
○ Regular (2 days) transport of laboratory waste (plastic, autoclaved fluids, etc) (can also be done by the national teams)
○ List of safe places, with electricity, sanitary facilities, in each country where to stay overnight in a caravan along the Danube

**SELECTED SITES**

- Due to the changed sampling strategy (cars vs ships) less sampling sites can be investigated during JDS4. The microbiology expert team defined the preliminary list of 38 sampling sites that shall be included in the investigations. The list is not definitive and will be updated in agreement with eDNA team and national experts. A nested sampling design will be applied: sampling sites will be divided into two categories: (i) “routine sites – marked with green” where a basic program is run and (ii) “hot-spot sites” – marked with yellow, where the full microbiology program is performed. Not more than 3 sites per day (max 2 yellow sites) will be processed. Experience from past JDS will guide the selection of the sampling sites. For each site, water samples and biofilms (form stones, according to phytobenthos sampling) will be taken. At most sites, samples from left, middle and right side will be analyzed. The basic program will at least consist of standard faecal indicators, DNA filtration for total microbiome and microbial faecal source tracking analysis and total cell numbers.

**SAMPLING**

- Due to mutual interests the sampling should be done together with eDNA team. The team for microbiology is currently discussing the possibility of using a sampling boat provided by eDNA team. Samples will be transported by a small car provided by microbiology team to the lab-bus by (hired lab container on truck or similar, stationed at a fixed place, can be shared with if necessary eDNA team e.g. storage).

**CENTRAL GOAL**

By the synergistic combination of different thematic and methodic microbiological approaches a comprehensive picture of the microbiology as regards patterns of microbial faecal pollution, antibiotic

resistances and the total bacterial population (microbiome and standing stock analysis) will be developed. The input of the national experts (parallel measurements, connection to national microbiology programs) and additional data made available by other expert groups (antibiotic concentrations, heavy metals, chemical anthropogenic wastewater markers, etc) will deliver important background information for a better interpretation of the observed microbiological patterns. An overview of the inter-connections between the different topics can be found in **Figure 1**.

**METHODS PROPOSED**

- **Standard faecal indicators:***E. coli* and intestinal Enterococci, ev. *Clostridium perfringens* (KIRSCHNER ET AL 2009, KIRSCHNER ET AL 2015, KIRSCHNER ET AL 2017); should be performed in the camper van or/and in the laboratories of the national partners (parallel measurements).
- **Cultivation of selected species relevant for antibiotic resistence:** *E. coli* , Enterococci, Klebsiella, Pseudomonas (KITTINGER ET AL 2016A, KITTINGER ET AL 2016B); cultivation in the home lab after cooling agar plates at 4°C and testing for a wide range of antibiotics (including multi-resistance, ESBL, CARB, VRE, etc.)
- **DNA-Filtration for**
  - **microbiome analysis:** 16S rDNA targeted (SAVIO ET AL 2015): development of the total bacterial community along the Danube; identification of microbial faecal pollution/source tracking
  - **resistome analysis:**qPCR-detection of specific antibiotic resistance factors in the bacterial genomes
  - **microbial source tracking:** qPCR detection of selected host-associated markers (human, ruminant, pig, etc.)
  - **detection of total faecal pollution:** qPCR for enterococci
- **Cell based methods:**
  - **On-line Flow Cytometry(FCM):** on-site (camper van) determination of bacterial numbers in an online flow cytometer
  - **Epifluorescence Microscopy (EFM):** calibration of FCM data, cell morphotype discrimination, cell volume determination for biomass calculation (VELIMIROV ET AL 2011)
  - **Fluorescence in situ hybridization(FISH):** FISH in combination with solid phase cytometry for cell-based determination of faecal indicators (faecal-FISH)
- **Comprehensive statistical analysis:** linking microbiological data sets among each other and with data sets from other JDS4 research groups (chemistry, biology)

**ADDITIONAL DEMANDS**

- **Advanced chemistry**

Parameters total P, total N, total organic C, chlorophyll a, heavy metals (at least one), selected antibiotics (Fluoroquinolones, Sulfonamides, Tetracyclines) need to be analyzed from the same samples which are used for microbiological analyses. So far Serbia (SEPA and IBISS) offered analyses for total P, total N, total organic C, and at least one metal in water as an in-kind. Detailed protocols for sampling/sample processing/storage and information on vessels are necessary.

- Basic hydrological data such as discharge, precipitation are needed from the hydro morphology experts from the national teams.

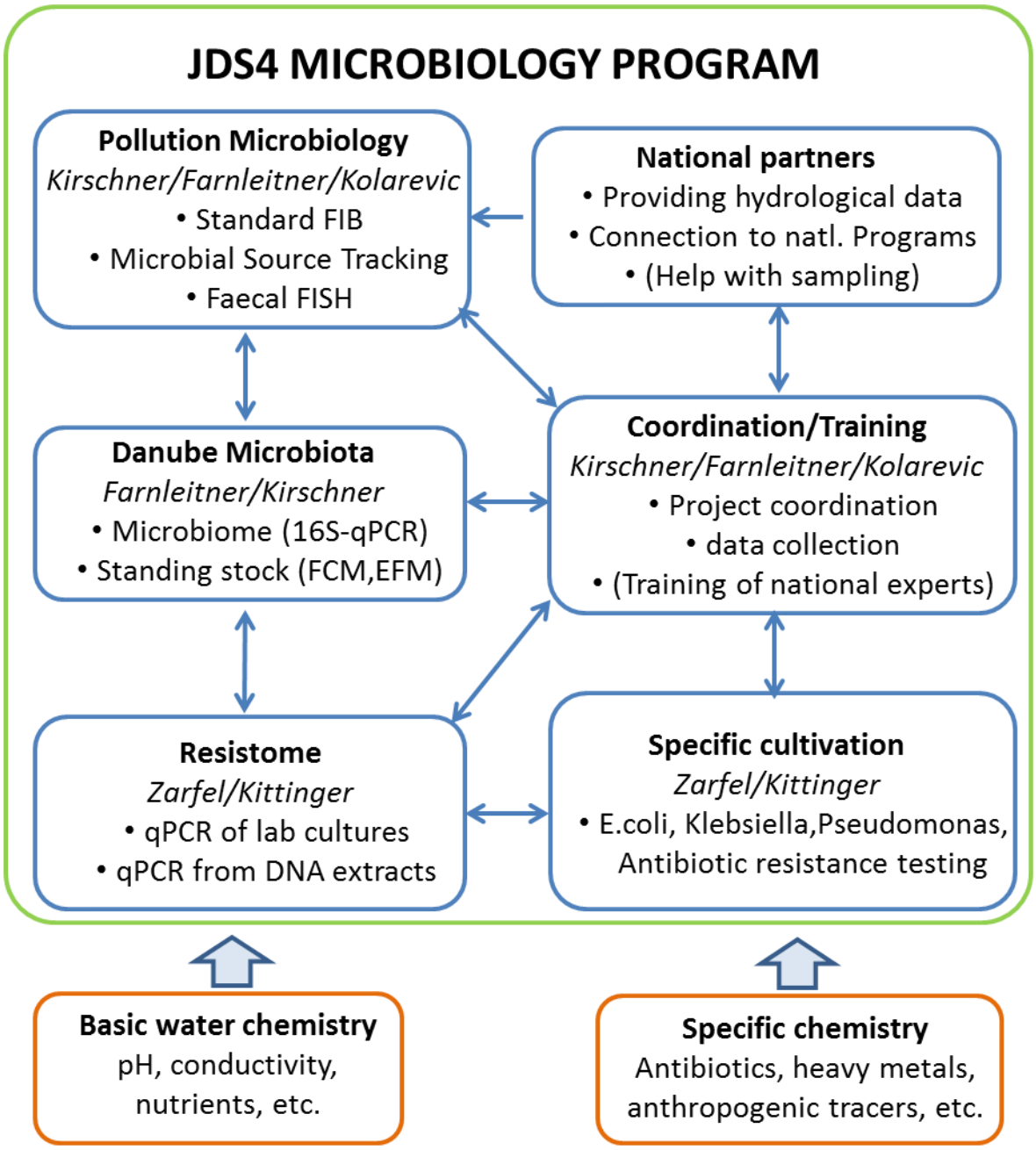



**Figure 1:**Draft of the planned JDS 4 Microbiology Program showing the integrated interaction of the three main topics (i) pollution microbiology, (ii) microbial population ecology and (iii) bacterial antibiotic resistance and virulence. FIB: faecal indicator bacteria, qPCR: quantitative PCR, FCM: flow cytometry; EFM: epifluorescence microscopy; FISH: fluorescence in situ hybridization; 16S: large subunit of bacterial ribosomes

**LITERATURE**

**Peer-review journals**

1. Kirschner AKT, Kavka GG, Velimirov B, Mach RL, Sommer R, Farnleitner AH (2009) Microbiological water quality along the Danube River: Integrating data from two whole-river surveys and a transnational monitoring network. *Water Research 43: 3673-3684*

2. Kirschner AKT, Reischer GH, Jakwerth S, Savio D, Toth E, Sommer R, Mach RL, Linke R, Kolarevic S&Farnleitner AH(2017) Multidimensional monitoring of microbial faecal pollution reveals dominance of human contamination along the whole Danube River. *Water Research 124: 543-555*
3. Kittinger C, Baumert R, Folli B, Lipp M, Liebmann A, Kirschner A, Farnleitner AH, Grisold AJ and Zarfel GE. Preliminary Toxicological Evaluation of the River Danube Using in Vitro Bioassays. Water-SUI 7, 1959-1968 (2015)
4. Kittinger C, Lipp M, Folli B, Kirschner A, Baumert R, Galler H, Grisold AJ, Luxner J, Weissenbacher M, Farnleitner AH, Zarfel G **(20**16a) Enterobacteriaceae Isolated from the River Danube: Antibiotic Resistances, with a Focus on the Presence of ESBL and Carbapenemases. *PLoS One 11(11): e0165820*
5. Kittinger C, Lipp M, Baumert R, Folli B, Koraimann G, Toplitsch D, Liebmann A, Grisold AJ, Farnleitner AH, Kirschner AKT, Zarfel **G** (2016b) Antibiotic resistance patterns of Pseudomonas spp. isolated from the River Danube. *Front. Microbiol. 7: 586*
6. Savio D, Sinclair L, Ijaz U, Parajka J, Reischer G, Stadler P, Blaschke AP, Bloeschl G, Mach R, Kirschner A, Farnleitner AH, Eiler A. Bacterial diversity along a 2600 km river continuum. *Environmental Microbiology 17(12): 4994-5007*
7. Velimirov B, Milosevic N, Kavka GG, Farnleitner AH, Kirschner AKT *(2011)* Development of the Bacterial Compartment Along the Danube River: A Continuum Despite Local Influences. *Microbial Ecology 61: 955-967*
8. Deutschmann B, Kolarević S, Brack W, Kaisarević S, Kostić J, Kračun-Kolarević M, Liska I, Paunović M, Seiler TB, Shao Y, Sipos S, Slobodnik J, Teodorović I, Vuković-Gačić B, Hollert H (2016) Longitudinal profile of the genotoxic potential of the River Danube on erythrocytes of wild common bleak (*Alburnus alburnus*) assessed using the comet and micronucleus assay. Science of The Total Environment*573*: 1441-1449.
9. Kolarević S, Kračun-Kolarević M, Kostić J, SlobodnikJ, LiškaI, Gačić Z, Paunović M, Knežević-Vukčević J, Vuković-Gačić B (2015) Assessment of the genotoxic potential along the Danube River by application of the comet assay on haemocytes of freshwater mussels: The Joint Danube Survey 3. Science of The Total Environment*540*: 377-385.

**Book article**

*1.* Kirschner AKT, Kavka GG, Reischer GH, Sommer R, Blaschke AP, Stevenson M, Vierheilig J, Mach RL, Farnleitner AH (2015) Microbiological Water Quality of the Danube River: Status Quo and Future Perspectives. *Handbook of Environmental Chemistry, special volume "The Danube River Basin"; Liska I &Slobodnik J (eds), Springer Verlag; Berlin, ISBN: 978-3-662-47739-7*

**JDS reports**

1. Kirschner A, Jakwerth S, Kolarevic S, Sommer R, Blaschke AP, Kavka G, Reischer GH & Farnleitner AH (2015) Bacterial Faecal Indicators. In: Liska I, Wagner F, Sengl M, Deutsch K, Slobodnik J (ed.) Joint Danube Survey 3 - A comprehensive Analysis of Danube Water Quality, Final scientific report. ICPDR, Vienna, Austria, ISBN 978-3-200-03795-3
2. Reischer GH, Kirschner A, Schnitzer G, Savio D, Mach RL, Bahlmann A, Schulze T, Brack W & Farnleitner AH (2015) Microbial Faecal Source Tracking. In: Liska I, Wagner F, Sengl M, Deutsch K,

Slobodnik J (ed.) Joint Danube Survey 3 - A comprehensive Analysis of Danube Water Quality, Final scientific report. ICPDR, Vienna, Austria, ISBN 978-3-200-03795-3

3. Zarfel G, Folli B, Lipp M, Pfeifer B, Baumert R, Farnleitner AH, Kirschner A & Kittinger C (2015) Spread of non-wild type antibiotic resistant phenotypes in the river Danube. In: Liska I, Wagner F, Sengl M, Deutsch K, Slobodnik J (ed.) Joint Danube Survey 3 - A comprehensive Analysis of Danube Water Quality, Final scientific report. ICPDR, Vienna, Austria, ISBN 978-3-200-03795-3
4. Kirschner A, Jakwerth S, Kolarevic S, Premm B, Reischer GH & Farnleitner AH (2015) Microbial Ecology. In: Liska I, Wagner F, Sengl M, Deutsch K, Slobodnik J (ed.) Joint Danube Survey 3 - A comprehensive Analysis of Danube Water Quality, Final scientific report. ICPDR, Vienna, Austria, ISBN 978-3-200-03795-3
5. Lettieri T, Ferrero V, Duque L, Lahm A, Kirschner A, Farnleitner AH & Carvalho RN (2015) Microbial Metagenomics. In: Liska I, Wagner F, Sengl M, Deutsch K, Slobodnik J (ed.) Joint Danube Survey 3 - A comprehensive Analysis of Danube Water Quality, Final scientific report. ICPDR, Vienna, Austria, ISBN 978-3-200-03795-3
6. Yeh TC, Bondar-Kunze E, Tomas D, Kirschner A, Marjanovic-Rajcic M, Welti N & Hein T (2015) Quality and quantity of dissolved organic matter. In: Liska I, Wagner F, Sengl M, Deutsch K, Slobodnik J (ed.) Joint Danube Survey 3 - A comprehensive Analysis of Danube Water Quality, Final scientific report. ICPDR, Vienna, Austria, ISBN 978-3-200-03795-3
7. Bahlmann A, Lochen T, Schulze T, Kirschner A, Brack W, Schneider RJ, Krauss M (2015) Chemical and immunochemical analysis of anthropogenic markers and organic contaminants. In: Liska I, Wagner F, Sengl M, Deutsch K, Slobodnik J (ed.) Joint Danube Survey 3 - A comprehensive Analysis of Danube Water Quality, Final scientific report. ICPDR, Vienna, Austria, ISBN 978-3-200-03795-3
8. Deutschmann B, Kolarević S, Hollert H, Kaisarević S, Kostić J, Seiler TB, Sipos S, Teodorović I, Vuković-Gačić B (2015) Biomarkers: In-situ detection of genotoxicity of the Danube River. In: Liska I, Wagner F, Sengl M, Deutsch K, Slobodnik J (ed.) Joint Danube Survey 3 - A comprehensive Analysis of Danube Water Quality, Final scientific report. ICPDR, Vienna, Austria, ISBN 978-3-200-03795-3
9. Kirschner AKT, Kavka GG, Velimirov B, Reischer GH, Mach RL, Farnleitner AH (2008) Microbiological water quality and DNA based quantitative microbial source tracking. Joint Danube Survey 2, Final scientific report. Liska I, Wagner F, Slobodnik J (eds.) ICPDR Vienna, Austria; pp 86-95
10. Velimirov B, Milosevic N, Hein T, Kavka G, Farnleitner A, Kirschner AKT (2008) Patterns of bacterial parameters in the Danube River: Are tributaries a determining factor? Joint Danube Survey 2, Final scientific report. Liska I, Wagner F, Slobodnik J (eds.) ICPDR Vienna, Austria; pp 96-105

# Annex IX

## JDS4 Survey Plan ANNEX B6: Zooplankton

**ZOOPLANKTON PROGRAM – JDS4**

**Sampling:**

For the zooplankton analysis samples are taken from the upper 50 cm water-layer. With a bucket (10 L) totals 100 litres water are filtered through plankton net at every sampling sites. The samples are preserved in the field with formaldehyde to 4-5 % concentration.

From the zooplankton community two main groups – Rotifera assemblage and Crustacea assemblage – will be investigated by different expert, therefore 2x100 litres samples are needed from every sampling site.

| | **1. Sample for Rotifera community analysis** | **2. Sample for Crustacea community analysis** |
|---|---|---|
| Instrument | Plankton net with **50 µm** mesh size | Plankton net with **70 µm** mesh size |
| Sample volume | 100 litres | 100 litres |
| Danube profile | Left side (L), Middle (M), Right side (R) | Left side (L), Middle (M), Right side (R) |
| Tributaries profile | Middle (M) | Middle (M) |
| Preservation | with formaldehyde to 4-5 % concentration | with formaldehyde to 4-5 % concentration |

**Sampling sites:**
The sampling sites are the same as Microbiology program + Baja at 1481 rkm. The bacteria are important food for many non-selective filter feeder zooplankton organisms. In this way may be a possibility to analyse the functional connections.
These are altogether 39 sampling site.

**Analysis:**
From the filtered sample a known part quality is investigated. The quantitative and qualitative composition of zooplankton community is determined with light- and stereo microscope. Exact identifying of many rotifer species (especially with soft or semi-hard cuticle) is possible only with studying of trophy. For this purpose is necessary to preparation the trophy from surrounding soft tissues, to which household bleach (NaOCl) is used.

**Evaluate of results:**
The changes of abundance and taxon composition along the longitudinal section of Danube and differences in the profile. Diversity analysis with Shannon-Wiener index. Effects of the tributaries and pollution on the zooplankton community and water quality by the indicator species. Presence and abundance of invasive species. Changes of the water quality compared with the ecological status in 2013.

# Annex X

## JDS4 Survey Plan ANNEX B7: Invasive alien species

**I Introduction**

The assessment of the ecological and economical/societal impacts of the introduction of non-indigenous species (NIS) became to be one of the primary focus areas of bioinvasion or biopollution science (Olenin et al., 2007; Panov et al., 2009).The emerging issue of invasive alien aquatic species in the Danube River Basin (DRB) was considered at the 11thMonitoring and Assessment Expert Group (MA EG) Meeting (Prague 18-19 March 2010), as well as during consequent MA EG meetings. The importance of the assessment of bioinvasion pressure to aquatic ecosystems was evidently recognized by the Member States, especially in terms of the WFD implementation. According to the opinion of the MA EG, it is important to collect the data on invasive species in order to properly manage the issue of pressures caused by biological invasions within the DRB.

The Danube expeditions have been recognized as valuable source of information related to biological invasions within the DRB, which is also expected from JDS4.

The aim of this document is to propose methodology of collecting and processing the data on IAS during JDS4.

**II Preparation phase**

For successful collection and processing of the data on IAS it is necessary to have checklist with basic authecological information and invasiveness of each species.

For that purpose, the list of alien species of the Danube River has been prepared (Paunović and Csányi, 2018).

Since the JDS4 will cover the main tributaries, the list should be further developed to cover selected water bodies at tributaries relevant for the DRBMP, as well (list of the ICPDR Guidance document for IAS (Paunović and Csányi, 2018):

1. March/Morava;
2. Drau/Drava;
3. Tysa/Tisza/Tisa,
4. Sava;
5. Tamiš/Timis and
6. Velika Morava.

On 28th MA EG in Ljubljana it was agreed to address IAS issue for all tributaries that are “of basin wide importance”, more precisely those that are the subject of the Danube River Basin Management Plan – tributaries with basin area larger than 4,000 sq. km. It was also concluded that additional WBs that are known to be of particular interest from the aspect of biological invasions should be addressed. Those WBs should be nominated by the countries.

The preliminary list of IAS for selected tributaries and WBs suggested by the countries should be finalized until the end of March 2019, in order to be discussed on MA EG prior to the start of the JDS4.

The template for data collection should be prepared for all Biological Quality Elements (BQE) covered by the JDS4.

The national coordinators should nominate the persons responsible for the IAS data collection (possibly until the end of2018 – in order to have enough time to update the list of the IAS for tributaries).

**III Procedure of data collection during JDS4**

The same dataset related for each Biological Quality Element will be used for collection of information on the IAS during the JDS4.

Persons nominated to be responsible in each national team will prepare the data (extracted from data on BQE) in agreed template and deliver to Core Team IAS expert and JDS4 Co-leader for biology.

Additional data will be collected by specific additional procedures for macroinvertebrate collection: K&S sampling on selected sites (to be agreed), additional monitoring for molluscs and by using LiNi traps.

**IV Data Processing**

Core Team IAS expert and JDS4 Co-leader for biology will process the data delivered by the national experts responsible for IAS based on agreed methodology.

We suggest using national WBs for assessment units for the pressure caused by IAS.

We suggest the use of the following indexes for the assessment of the pressures caused to the WB.

- SBC Index(Arbačiauskas et al., 2008; Panov et al., 2009) - macroinvertebrates and fish;
- BPL index (Olenin et al., 2007) – all BQE and
- BAI index (Paunović and Csányi, 2018) - all BQE.

The specific input of national experts will be needed for application of the BPL index for tributaries (Olenin et al., 2007), since it requires specific expert knowledge.

For more information, please see http://www.corpi.ku.lt/databases/index.php/binpas/

On this web page, you can find the information about the use of the index, as well as create account for on-line use of existing data and creating your own assessment.

TO DESCRIBE PROCEDURE OF USE OF EACH INDEX IN DETAIL – TO SUMMARIZE FROM IAS GUIDANCE DOCUMENT AND TO PROVIDE "COOK BOOK"

The SBC assessment is derived from data on number of non-indigenous species and their abundance in comparison to a total number of species and community abundance, using the abundance (ACI) and richness contamination index (RCI), using the following equations:

$$ACI = Na/Nt,$$

where Na and Nt are numbers of specimens of alien taxa and total specimens in a sample, respectively, and

$$RCI = na/nt,$$

where na is the total number of alien taxa, and nt is the total number of identified taxa.

With values of ACI and RCI, the site-specific biocontamination index (SBC) can then be derived from matrix:

| RCI % | ACI % | | | | |
|---|---|---|---|---|---|
| | 0 | >0 - <10 | >10-20 | 21-50 | >50 |
| 0 | 0 | | | | |
| >0 - <10 | | 1 | 2 | 3 | 4 |
| >10-20 | | 2 | 2 | 3 | 4 |
| 21-50 | | 3 | 3 | 3 | 4 |

| | | | | | |
|---|---|---|---|---|---|
| >50 | | 4 | 4 | 4 | 4 |

The index value ranges from 0 (“no” biocontamination) to 4 (“severe” biocontamination).

Bioinvasion Assessment Index (BAI) - final score for each species assessed by the IAS-RAP-Danube(Paunović and Csányi, 2018) is linked with abundance of non-indigenous taxa, by applying the following calculation:

$$BAI=(N1*P1+N2*P2...Nn*Pn)/A,$$

where N is the abundance of each recorded alien species, P is “Relative contribution – Pondering Value” from IAS-RAP-Danube for each recorded alien species and A is total abundance of assessed community.
The described BAI index take into the consideration both, abundance and characteristics of each particular taxa (by involving the “Relative contribution – Pondering Value”).
In theory, index range is between 0 and 1.

**V Reporting**

Core Team IAS expert and JDS4 Co-leader for biology will be responsible for report preparation.
The report should be of similar structure as Chapter on the IAS in the JDS4 Scientific Report (Paunović et al., 2015). It should contain assessment of the status of the IAS recorded during JDS4, comparisons with previous surveys, as well as clear recommendations about needs for mitigation measures and technical possibilities.

**VI References**

Arbačiauskas, K., Semenchenko, V., Grabowski, M., Leuven, R.S.E.W., Paunović, M., Son, M.O., Csányi, B., Gumuliauskaite, S., Konopacka, A., Nehring, S., van der Velde, G., Vezhnovetz, V., Panov, V.E., 2008. Assessment of biocontamination of benthic macroinvertebrate communities in European inland waterways. Aquat. Invasions. https://doi.org/10.3391/ai.2008.3.2.12

Olenin, S., Minchin, D., Daunys, D., 2007. Assessment of biopollution in aquatic ecosystems. Mar. Pollut. Bull. 55, 379–394. https://doi.org/10.1016/j.marpolbul.2007.01.010

Panov, V.E., Alexandrov, B., Arbaciauskas, K., Binimelis, R., Copp, G.H., Grabowski, M., Lucy, F., Leuven, R.S.E.W., Nehring, S., Paunović, M., Semenchenko, V., Son, M.O., 2009. Assessing the risks of aquatic species invasions via European inland waterways: from concepts to environmental indicators. Integr. Environ. Assess. Manag. 5, 110–126. https://doi.org/10.1897/IEAM_2008-034.1

Paunović, M., Csányi, B., 2018. Guidance document on Invasive Alien Species (IAS) in the Danube River Basin, ICPDR.

Paunović, M., Csányi, B., Stanković, I., Graf, W., Leitner, P., Brammer, V., Huber, T., Szekeres, J., Borza, P., 2015. Invasive Species, in: Liška, I., Wagner, F., Sengl, M., Deutsch, K., Slobodník, J. (Eds.), Joint Danube Survey 3: A Comprehensive Analysis of Danube Water Quality. International Commission for the Protection of the Danube River - ICPDR, Vienna, p. 140’147.

# Annex XI

## The EU Standard Number of methods for the investigated compounds in surface water

| Compound | dimension | EU Standard Number |
|---|---|---|
| **Physico-chemical parameters** | | |
| pH | -log($H^+$) | EN ISO 10523:2012 |
| specific electronic conductivity | µS/cm | EN 27888:1993, ISO 7888:1985 |
| total suspending matter (TSM) | mg/L | ISO 12750-6:1971, ISO 448-33:1985 |
| total dissolved material | mg/L | ISO 12750-6:1971, ISO 448-19:1986 |
| potassium ion ($K^+$) | mg/L | ISO 9964-3:1993 |
| sodium ion ($Na^+$) | mg/L | ISO 9964-3:1993 |
| calcium ion ($Ca^{+2}$) | mg/L | ISO 7980:1986 |
| magnesium ion ($Mg^{+2}$) | mg/L | ISO 7980:1986 |
| chloride ion ($Cl^-$) | mg/L | ISO 9297:1989 |
| sulphate ion ($SO_4^{-2}$) | mg/L | ISO 9280:1990 |
| hydrogen carbonate ion ($HCO_3^-$) | mg/L | ISO 448-11:1986, EN ISO 9963-1,2:1995 |
| carbonate ion ($CO_3^{-2}$) | mg/L | ISO 448-11:1986, EN ISO 9963-1,2:1995 |
| dissolved oxygen | mg/L | EN 25813:1992, ISO 5813:1983 |
| oxygen saturation | % | EN ISO 5814:2012 |
| ($BOI_5$) $BOD_5$ biological oxygen demand | mg/L | EN 1899-2:1998, ISO 5815:1989 |
| (KOIek) CODek chemical oxigen demand (Cr) | mg/L | ISO 6060:1989, ISO 15705:2002 |
| (KOIep) CODep chemical oxigen demand ($KMnO_4$) | mg/L | EN ISO 8467:1995 |
| total organic carbon (TOC) | mg/L | EN 1484:1997 |
| organic bonding N | mg/L | ISO 448-27:1985 |
| inorganic N | mg/L | ISO 260-12:1987, ISO 448-27:1985 |
| total organic formed-P | mg/L | ISO 15681-2:2003 |
| ammonium-N ($H_3N$) | mg/L | ISO 7150-1:1984 |
| nitrite-N ($NO_2^-$-N) | mg/L | EN ISO 13395:1996 |
| nitrate-N ($NO_3^-$-N) | mg/L | EN ISO 13395:1996 |

| | | |
|---|---|---|
| dissolved ortophosphate-P (o-$PO_4^{-3}$-P) | mg/L | EN ISO 6878:2004 |
| total-N | mg/L | ISO 29441:2010 |
| total-P | mg/L | ISO 260-20:1980, ISO 12750-17:1974 |
| a-chlorophyll | µg/L | ISO 10260:1992 |
| total and dissolved iron (Fe) | mg/L | ISO 1484-3:2006, EN ISO 17294-2:2016 |
| total and dissolved manganese (Mn) | mg/L | ISO 1484-3:2006, EN ISO 17294-2:2016 |
| total and dissolved copper (Cu) | µg/L | ISO 1484-3:2006, EN ISO 17294-2:2016 |
| total and dissolved cadmium (Cd) | µg/L | ISO 1484-3:2006, EN ISO 17294-2:2016 |
| total and dissolved nickel (Ni) | µg/L | ISO 1484-3:2006, EN ISO 17294-2:2016 |
| total and dissolved zinc (Zn) | µg/L | ISO 1484-3:2006, EN ISO 17294-2:2016 |
| total and dissolved chromium (Cr) | µg/L | ISO 1484-3:2006, EN ISO 17294-2:2016 |
| total and dissolved lead (Pb) | µg/L | ISO 1484-3:2006, EN ISO 17294-2:2016 |
| total and dissolved arsenic (As) | µg/L | ISO 1484-3:2006, EN ISO 17294-2:2016 |
| total and dissolved mercury (Hg) | µg/L | EN ISO 17852:2008 |
| chromium(VI), Cr(VI) | µg/L | ISO 1484-3:2006, EN ISO 17294-2:2016 |
| EPH-GC (Extractible of Petrol Hydrocarbons) | µg/L | EN ISO 9377-2:2000 |
| ***Biological parameres*** | | |
| phytobenthos | | EN 14407:2014, EN 115708:2009 |
| phyitoplankton | | EN 15204:2006 |
| fish | | EN 14962:2006, EN 14011:2003, unique method |
| macrozoobenthos | | EN10870:2012, EN 16150:2012, unique method |
| macrophytes - irrelevant (rivers with deep water, frequent water level changes, high turbidity) | | |
| ***Priority substances*** | | |
| Alachlor | ng/L | EN ISO 10695:2000 |
| Anthracene | ng/L | EN ISO 17993:2003 |
| Atrazine | ng/L | EN ISO 10695:2000 |
| Benzene | ng/L | EN ISO 15680:2003 |
| Brominated diphenylethers | ng/L | EN 16694:2015 |
| Carbon tetrachloride | ng/L | EN ISO 15680:2004 |
| Chloroalkanes, C10-13 | ng/L | EN ISO 12010:2014 |

| | | |
|---|---|---|
| Chlorfenvinphos | ng/L | EN 12918:1999 |
| Chlorpyrifos (Chlorpyrifos-ethyl) | ng/L | EN 12918:1999 |
| Cyclodiene pesticides (aldrin, dieldrin, endrin, isodrin) | ng/L | EN ISO 6468:1996 |
| All DDTs | ng/L | EN ISO 6468:1996 |
| p,p-DDT | ng/L | EN ISO 6468:1996 |
| 1,2-dichloroethane | ng/L | EN ISO 10301:1997 |
| Dichloromethane | ng/L | EN ISO 10301:1997 |
| Di(2-ethylhexyl)phthalate (DEHP) | ng/L | EN ISO 18856:2005 |
| Diuron | ng/L | EN ISO 6468:1996, EN ISO 10695:2000 |
| Endosulfan | ng/L | EN ISO 6468:1996 |
| Fluoranthene | ng/L | EN ISO 17993:2003 |
| Hexachlorobenzene | ng/L | EN ISO 6468:1996 |
| Hexachlorobutadiene | ng/L | EN ISO 6468:1996 |
| Hexachlorocyclohexane | ng/L | EN ISO 6468:1996 |
| Isoproturon | ng/L | EN ISO 10695:2000 |
| Naphthalene | ng/L | EN ISO 15680:2003 |
| Nonylphenols | ng/L | EN ISO 18857-1:2006 |
| Octylphenols (6) | ng/L | EN ISO 18857-1:2006 |
| Pentachlorobenzene | ng/L | EN ISO 10301:1997 |
| Pentachlorophenol | ng/L | EN 12673:1998 |
| Polyaromatic hydrocarbons (PAH) | ng/L | EN ISO 17993:2003, EN 16691:2015 |
| Simazine | ng/L | EN ISO 10695:2000 |
| Tetrachlorethylene | ng/L | EN ISO 10301:1997 |
| Trichlorethylene | ng/L | EN ISO 15680:2003 |
| Tributyltin compounds | ng/L | EN ISO 17353:2005 (ISO 17353:2004) |
| Trichlorobenzenes | ng/L | EN ISO 15680:2003 |
| Trichloromethane (chloroform) | ng/L | EN ISO 10301:1997 |
| Trifluralin | ng/L | EN ISO 10695:2000 |
| Dicofol | ng/L | EN ISO 6468:1996 |
| Perfluorooctane sulfonic acid and its derivatives (PFOS) | ng/L | ISO 25101:2009 |
| Quinoxyfen | ng/L | EN ISO 6468:1996, EN ISO 10695:2000 |
| Dioxins and dioxin-like compounds | ng/L | ISO 1484-10:2004 |
| Aclonifen | ng/L | EN ISO 6468:1996, EN ISO 10695:2000 |
| Bifenox | ng/L | EN ISO 10695:2000 |
| Cybutryne | ng/L | EN ISO 27108:2013 |
| Cypermethrin | ng/L | EN ISO 10695:2000 |

| | | |
|---|---|---|
| Dichlorvos | ng/L | EN 12918:1999 |
| Hexabromocyclododecanes (HBCDD) | ng/L | EN ISO 6468:1996, EN ISO 10695:2000 |
| Heptachlor and heptachlor epoxide | ng/L | EN ISO 6468:1996 |
| Terbutryn | ng/L | EN ISO 10695:2000 |
| microplastic | | unique method (Hungary) |