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# Technical report with results from the fish sampling and analyses from the Joint Danube Survey 2007

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# Joint Danube Survey 2

## Final Scientific Report

This Final Report contains an overview of the scientific findings of the Joint Danube Survey 2 (JDS2).
For most chapters, more detailed information can be found on the attached CD-ROM, where full versions of the scientific papers are presented.
Map showing all JDS2 sampling sites is at the end of the report.

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# 1 Assessment of water quality in the Danube River Basin – the need for and aims of a Joint Danube Survey

Igor Liška

In June 1994, the Convention on Cooperation for the Protection and Sustainable Use of the Danube River (DRPC) was signed in Sofia (coming into force in October 1998), with the objective of achieving sustainable and equitable water management of surface and ground waters in the Danube catchment area. Provisions of the DRPC include the need for cooperation in the field of monitoring and assessment, which is accomplished through the operation of the Trans National Monitoring Network (TNMN) in the Danube River Basin.

The TNMN has been in the operation since 1996 but the first steps towards it were taken ten years earlier under the Bucharest Declaration, when a monitoring programme was established containing 11 transboundary cross sections on the Danube River.

Implementation of the EU Water Framework Directive - WFD (2000/60/EC) after 2000 necessitated the revision of the TNMN in the Danube River Basin District. In line with the WFD implementation timeline, a revised TNMN has been under operation since 2007 and its details are described in the ICPDR Monitoring strategy.

The TNMN operation is supported by the basin-wide network of National Reference Laboratories, which amongst other things supervises all water quality measuring activities under the ICPDR and guarantees the quality of results of the analyses. However, the harmonization of methods and improvement of comparability of analytical results is an ongoing process. Although major achievements in this respect demonstrate the progress made (running a basin-wide analytical quality control programme), much more effort is needed to achieve full comparability of results between various institutions and between respective riparian countries.

To get a better overview of water quality (be it an occurrence of specific dangerous substances or an in-depth characterization of river biology), a complementary monitoring activity has been established in the Danube River Basin. This has been organised in the form of scientific longitudinal surveys on the whole stretch of the river. The first Joint Danube Survey (JDS1) was carried out in 2001 and its results were a key information source for characterization of the Danube River Basin District as required by the EU WFD. Having recognised the success of the JDS1, the Danube Declaration (adopted by the ICPDR Contracting Parties at the Ministerial Meeting in December 2004) expressed the need to organise a second Joint Danube Survey.

The concept of Joint Danube Surveys has been formally included in the WFD compliant monitoring strategy of the ICPDR as a tool for investigative monitoring. The Summary Report to the EU on monitoring programmes in the Danube River Basin District (designed under Article 8 - TNMN in the Danube River Basin District) declares that the basin-wide Joint Danube Surveys will be used to carry out investigative monitoring as required e.g. for testing new methods; checking the impact of "new" chemical substances etc. Joint Danube Surveys will be carried out every 6 years.

In line with the ICPDR monitoring strategy and to continue the success of the first survey, the second Joint Danube Survey was organised in August and September 2007. This survey was the world's biggest river research expedition in 2007.

The overall objective of the second Joint Danube Survey (JDS2) was to undertake an international longitudinal ship survey that would produce comparable and reliable information on water quality for

the entire length of the Danube River (including the major tributaries) on a short-term basis. It was envisaged that the outcomes of the JDS2 should include information needs arising from the implementation of the EU WFD.

Specific objectives and technical goals of JDS2 included:

– Producing a homogenous data set for the Danube River based on a single sampling procedure and laboratory analysis of specified determinands and biological quality elements;

– Screening of WFD priority pollutants and other relevant hazardous substances;

– General overview of the habitat morphology of the Danube River;

– Providing a forum for riparian/river basin country participation for sampling and intercomparison exercises;

– Facilitating specific training needs and improving in-country experience;

– Comparing the results of JDS2 with the outcomes of JDS1;

– Assessment and confirmation of the pressures and impacts as stated in the Roof Report 2004;

– Biological validation of the Danube typology;

– Supporting ecological and chemical assessment of the Danube River in line with the WFD;

– Contribution to the Danube Intercalibration Exercise;

– Microbiological analysis;

– Analysis of radioisotopes;

– Promoting public awareness.

During the survey, a Danube-wide investigation of fish and river hydromorphology was performed for the first time. Novel sampling and analytical techniques were applied for a more efficient in-depth pollution analysis. Special attention was given to the analysis of a wide range of persistent organic and inorganic micropollutants in sediment, biota and suspended solids, with a view to improving knowledge of substances not regularly monitored by the TNMN.

The JDS2 was not only of scientific value but also made a major contribution to disseminating information on water quality to the citizens of the Danube countries and helped raise public concern for water protection issues.

# 2 Survey preparation and programme

Jaroslav Slobodník

## 2.1 Survey programme

The Second Joint Danube Survey (JDS2) was undertaken from the 13 August to 26 September 2007. 96 sites were sampled by the JDS2 Core Team along a 2600 km stretch of the Danube, 24 of which were located in the mouths of tributaries or side arms. Samples from the first station Upstream Iller in Germany were collected using cars, the remaining 2415 km were sampled by ships. An additional 28 sites were sampled by National Teams during longitudinal surveys on selected Danube tributaries.

Sampling at the JDS2 stations included five different sample types - water, sediment, biology, suspended particulate matter (SPM) and biota (mussels and fish) - each with a different determinand list. Each sample was taken at various sampling points across the station cross-section of the main river (left, middle, right) and in the middle of the cross-section of the tributaries.

A fish survey was performed at 45 sampling sites on the Danube and 21 sites at the mouths of tributaries using a separate sampling strategy; logistically it was kept as a parallel activity using an independent vessel (Vienna 115) along with an electrofishing boat. The fish survey was managed by the Joint Research Centre of the European Commission (EC JRC) in Ispra in cooperation with an international team of fish experts. The selection of sampling sites and timing schedule of the survey were harmonised with the programme of sampling for chemical and biological analyses.

A continuous observation of hydromorphological parameters (in ca. 50 km stretches) was carried out during sailing, with more detailed screening carried out at each of the 96 sampling sites.

Detailed information on the actual sampling programme is shown in Table 1.

## 2.2 Survey preparation / Cruise Manual

Preparations for the JDS2 as regards the definition of survey objectives; selection of parameters to be measured; identification of sampling and analysis methods; identification of sampling sites and selection of experts was carried out by the Monitoring and Assessment Expert Group (MAEG) of the ICPDR. During the preparatory phase, the JDS2 Cruise Manual was developed, containing a detailed description of the tasks to be accomplished during preparation of the survey, sampling and analyses programme and reporting. A set of Standard Operational Procedures (SOPs) was developed, describing in detail sampling procedures and on-board analyses.

A series of meetings was held before the survey to agree upon logistical issues, equipment preparation and the methods to be used. Consumables, sample containers, chemicals and smaller equipment were purchased and delivered to the survey ships in July and August 2007. A significant part of the equipment was loaned by the JDS2 cooperating laboratories.

## 2.3 JDS2 Core Team

Members of the JDS2 Core Team and Reserve Team were nominated by the Danube states (Contracting Parties of the ICPDR) and selected by the ICPDR Monitoring and Assessment Expert group. Core Team members, responsible for sampling and on-board analyses, were on-board three ships (the Argus, Szechenyi and Vienna 115) during the survey.

| JDS2 Core Team | |
| --- | --- |
| Igor Liska, JDS2 Manager | Gabriel Chiriac, Biologist – general hydrobiology |
| Jaroslav Slobodnik, Technical coordinator | Mary Craciun, Chemist - suspended solids sampling |
| Bela Csanyi, Team leader | Carmen Hamchevici, Chemist - on-board analyses |
| Wolfram Graf, Biologist – macrozoobenthos expert (Upper Danube) | Hana Hudcova, Chemist - water sampling, sediment processing, radiology |
| Patrick Leitner, Biologist – macrozoobenthos expert (Upper Danube) | Wolfgang Kraier, Expert in hydromorphology |
| Momir Paunovic, Biologist – macrozoobenthos expert (Lower Danube) | Ulrich Schwarz, Expert in hydromorphology |
| Jarmila Makovinska, Biologist – phytobenthos expert | Alexander Kirschner, Microbiologist |
| Martin Dokulil, Biologist – phytoplankton expert | Branko Velimirov, Microbiologist |
| Christina Kaiblinger, Biologist – phytoplankton expert | Richard Niederreiter, Air-lift sampling expert |
| Brigitte Schmidt, Biologist – macrophytes expert | Stefan Steiner, Air-lift sampling expert |

| Fish Team | |
| --- | --- |
| Christian Wiesner, Team leader and fish expert | Gabor Guti, Fish expert |
| Niels Jepsen, Team leader and fish expert – EC JRC Ispra | Grigore Davideanu, Fish expert (Lower Danube) |
| Nikolaus Schotzko, Fish expert | Jaroslav Cerny, Fish expert |

| Reserve Team | |
| --- | --- |
| Matus Haviar, Biologist – macrozoobenthos expert | Zoran Stojanovic, Chemist |
| Thomas Ofenbock, Biologist – macrozoobenthos expert | Violeta Astratinei, Microbiologist |
| Laurentia Ungureanu, Biologist – phytoplankton expert | Nemanja Milosevic, Microbiologist |
| Eva Lanz, Biologist – macrophytes expert | |

| JDS2 Managerial and Administrative Team | |
| --- | --- |
| Jasmine Bachmann, Public participation and public relation | Alexander Hoebart, Information manager |
| Paul Csagoly, Communication and editing | Patricia Faltusova, Administrative support |
| Anna Koch, Financial officer | |

## 2.4 JDS2 National Teams

National Teams joined the JDS2 ships upon entering the territory of their country. They cooperated with the JDS2 Core Team in collecting and processing the samples. Participation of National Teams was not only a great help in accomplishing the ambitious technical programme of the survey but it was a unique opportunity for exchange of experience and harmonization of the sampling and analytical methodologies throughout the Danube Basin. Such activity was essential to the implementation of the WFD and represented a particular support to the intercalibration activities.

The network of National JDS2 Coordinators helped the Core Team with all necessary logistical arrangements in their home countries.

**JDS2 National Coordinators**

| | | | | |
|---|---|---|---|---|
| DE | Ursula Schmedtje, National Coordinator | BG | Tzvetanka Dimitrova, National Coordinator |
| DE | Jürgen Seibold, Deputy | BG | Dimitar Vergiev, National Coordinator |
| DE | Birgit Wolf, Deputy | BG | Mina Asenova, Deputy |
| AT | Wolfgang Rodinger, National Coordinator | BG | Hristo Kasadzhikov, Deputy |
| AT | Franz Wagner, Deputy | BG | Ivan Ivanov, Deputy |
| CZ | Ilja Bernardova, National Coordinator | RO | Gabriel Chiriac, National Coordinator |
| CZ | Monika Machkova, Deputy | RO | Liviu Popescu, Deputy |
| SK | Jarmila Makovinska, National Coordinator | RO | Serban Iliescu, Deputy |
| SK | Livia Tothova, Deputy | RO | Teodor Lucian Constantinescu, Deputy |
| HU | Ferenc Laszlo, National Coordinator | MD | Gavril Gilca, National Coordinator |
| HU | Ildiko Horvathne-Kiss, Deputy | UA | Oleksander Deziron, National Coordinator |
| HR | Dagmar Surmanovic, National Coordinator | UA | Yuriy Nabyvanets, Deputy |
| HR | Marija Marjanovic-Rajcic, Deputy | UA | Valerii Kasianchuk, Deputy |
| RS | Marija Kostic, National Coordinator | | |
| RS | Momir Paunovic, Deputy | | |

**JDS2 National Key Fish Experts**

| | | | | |
|---|---|---|---|---|
| DE | Andreas Kolbinger | HR | Perica Mustafic, Marko Caleta |
| AT | Nikolaus Schotzko, Christian Wiesner | RS | Predrag Simonovic |
| CZ | Zdenek Adamek | RO | Grigore Davideanu |
| SK | Jaroslav Cerny, Vladimir Kovac | BG | Stoyan Mihov |
| HU | Gabor Guti | UA | Yevgen Savchenko |

In total, more than 110 national experts participated in the survey, with Romania assembling the largest team consisting of 41 experts and support staff.

## 2.5  JDS2 Determinands

Altogether more than 280 individual parameters were investigated within the JDS2. This number includes parameters determined on-board during the survey and also the chemical, microbiological, ecotoxicological, radiological and biological parameters analysed after the cruise. Special care was taken to include analysis of all quality elements needed for the assessment of the status of the Danube River according to the WFD. The only exception were $C_{10}$-$C_{13}$ chlorinated paraffins (WFD priority substances) due to the fact that harmonised methodologies for their determination at the EU level are not yet available.

## 2.6  JDS2 Laboratories

A large proportion of the laboratory services required for the JDS2 were secured through in-kind contributions by the ICPDR Contracting Parties, who provided their top laboratory facilities. Supplementary analyses were contracted to the JDS2 laboratories. Leading national laboratories from Germany, Austria, Czech Republic, Slovakia, Hungary and Romania performed the chemical analyses. The post-survey biological, microbiological and ecotoxicological analyses were provided as in-kind contributions by numerous institutions in Austria and a laboratory in Slovakia. Supplementary biological analyses were carried out in Hungary, Slovakia and Serbia. Analysis of isotopes was carried

out for free by the International Atomic Energy Agency (IAEA), two institutions in Austria and one in Ukraine. The EC JRC in Ispra, Italy, provided valuable support to the JDS2 through coordination of the fish survey and analyses of a wide range of priority and other substances in various matrices. For a full list of laboratories involved, see Table 2. TZW Karlsruhe / IAWD provided analyses of organic substances for free.

## 2.7 The ships

The survey was carried out using three ships: the Szechenyi, Argus and Vienna 115, sailing under Hungarian, Serbian and Slovakian flags respectively. Technical information on the ships is as follows:

|  | Szechenyi | Argus | Vienna 115 |
|---|---|---|---|
| Type of boat | Motor boat - an icebreaker with accommodation facilities, dining and meeting room | Motor boat, mounted grab - a research vessel used for water quality surveys, equipped with sampling devices, in-built field instrumentation and laboratory desks | Motor boat with electrofishing equipment and accommodation facilities |
| Captain | Dezso Kovacs | Jovica Golubovic and Ilja Barut | Tibor Kiss |
| Cruising speed | 18 km/h | 25 km/h | 15 km/h |
| Dimensions | Length: 40.5 m; width: 9.3 m; draught: 1.8 m; height: 8.15 m; | Length: 33.0 m; width: 4.5 m; draught: 1.3 m; height: 5 m | Length: 12.5 m; width: 3.5 m; draught: 1.1 m |
| Crew | 11 persons | 3 persons | 2 persons |

The Argus was used for sampling and on-board laboratory analyses, while the Szechenyi provided accommodation for the Core Team and National Team members as well as storage. Two small boats from the ships were used for parallel biological and chemical on-shore sampling. The fish team on Vienna 115 followed a separate sampling schedule using an additional electrofishing boat.

## 2.8 The survey

### 2.8.1 Sampling

A small proportion of the chemical, biological and microbiological determinations were carried out directly on board the Argus; the majority of samples were transported under controlled conditions to the JDS2 laboratories for analysis.

Measurements made directly on-board during the survey included:

– Ten general physico-chemical parameters - alkalinity, ammonium ($NH_4$-N), conductivity, dissolved oxygen, nitrates ($NO_3$-N), nitrites ($NO_2$-N), orthophosphate-phosphorus ($PO_4$-P), pH, water temperature, transparency;

– Three microbiological parameters - Intestinal Enterococci (MU/SF Microtiterplates), *Escherichia coli* (Colilert), Total Coliforms (Colilert);

– Phytoplankton chlorophyll-a;

– Radon isotope ($^{222}$Rn).

Water samples for analysis of heavy metals were filtered through 0.45 µm pore size membrane filters using a portable filtration device.

Sediment samples were taken from the left and right banks of the river (1-1.5 m depth) with a sampling net. This was followed by on-board grain size fractioning with wet sieving in order to get a

less than 63 μm fraction for later analysis in the JDS2 laboratories. Undisturbed sediments were collected for ecotoxicological analysis of extracted pore water.

SPM samples were collected from the middle of the river by pumping and centrifugation of water starting at a JDS2 station and continuing when sailing until the sufficient amount of the sample material had been recovered.

Mussels and fish tissue collected from selected sites were collected and preserved (deep frozen) for analysis of trace metals and persistent organic pollutants (POPs).

Sediments, SPM and mussels samples were first freeze-dried in the laboratory of Umweltbundesamt GmbH in Vienna, Austria and then distributed for analysis to the individual laboratories. All remaining sediment, SPM and mussel samples were stored in the JDS2 Central Storage Facility at the Water Research Institute in Bratislava, Slovakia for future analysis.

EC JRC performed an extended analysis of POPs at the selected "top 23" sites for water, SPM, sediment (composite of right and left-hand side), mussels and fish tissue including QA/QC checks. In a special set-up, the water was pumped through polymeric XAD cartridges where the POPs were collected for later analysis. The experiment was synchronised with the pumping time of water for collection of SPM samples.

Sampling of benthic invertebrates was conducted using two parallel techniques – Air-lift sampling and combined Kick & Sweep / dredging sampling. Phytobenthos sampling was accompanied by direct on-site biomass determination by fluorescence detection. Macrophytes were collected at ca. 3 km stretches on both sides of the Danube. The electric fishing CEN-standardised methodology was used for fish survey purposes.

A radiological sampling programme was carried out for analyses of a wide range of isotopes in water and sediment matrices.

The hydromorphological survey included collection of basic hydromorphological data for each station such as the main hydrological values; catchment size upstream; river kilometre (rkm); for the Danube, basic cross-section sketches; river and valley slope; a zoom-in of the navigation map; satellite images; a simple historical comparison and additional overview parameters required for the fish survey.

### 2.8.2  Hydrological conditions
A brief information on hydrological conditions during the survey is provided in Chapter 3.3.4.

## 2.9 Financial arrangements
The JDS2 budget exceeded 1 million EUR, more than 90% of which was financed by the ICPDR Contracting Parties through cash or in-kind contributions. An important aspect was support by corporate entities: Alcoa Foundation and Dexia Kommunalkredit Bank provided financial support; Coca-Cola HBC assisted with food products for JDS2 staff and with the organisation and financing of related public awareness events.

Additional in-kind contribution came from the Contracting Parties through the participation of National Teams and the organisation of longitudinal surveys on tributaries.

An important contribution was provided by the Bundesamt für Wasserwirtschaft of Austria.

## 2.10    Public awareness
During the JDS2, press conferences were organised along the route of the cruise: the official public launch was organised in Regensburg (Germany) with consecutive press events in Vienna (Austria), Bratislava (Slovakia), Budapest (Hungary), Osijek (Croatia), Belgrade (Serbia), Turnu Severin

(Romania), Ruse (Bulgaria) and Vilkovo (Ukraine). The closing press conference was held in Tulcea (Romania).

A special website dedicated to the JDS2 ( http://www.icpdr.org/jds) was created providing daily on-line information about the survey in the form of a diary supported with relevant information about the sampling sites, public events, press releases and contact persons. Also results from the on-board analyses of 11 parameters were presented at the end of each day in a comprehensive chart form.

Numerous leaflets and fact sheets with relevant information about the JDS2 (such as the route, experts and institutions involved etc.) were developed in English as well as some national languages and distributed at respective press conferences. Additional promotional material targeting children (including T-shirts, caps and magnifying glasses) was distributed throughout the survey.

## 2.11    Reporting

The JDS2 report is available on the website of the ICPDR (www.icpdr.org). All data and relevant metadata from the JDS2 were collected in the ICPDR database system. The existing Water Quality Database was extended for the JDS2 component. Special care was taken to ensure the compatibility of the obtained results with the EC environmental databases (WISE) format.

New parts of the database for storage of hydromorphology and fish parameters were developed and all database components (chemistry, biology, hydromorphology, ecotoxicology) were integrated. Results from on-board analyses and hydromorphological observations were directly uploaded into the database during the survey (www.icpdr.org/jds).

## 2.12    Acknowledgements

The survey was supported by the Alcoa Foundation, Dexia Kommunalkredit Bank and Coca-Cola HBC. Special thanks are owed to Via Donau for providing the survey ships with navigation and positioning systems.

## Table 1: List of sampling locations and samples collected during the JDS2

| JDS2 No. | River km | Location | Danube type | Sampling site name | Country code(s) | | Water | SPM | Sediments | Biota - Mussels | Biota - Fish | Macrozoobenthos | Phytobenthos | Phytoplankton | Water - XAD cartridges | Sediment - Pore water | Date of sampling |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2600 | M | 1 | Upstream Iller | DE | | Y | | | | | Y | Y | Y | | | 13/08/07 |
| 2 | 2415 | L | 2 | Kelheim | DE | | | | Y | | | | Y | | | Y | 15/08/07 |
| 2 | 2415 | R | 2 | Kelheim | DE | | | | | | | | Y | | | | 15/08/07 |
| 2 | 2415 | M | 2 | Kelheim | DE | | Y | | Y | | Y | Y | | Y | | | 15/08/07 |
| 2 | 2415 | M | 2 | River km 2415 - 2360 | DE | | | Y | | | | | | | Y | | 15/08/07 |
| 3 | 2354 | L | 2 | Geisling power plant | DE | | | | Y | | | | Y | | | | 15/08/07 |
| 3 | 2354 | R | 2 | Geisling power plant | DE | | | | Y | | | | Y | | | | 15/08/07 |
| 3 | 2354 | M | 2 | Geisling power plant | DE | | Y | | Y | | | Y | | Y | | | 15/08/07 |
| 3 | 2354 | M | 2 | River km 2353.5 - 2312 | DE | | | Y | | | | | | | | | 15/08/07 |
| 4 | 2285 (2287) | L | 2 | Deggendorf | DE | | | | Y | | | | Y | | | | 16/08/07 |
| 4 | 2285 (2287) | R | 2 | Deggendorf | DE | | | | Y | | | | Y | | | | 16/08/07 |
| 4 | 2285 (2287) | M | 2 | Deggendorf | DE | | Y | | Y | | | | Y | Y | | | 16/08/07 |
| 4 | 2285 | M | 2 | River km 2285 - 2284 | DE | | | Y | | | | | | | | | 16/08/07 |
| 5 | 2278 | L | 2 | Niederalteich | DE | | | | Y | | | | Y | | | | 16/08/07 |
| 5 | 2278 | R | 2 | Niederalteich | DE | | | | Y | | | | Y | | | | 16/08/07 |
| 5 | 2278 | M | 2 | Niederalteich | DE | | Y | | Y | | | Y | | Y | | | 16/08/07 |
| 5 | 2278 | M | 2 | River km 2278 - 2278 | DE | | | Y | | | | | | | | | 16/08/07 |
| 6 | 2225 | L | | /Inn, rkm 4.3 | DE | AT | | | Y | | | Y | | | | | 16/08/07 |
| 6 | 2225 | R | | /Inn, rkm 4.3 | DE | AT | | | | | | Y | Y | | | | 16/08/07 |
| 6 | 2225 | M | | /Inn, rkm 4.3 | DE | AT | Y | | | | | | | Y | | | 16/08/07 |
| 7 | 2205 | L | 3 | Jochenstein | DE | AT | | | Y | | | | Y | | | | 17/08/07 |
| 7 | 2205 | R | 3 | Jochenstein | DE | AT | | | Y | | | | Y | | | Y | 17/08/07 |
| 7 | 2205 | M | 3 | Jochenstein | DE | AT | Y | | | | Y | Y | | Y | | | 17/08/07 |
| 7 | 2163 | M | 3 | River km 2162.5 - 2120 | DE | AT | | Y | | | | | | | Y | | 17/08/07 |
| 8 | 2120 | L | 3 | Upstream dam Abwinden-Asten | AT | | | | Y | | | | | | | | 18/08/07 |
| 8 | 2120 | R | 3 | Upstream dam Abwinden-Asten | AT | | | | Y | | | | | | | | 18/08/07 |
| 8 | 2120 | M | 3 | Upstream dam Abwinden-Asten | AT | | Y | | | | | | Y | Y | | | 18/08/07 |
| 8 | 2116 | M | 3 | River km 2116 - 2062 | AT | | | Y | | | | | | | | | 18/08/07 |
| 9 | 2061 | L | 3 | Upstream dam Ybbs-Persenbeug | AT | | | | Y | | | | | | | | 18/08/07 |
| 9 | 2062 | R | 3 | Upstream dam Ybbs-Persenbeug | AT | | | | Y | | | | | | | | 18/08/07 |

| JDS2 No. | River km | Location | Danube type | Sampling site name | Country code(s) | | Water | SPM | | Sediments | Biota - Mussels | Biota - Fish | Macrozoobenthos | Phytobenthos | Phytoplankton | Water - XAD cartridges | Sediment - Pore water | Date of sampling |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9 | 2061 | M | 3 | Upstream dam Ybbs-Persenbeug | AT | | Y | | | Y | | | Y | | Y | | | 18/08/07 |
| 10 | 2008 | L | 3 | Oberloiben | AT | | | | | Y | | | | | | | | 18/08/07 |
| 10 | 2008 | R | 3 | Oberloiben | AT | | | | | Y | | | | | | | | 18/08/07 |
| 10 | 2008 | M | 3 | Oberloiben | AT | | Y | | | Y | | | Y | | Y | | | 18/08/07 |
| 10 | 2013 | M | 3 | River km 2013 - 2000 | AT | | | | Y | | | | | | | | | 18/08/07 |
| 11 | 1950 | L | 4 | Upstream dam Greifenstein | AT | | | | | Y | | | | | | | | 19/08/07 |
| 11 | 1950 | R | 4 | Upstream dam Greifenstein | AT | | | | | Y | | | | | | | | 19/08/07 |
| 11 | 1950 | M | 4 | Upstream dam Greifenstein | AT | | Y | | | Y | | | Y | | Y | | | 19/08/07 |
| 11 | 1991 | M | 4 | River km 1991 - 1950 | AT | | | | Y | | | | | | | | | 19/08/07 |
| 12 | 1942 | L | 4 | Klosterneuburg | AT | | | | | Y | | | | | | | Y | 19/08/07 |
| 12 | 1942 | R | 4 | Klosterneuburg | AT | | | | | Y | | | | | | | Y | 19/08/07 |
| 12 | 1942 | M | 4 | Klosterneuburg | AT | | Y | | | | | | Y | | Y | | | 19/08/07 |
| 12 | 1942 | M | 4 | River km 1942 - 1933 | AT | | | | Y | | | | | | | Y | | 19/08/07 |
| 13 | 1895 | L | 4 | Wildungsmauer | AT | | | | | Y | | | | Y | | | | 21/08/07 |
| 13 | 1895 | R | 4 | Wildungsmauer | AT | | | | | | | | | Y | | | | 21/08/07 |
| 13 | 1895 | M | 4 | Wildungsmauer | AT | | Y | | | | | Y | Y | | Y | | | 21/08/07 |
| 13 | 1921 | M | 4 | River km 1921 - 1884 | AT | | | | Y | | | | | | | | | 21/08/07 |
| 14 | 1881 | L | 4 | Upstream Morava (Hainburg) | AT | | | | | Y | | | | Y | | | | 21/08/07 |
| 14 | 1881 | R | 4 | Upstream Morava (Hainburg) | AT | | | | | Y | | | | Y | | | | 21/08/07 |
| 14 | 1881 | M | 4 | Upstream Morava (Hainburg) | AT | | Y | | | Y | | | Y | | Y | | | 21/08/07 |
| 14 | 1888 | M | 4 | River km 1888 - 1880 | AT | | | | Y | | | | | | | | | 21/08/07 |
| 15 | 1880 | L | | /Morava (rkm 0.08) | AT | SK | | | | | | | | | | | | 21/08/07 |
| 15 | 1880 | R | | /Morava (rkm 0.08) | AT | SK | | | | Y | | | | | | | | 21/08/07 |
| 15 | 1880 | M | | /Morava (rkm 0.08) / (rkm 0.5) | AT | SK | Y | | | | | | Y | | Y | | | 21/08/07 |
| 16 | 1869 | L | 4 | Bratislava | SK | | | | | Y | | | | Y | | | Y | 22/08/07 |
| 16 | 1865 | R | 4 | Bratislava | SK | | | | | Y | | | | Y | | | Y | 22/08/07 |
| 16 | 1869 | M | 4 | Bratislava | SK | | Y | | | | | Y | Y | | Y | | | 22/08/07 |
| 16 | 1870 | M | 4 | River km 1870 - 1864 | SK | | | | Y | | | | | | | Y | | 22/08/07 |
| 17 | 1852 | L | 4 | Gabcikovo reservoir | SK | HU | | | | | | | Y | | | | | 23/08/07 |
| 17 | 1852 | R | 4 | Gabcikovo reservoir | SK | HU | | | | | Y | | Y | | | | | 23/08/07 |
| 17 | 1852 | M | 4 | Gabcikovo reservoir | SK | HU | Y | | | Y | Y | | | Y | Y | | Y | 23/08/07 |
| 17 | 1862 | M | 4 | River km 1862 - 1819 | SK | HU | | | Y | | | | | | | | | 23/08/07 |
| 18 | 1806 | L | 4 | Medvedov/Medve | SK | HU | | | | Y | Y | | | Y | | | Y | 23/08/07 |
| 18 | 1806 | R | 4 | Medvedov/Medve | SK | HU | | | | Y | | | | Y | | | Y | 23/08/07 |
| 18 | 1806 | M | 4 | Medvedov/Medve | SK | HU | Y | | | Y | Y | | Y | | Y | | | 23/08/07 |
| 19 | 1794 | L | 4 | /Moson Danube Arm – end (rkm 0.1) | HU | | | | | Y | | | Y | | | | | 23/08/07 |

| JDS2 No. | River km | Location | Danube type | Sampling site name | Country code(s) | | Water | SPM | Sediments | Biota - Mussels | Biota - Fish | Macrozoobenthos | Phytobenthos | Phytoplankton | Water - XAD cartridges | Sediment - Pore water | Date of sampling |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 19 | 1794 | R | 4 | /Moson Danube Arm – end (rkm 0.1) | HU | | | | | | | | | | | | 23/08/07 |
| 19 | 1794 | M | 4 | /Moson Danube Arm – end (rkm 0.1) | HU | | Y | | | | | | | Y | | | 23/08/07 |
| 19 | 1777 | M | 4 | River km 1777 - 1770 | HU | | | | Y | | | | | | | | 23/08/07 |
| 20 | 1768 | L | 5 | Komarno/Komarom | SK | HU | | | Y | Y | | | Y | | | | 24/08/07 |
| 20 | 1768 | R | 5 | Komarno/Komarom | SK | HU | | | Y | | | | Y | | | | 24/08/07 |
| 20 | 1768 | M | 5 | Komarno/Komarom | SK | HU | Y | | Y | Y | | Y | | Y | | | 24/08/07 |
| 21 | 1766 | L | | /Vah (rkm 0.8) | SK | | | | Y | | | | | | | | 24/08/07 |
| 21 | 1766 | R | | /Vah (rkm 0.8) | SK | | | | | Y | | | Y | | | | 24/08/07 |
| 21 | 1766 | M | | /Vah (rkm 0.8) | SK | | Y | | | Y | | Y | | Y | | | 24/08/07 |
| 21 | 1766 | M | | River km 1766 - 1767 | SK | | | | Y | | | | | | | | 24/08/07 |
| 22 | 1761 | L | 5 | Iza/Szony | SK | HU | | | Y | Y | | | Y | | | Y | 24/08/07 |
| 22 | 1761 | R | 5 | Iza/Szony | SK | HU | | | Y | Y | | | Y | | | Y | 24/08/07 |
| 22 | 1761 | M | 5 | Iza/Szony | SK | HU | Y | | | | | Y | | Y | | | 24/08/07 |
| 22 | 1768 | M | 5 | River km 1768 - 1752 | SK | HU | | | Y | | | | | | Y | | 24/08/07 |
| 22 | 1725 | M | 5 | River km 1724.5 - 1724.5 | SK | HU | | | Y | | | | | | Y | | 24/08/07 |
| 23 | 1719 | L | 5 | Sturovo/Esztergom | SK | HU | | | Y | | | | Y | | | | 25/08/07 |
| 23 | 1719 | R | 5 | Sturovo/Esztergom | SK | HU | | | Y | | | | Y | | | | 25/08/07 |
| 23 | 1719 | M | 5 | Sturovo/Esztergom | SK | HU | Y | | Y | | | Y | | Y | | | 25/08/07 |
| 23 | 1716 | M | 5 | River km 1716 - 1708 | SK | HU | | | Y | | | | | | | | 25/08/07 |
| 24 | 1716 | M | | /Hron (rkm 0.5) | SK | | Y | | Y | | | Y | Y | Y | | | 25/08/07 |
| 25 | 1708 | M | | /Ipoly (rkm 0.7) | SK | HU | Y | | Y | | | Y | Y | Y | | | 25/08/07 |
| 26 | 1707 | L | 5 | Szob | HU | | | | Y | Y | | | Y | | | | 25/08/07 |
| 26 | 1707 | R | 5 | Szob | HU | | | | Y | | | | Y | | | Y | 25/08/07 |
| 26 | 1707 | M | 5 | Szob | HU | | Y | | | Y | Y | Y | | Y | | | 25/08/07 |
| 26 | 1707 | M | 5 | River km 1707 - 1693 | HU | | | | Y | | | | | | Y | | 25/08/07 |
| 27 | 1692 | L | 5 | Upstream end of Szentendre Island | HU | | | | Y | | | | Y | | | | 26/08/07 |
| 27 | 1692 | R | 5 | Upstream end of Szentendre Island | HU | | | | | | | | | | | | 26/08/07 |
| 27 | 1692 | M | 5 | Upstream end of Szentendre Island | HU | | Y | | | | | Y | | Y | | | 26/08/07 |
| 27 | 1693 | M | 5 | River km 1693 - 1669 | HU | | | | Y | | | | | | | | 26/08/07 |
| 28 | 1692 | L | 5 | /Upstream end of Szentendre Island (arm) | HU | | | | | | | | | | | | 26/08/07 |
| 28 | 1692 | R | 5 | /Upstream end of Szentendre Island (arm) | HU | | | | Y | | | | Y | | | | 26/08/07 |
| 28 | 1692 | M | 5 | /Upstream end of Szentendre Island (arm) | HU | | Y | | | | | Y | | Y | | | 26/08/07 |
| 29 | 1659 | L | 5 | Budapest upstream | HU | | | | Y | | | | Y | | | Y | 27/08/07 |

| JDS2 No. | River km | Location | Danube type | Sampling site name | Country code(s) | Water | SPM | Sediments | Biota - Mussels | Biota - Fish | Macrozoobenthos | Phytobenthos | Phytoplankton | Water - XAD cartridges | Sediment - Pore water | Date of sampling |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 29 | 1659 | R | 5 | Budapest upstream | HU | | | | Y | Y | | | Y | | | Y | 27/08/07 |
| 29 | 1659 | M | 5 | Budapest upstream | HU | Y | | | | Y | | Y | | Y | | | 27/08/07 |
| 29 | 1655 | M | 5 | River km 1655 - 1659 | HU | | | Y | | | | | | | | | 27/08/07 |
| 30 | 1658 | L | 5 | /Budapest (old Danube) end of S.arm | HU | | | | Y | | | | Y | | | | 27/08/07 |
| 30 | 1658 | R | 5 | /Budapest (old Danube) end of S.arm | HU | | | | Y | | | | Y | | | | 27/08/07 |
| 30 | 1658 | M | 5 | /Budapest (old Danube) end of S.arm | HU | Y | | | Y | | | Y | | Y | | | 27/08/07 |
| 31 | 1642 | L | 5 | /Rackeve-Soroksar Danube Arm - start | HU | | | | | | | | | | | | 29/08/07 |
| 31 | 1642 | R | 5 | /Rackeve-Soroksar Danube Arm - start | HU | | | | Y | | | | Y | | | Y | 29/08/07 |
| 31 | 1642 | M | 5 | /Rackeve-Soroksar Danube Arm - start | HU | Y | | | | | | Y | | Y | | | 29/08/07 |
| 32 | 1632 | L | 5 | Budapest downstream | HU | | | | Y | | | | Y | | | Y | 29/08/07 |
| 32 | 1632 | R | 5 | Budapest downstream | HU | | | | Y | Y | | Y | Y | | | Y | 29/08/07 |
| 32 | 1632 | M | 5 | Budapest downstream | HU | Y | | | | Y | Y | | | Y | | | 29/08/07 |
| 32 | 1640 | M | 5 | River km 1640 - 1598 | HU | | | Y | | | | | | | | | 29/08/07 |
| 33 | 1605 | L | 5 | Adony/Lórév | HU | | | | Y | | | | Y | | | | 29/08/07 |
| 33 | 1605 | R | 5 | Adony/Lórév | HU | | | | Y | Y | | | Y | | | | 29/08/07 |
| 33 | 1605 | M | 5 | Adony/Lórév | HU | Y | | | Y | Y | | Y | | Y | | | 29/08/07 |
| 34 | 1586 | L | | /Rackeve-Soroksar Danube Arm - end | HU | | | | | | | | Y | | | | 30/08/07 |
| 34 | 1586 | R | | /Rackeve-Soroksar Danube Arm - end | HU | | | | Y | | | | Y | | | Y | 30/08/07 |
| 34 | 1586 | M | | /Rackeve-Soroksar Danube Arm - end | HU | Y | | | | | | | | Y | | | 30/08/07 |
| 35 | 1560 | L | 5 | Dunafoldvar | HU | | | | Y | | | | Y | | | Y | 30/08/07 |
| 35 | 1560 | R | 5 | Dunafoldvar | HU | | | | Y | Y | | | Y | | | Y | 30/08/07 |
| 35 | 1560 | M | 5 | Dunafoldvar | HU | Y | | | | Y | | Y | | Y | | | 30/08/07 |
| 35 | 1577 | M | 5 | River km 1577 - 1552 | HU | | | Y | | | | | | | Y | | 30/08/07 |
| 36 | 1533 | L | 5 | Paks | HU | | | | Y | | | | Y | | | | 30/08/07 |
| 36 | 1533 | R | 5 | Paks | HU | | | | Y | | | | Y | | | | 30/08/07 |
| 36 | 1533 | M | 5 | Paks | HU | Y | | | Y | | | Y | | Y | | | 30/08/07 |
| 37 | 1497 | M | | /Sio (rkm 1.0) | HU | Y | | | Y | | | Y | Y | Y | | | 31/08/07 |
| 38 | 1481 | L | 6 | Baja | HU | | | | Y | | | | Y | | | | 31/08/07 |
| 38 | 1481 | R | 6 | Baja | HU | | | | Y | | | | Y | | | | 31/08/07 |
| 38 | 1481 | M | 6 | Baja | HU | Y | | | Y | | | Y | | Y | | | 31/08/07 |
| 38 | 1517 | M | 6 | River km 1517 - 1481 | HU | | | Y | | | | | | | | | 31/08/07 |
| 39 | 1434 | L | 6 | Hercegszanto | HU | | | | Y | | | | Y | | | Y | 01/09/07 |
| 39 | 1434 | R | 6 | Hercegszanto | HU | | | | Y | Y | | | Y | | | Y | 01/09/07 |
| 39 | 1434 | M | 6 | Hercegszanto | HU | Y | | | | Y | Y | Y | | Y | | | 01/09/07 |
| 39 | 1477 | M | 6 | River km 1477 - 1443 | HU | | | Y | | | | | | | Y | | 01/09/07 |

| JDS2 No. | River km | Location | Danube type | Sampling site name | Country code(s) | | Water | SPM | Sediments | Biota - Mussels | Biota - Fish | Macrozoobenthos | Phytobenthos | Phytoplankton | Water - XAD cartridges | Sediment - Pore water | Date of sampling |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 40 | 1424 | L | 6 | Batina | HR | RS | | | Y | | | | Y | | | | 01/09/07 |
| 40 | 1424 | R | 6 | Batina | HR | RS | | | Y | | | | Y | | | | 01/09/07 |
| 40 | 1424 | M | 6 | Batina | HR | RS | Y | | Y | | | Y | | Y | | | 01/09/07 |
| 40 | 1425 | M | 6 | River km 1425 - 1380 | HR | RS | | Y | | | | | | | | | 01/09/07 |
| 41 | 1384 | L | 6 | Upstream Drava | HR | RS | | | Y | Y | | | Y | | | | 02/09/07 |
| 41 | 1384 | R | 6 | Upstream Drava | HR | RS | | | Y | | | | Y | | | | 02/09/07 |
| 41 | 1384 | M | 6 | Upstream Drava | HR | RS | Y | | Y | Y | | Y | | Y | | | 02/09/07 |
| 42 | 1379 | L | | /Drava (rkm 1.4) | HR | RS | | | | Y | | | | | | | 02/09/07 |
| 42 | 1382 | R | | /Drava (rkm 1.4) | HR | RS | | | Y | | | | Y | | | Y | 02/09/07 |
| 42 | 1379 | M | | /Drava (rkm 1.4) | HR | RS | Y | | | Y | | Y | | Y | | | 02/09/07 |
| 42 | 1379 | M | | /Drava - SPM River km 1 - 1 | HR | RS | | Y | | | | | | | Y | | 02/09/07 |
| 43 | 1367 | L | 6 | Downstream Drava (Erdut/Bogojevo) | HR | RS | | | Y | | | | Y | | | | 03/09/07 |
| 43 | 1367 | R | 6 | Downstream Drava (Erdut/Bogojevo) | HR | RS | | | Y | | | | Y | | | | 03/09/07 |
| 43 | 1367 | M | 6 | Downstream Drava (Erdut/Bogojevo) | HR | RS | Y | | Y | | | Y | | Y | | | 03/09/07 |
| 43 | 1367 | M | 6 | River km 1367 - 1355 | HR | RS | | Y | | | | | | | | | 03/09/07 |
| 44 | 1355 | L | 6 | Dalj | HR | RS | | | Y | | | | Y | | | | 03/09/07 |
| 44 | 1355 | R | 6 | Dalj | HR | RS | | | Y | | | | Y | | | | 03/09/07 |
| 44 | 1355 | M | 6 | Dalj | HR | RS | Y | | Y | | | Y | | Y | | | 03/09/07 |
| 45 | 1300 | L | 6 | Ilok/Backa Palanka | HR | RS | | | Y | | | | Y | | | Y | 03/09/07 |
| 45 | 1300 | R | 6 | Ilok/Backa Palanka | HR | RS | | | Y | Y | | | Y | | | Y | 03/09/07 |
| 45 | 1300 | M | 6 | Ilok/Backa Palanka | HR | RS | Y | | | Y | | Y | | Y | | | 03/09/07 |
| 45 | 1326 | M | 6 | River km 1326 - 1299 | HR | RS | | Y | | | | | | | Y | | 03/09/07 |
| 46 | 1262 | L | 6 | Upstream Novi-Sad | RS | | | | Y | | | | Y | | | Y | 04/09/07 |
| 46 | 1262 | R | 6 | Upstream Novi-Sad | RS | | | | Y | | | | Y | | | Y | 04/09/07 |
| 46 | 1262 | M | 6 | Upstream Novi-Sad | RS | | Y | | Y | | | Y | | Y | | | 04/09/07 |
| 47 | 1252 | L | 6 | Downstream Novi-Sad | RS | | | | Y | | | | Y | | | Y | 04/09/07 |
| 47 | 1252 | R | 6 | Downstream Novi-Sad | RS | | | | Y | Y | | | Y | | | Y | 04/09/07 |
| 47 | 1252 | M | 6 | Downstream Novi-Sad | RS | | Y | | | Y | | Y | | Y | | | 04/09/07 |
| 47 | 1252 | M | 6 | River km 1252 - 1252 | RS | | | | | Y | | | | | Y | | 04/09/07 |
| 48 | 1216 | L | 6 | Upstream Tisa (Stari Slankamen) | RS | | | | Y | | | | Y | | | Y | 05/09/07 |
| 48 | 1216 | R | 6 | Upstream Tisa (Stari Slankamen) | RS | | | | Y | | | | Y | | | Y | 05/09/07 |
| 48 | 1216 | M | 6 | Upstream Tisa (Stari Slankamen) | RS | | Y | | Y | | | Y | | Y | | | 05/09/07 |
| 49 | 1216 | L | | /Tisa (rkm 1.0) | RS | | | | | | | | Y | | | | 05/09/07 |
| 49 | 1216 | R | | /Tisa (rkm 1.0) | RS | | | | Y | | | | | | | | 05/09/07 |
| 49 | 1215 | M | | /Tisa (rkm 1.0) | RS | | Y | | | | | Y | | Y | | | 05/09/07 |
| 49 | 1215 | M | | /Tisa - SPM River km 1 - 1 | RS | | | Y | | | | | | | | | 05/09/07 |

| JDS2 No. | River km | Location | Danube type | Sampling site name | Country code(s) | Water | SPM | | Sediments | Biota - Mussels | Biota - Fish | Macrozoobenthos | Phytobenthos | Phytoplankton | Water - XAD cartridges | Sediment - Pore water | Date of sampling |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 50 | 1200 | L | 6 | Downstream Tisa/Upstream Sava (Belegis) | RS | | | | Y | | | | Y | | | Y | 05/09/07 |
| 50 | 1200 | R | 6 | Downstream Tisa/Upstream Sava (Belegis) | RS | | | | Y | | | | Y | | | Y | 05/09/07 |
| 50 | 1200 | M | 6 | Downstream Tisa/Upstream Sava (Belegis) | RS | Y | | | | Y | | Y | | Y | | | 05/09/07 |
| 50 | 1200 | M | 6 | River km 1200 - 1174 | RS | | | Y | | | | | | | | | 05/09/07 |
| 51 | 1170 | L | | /Sava (rkm 7.0) | RS | | | | Y | Y | | | Y | | | Y | 07/09/07 |
| 51 | 1170 | R | | /Sava (rkm 7.0) | RS | | | | | | | | | | | | 07/09/07 |
| 51 | 1170 | M | | /Sava (rkm 7.0) | RS | Y | | | | | | Y | | Y | | | 07/09/07 |
| 51 | 1170 | M | | /Sava - SPM River km 7 - 7 | RS | | | Y | | | | | | | Y | | 07/09/07 |
| 52 | 1159 | L | 6 | Upstream Pancevo/Downstream Sava | RS | | | | Y | | | | Y | | | Y | 07/09/07 |
| 52 | 1159 | R | 6 | Upstream Pancevo/Downstream Sava | RS | | | | Y | Y | | | Y | | | Y | 07/09/07 |
| 52 | 1159 | M | 6 | Upstream Pancevo/Downstream Sava | RS | Y | | | | Y | | Y | | Y | | | 07/09/07 |
| 52 | 1164 | M | 6 | River km 1164 - 1156 | RS | | | Y | | | | | | | | | 07/09/07 |
| 53 | 1151 | L | 6 | Downstream Pancevo | RS | | | | Y | | | | | | | Y | 07/09/07 |
| 53 | 1151 | R | 6 | Downstream Pancevo | RS | | | | Y | Y | | | | | | Y | 07/09/07 |
| 53 | 1151 | M | 6 | Downstream Pancevo | RS | Y | | | | Y | | Y | | Y | | | 07/09/07 |
| 53 | 1151 | M | 6 | River km 1151 - 1115 | RS | | | Y | | | | | | | Y | | 07/09/07 |
| 54 | 1132 | L | 6 | Grocka | RS | | | | Y | | | | | | | | 08/09/07 |
| 54 | 1132 | R | 6 | Grocka | RS | | | | Y | | | | Y | | | | 08/09/07 |
| 54 | 1132 | M | 6 | Grocka | RS | Y | | | Y | | | Y | | Y | | | 08/09/07 |
| 55 | 1107 | L | 6 | Upstream Velika Morava | RS | | | | Y | | | Y | | | | Y | 08/09/07 |
| 55 | 1107 | R | 6 | Upstream Velika Morava | RS | | | | Y | | | Y | | | | Y | 08/09/07 |
| 55 | 1107 | M | 6 | Upstream Velika Morava | RS | Y | | | Y | | | | | Y | | | 08/09/07 |
| 56 | 1103 | L | | /Velika Morava | RS | | | | Y | | | | | | | Y | 08/09/07 |
| 56 | 1103 | R | | /Velika Morava | RS | | | | | | | | Y | | | | 08/09/07 |
| 56 | 1103 | M | | /Velika Morava | RS | Y | | | | Y | | Y | | Y | | | 08/09/07 |
| 56 | 1103 | M | | /Velika Morava - SPM River km 1 - 1 | RS | | | Y | | | | | | | Y | | 08/09/07 |
| 57 | 1097 | L | 6 | Downstream Velika Morava | RS | | | | Y | | | | Y | | | Y | 09/09/07 |
| 57 | 1097 | R | 6 | Downstream Velika Morava | RS | | | | Y | | | | | | | Y | 09/09/07 |
| 57 | 1097 | M | 6 | Downstream Velika Morava | RS | Y | | | | | | Y | | Y | | | 09/09/07 |

| JDS2 No. | River km | Location | Danube type | Sampling site name | Country code(s) | | Sample matrices | | | | | | | | | | | Date of sampling |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Water | SPM | Sediments | Biota - Mussels | Biota - Fish | Macrozoobenthos | Phytobenthos | Phytoplankton | Water - XAD cartridges | Sediment - Pore water | | |
| 57 | 1097 | M | 6 | River km 1097 - 1077 | RS | | | | Y | | | | | | | | | 09/09/07 |
| 58 | 1077 | L | 6 | Starapalanka – Ram | RS | | | | | Y | | | Y | Y | | | Y | 09/09/07 |
| 58 | 1077 | R | 6 | Starapalanka – Ram | RS | | | | | Y | Y | | Y | Y | | | Y | 09/09/07 |
| 58 | 1077 | M | 6 | Starapalanka – Ram | RS | | | Y | | | Y | | | | Y | | | 09/09/07 |
| 58 | 1077 | M | 6 | River km 1077 - 1060 | RS | | | | Y | | | | | | | Y | | 09/09/07 |
| 59 | 1071 | L | 7 | Banatska Palanka/Bazias | RS | RO | | | Y | | | | Y | | | | | 09/09/07 |
| 59 | 1071 | R | 7 | Banatska Palanka/Bazias | RS | RO | | | Y | | | | Y | | | | | 09/09/07 |
| 59 | 1071 | M | 7 | Banatska Palanka/Bazias | RS | RO | Y | | Y | | | | Y | | Y | | | 09/09/07 |
| 60 | 1040 | L | 7 | Irongate reservoir (Golubac/Koronin) | RS | RO | | | Y | | | | Y | | | | Y | 10/09/07 |
| 60 | 1040 | R | 7 | Irongate reservoir (Golubac/Koronin) | RS | RO | | | Y | | | | Y | | | | Y | 10/09/07 |
| 60 | 1040 | M | 7 | Irongate reservoir (Golubac/Koronin) | RS | RO | Y | | | | | | Y | | Y | | | 10/09/07 |
| 60 | 1067 | M | 7 | River km 1067 - 991 | RS | RO | | Y | | | | | | | | | | 10/09/07 |
| 61 | 991 | L | 7 | Donji Milanovac | RS | RO | | | Y | | | | Y | Y | | | | 10/09/07 |
| 61 | 991 | R | 7 | Donji Milanovac | RS | RO | | | Y | | | | Y | Y | | | | 10/09/07 |
| 61 | 991 | M | 7 | Donji Milanovac | RS | RO | Y | | Y | | | | | | Y | | | 10/09/07 |
| 61 | 989 | M | 7 | River km 989 - 989 | RS | RO | | Y | | | | | | | | | | 10/09/07 |
| 62 | 954 | L | 7 | Irongate reservoir (Tekija/Orsova) | RS | RO | | | Y | | | | Y | Y | | | | 11/09/07 |
| 62 | 954 | R | 7 | Irongate reservoir (Tekija/Orsova) | RS | RO | | | Y | | | | Y | Y | | | | 11/09/07 |
| 62 | 954 | M | 7 | Irongate reservoir (Tekija/Orsova) | RS | RO | Y | | Y | | | | | Y | | | | 11/09/07 |
| 62 | 956.2 | M | 7 | River km 956.2 - 943 | RS | RO | | Y | | | | | | | | | | 11/09/07 |
| 63 | 926 | L | 8 | Vrbica/Simijan | RS | RO | | | Y | | | | Y | Y | | | | 11/09/07 |
| 63 | 926 | R | 8 | Vrbica/Simijan | RS | RO | | | Y | | | | Y | Y | | | | 11/09/07 |
| 63 | 926 | M | 8 | Vrbica/Simijan | RS | RO | Y | | Y | | | | | Y | | | | 11/09/07 |
| 64 | 865 | L | 8 | Iron Gate II | RS | RO | | | Y | | | | Y | | | | Y | 13/09/07 |
| 64 | 865 | R | 8 | Iron Gate II | RS | RO | | | Y | | | | Y | Y | | | Y | 13/09/07 |
| 64 | 865 | M | 8 | Iron Gate II | RS | RO | Y | | | | | | | Y | | | | 13/09/07 |
| 64 | 926 | M | 8 | River km 926 - 849 | RS | RO | | Y | | | | | | | | | | 13/09/07 |
| 65 | 849 | L | 8 | Upstream Timok (Rudujevac/Gruia) | RS | RO | | | Y | | | | Y | | | | | 13/09/07 |
| 65 | 849 | R | 8 | Upstream Timok (Rudujevac/Gruia) | RS | RO | | | | | | | Y | | | | | 13/09/07 |
| 65 | 849 | M | 8 | Upstream Timok (Rudujevac/Gruia) | RS | RO | Y | | | | | | Y | | Y | | | 13/09/07 |
| 66 | 845 | M | | /Timok (rkm 0.2) | RS | BG | Y | | Y | | | | Y | | Y | | | 13/09/07 |
| 66 | 845 | M | | River km 845 - 842 | RS | BG | | Y | | | | | | | | | | 13/09/07 |
| 67 | 834 | L | 8 | Pristol/Novo Selo Harbour | RO | BG | | | Y | | | | Y | | | | | 14/09/07 |

| JDS2 No. | River km | Location | Danube type | Sampling site name | Country code(s) | | Sample matrices | | | | | | | | | | | Date of sampling |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Water | SPM | Sediments | Biota - Mussels | Biota - Fish | Macrozoobenthos | Phytobenthos | Phytoplankton | Water - XAD cartridges | Sediment - Pore water | | |
| 67 | 834 | R | 8 | Pristol/Novo Selo Harbour | RO | BG | | | | | | | Y | | | | 14/09/07 |
| 67 | 834 | M | 8 | Pristol/Novo Selo Harbour | RO | BG | Y | | | | | Y | | Y | | | 14/09/07 |
| 67 | 840 | M | 8 | River km 840 - 795 | RO | BG | | | Y | | | | | | | | 14/09/07 |
| 68 | 795 | L | 8 | Calafat | RO | BG | | | | | | Y | Y | | | | 14/09/07 |
| 68 | 795 | R | 8 | Calafat | RO | BG | | | Y | | | Y | | | | Y | 14/09/07 |
| 68 | 795 | M | 8 | Calafat | RO | BG | Y | | | | | | | Y | | | 14/09/07 |
| 68 | 790 | M | 8 | River km 790 - 743 | RO | BG | | | Y | | | | | | | | 14/09/07 |
| 69 | 685 | L | 8 | Downstream Kozloduy | BG | RO | | | Y | | | Y | | | | | 15/09/07 |
| 69 | 685 | R | 8 | Downstream Kozloduy | BG | RO | | | Y | | | Y | | | | | 15/09/07 |
| 69 | 685 | M | 8 | Downstream Kozloduy | BG | RO | Y | | Y | | | | | Y | | | 15/09/07 |
| 69 | 736 | M | 8 | River km 736 - 670 | BG | RO | | | Y | | | | | | | | 15/09/07 |
| 70 | 640 | L | 8 | Upstream Iskar (Bajkal) | BG | RO | | | Y | | | | | | | | 15/09/07 |
| 70 | 640 | R | 8 | Upstream Iskar (Bajkal) | BG | RO | | | Y | | | | | | | | 15/09/07 |
| 70 | 640 | M | 8 | Upstream Iskar (Bajkal) | BG | RO | Y | | | | | Y | | Y | | | 15/09/07 |
| 70 | 666 | M | 8 | River km 666 - 630 | BG | RO | | | Y | | | | | | | | 15/09/07 |
| 71 | 637 | M | | /Iskar (rkm 0.3) | BG | | Y | | Y | | | Y | | Y | | Y | 15/09/07 |
| 72 | 629 | L | 8 | Downstream Iskar | BG | RO | | | Y | | | Y | | | | | 16/09/07 |
| 72 | 629 | R | 8 | Downstream Iskar | BG | RO | | | Y | | | Y | | | | | 16/09/07 |
| 72 | 629 | M | 8 | Downstream Iskar | BG | RO | Y | | | | | | | Y | | | 16/09/07 |
| 72 | 627 | M | 8 | River km 627 - 624 | BG | RO | | | Y | | | | | | | | 16/09/07 |
| 73 | 606 | L | 8 | Upstream Olt | RO | BG | | | Y | | | Y | | | | | 16/09/07 |
| 73 | 606 | R | 8 | Upstream Olt | RO | BG | | | | | | Y | Y | | | | 16/09/07 |
| 73 | 606 | M | 8 | Upstream Olt | RO | BG | Y | | | | | | | Y | | | 16/09/07 |
| 74 | 605 | L | | /Olt (rkm 0.4) | RO | | | | Y | | | | | | | Y | 16/09/07 |
| 74 | 605 | R | | /Olt (rkm 0.4) | RO | | | | | | | | | | | | 16/09/07 |
| 74 | 605 | M | | /Olt (rkm 0.4) | RO | | Y | | | | | Y | | Y | | | 16/09/07 |
| 75 | 602 | L | 8 | Downstream Olt | RO | BG | | | Y | | | | | | | | 16/09/07 |
| 75 | 602 | R | 8 | Downstream Olt | RO | BG | | | Y | | | | Y | | | | 16/09/07 |
| 75 | 602 | M | 8 | Downstream Olt | RO | BG | Y | | Y | | | Y | | Y | | | 16/09/07 |
| 76 | 579 | L | 8 | Downstream Turnu-Magurele/Nikopol | RO | BG | | | Y | | | Y | | | | Y | 17/09/07 |
| 76 | 579 | R | 8 | Downstream Turnu-Magurele/Nikopol | RO | BG | | | | | | Y | Y | | | | 17/09/07 |
| 76 | 579 | M | 8 | Downstream Turnu-Magurele/Nikopol | RO | BG | Y | | | | | | | Y | | | 17/09/07 |
| 76 | 579 | M | 8 | River km 579 - 550 | RO | BG | | | Y | | | | | | Y | | 17/09/07 |
| 77 | 550 | L | 8 | Downstream Zimnicea/Svishtov | RO | BG | | | Y | | | Y | | | | | 17/09/07 |
| 77 | 550 | R | 8 | Downstream Zimnicea/Svishtov | RO | BG | | | | | | Y | Y | | | | 17/09/07 |

| JDS2 No. | River km | Location | Danube type | Sampling site name | Country code(s) | | Water | SPM | | Sediments | Biota - Mussels | Biota - Fish | Macrozoobenthos | Phytobenthos | Phytoplankton | Water - XAD cartridges | Sediment - Pore water | Date of sampling |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | Sample matrices | | | | | | |
| 77 | 550 | M | 8 | Downstream Zimnicea/Svishtov | RO | BG | Y | | | Y | | Y | | | Y | | | 17/09/07 |
| 78 | 537 | M | | /Jantra (rkm 1.0) | BG | | Y | | | Y | | | Y | | Y | | | 18/09/07 |
| 78 | 554 | M | | River km 554 - 542 | BG | | | | | Y | | | | | | | | 18/09/07 |
| 79 | 532 | L | 8 | Downstream Jantra | RO | BG | | | | Y | | | Y | | | | | 18/09/07 |
| 79 | 532 | R | 8 | Downstream Jantra | RO | BG | | | | Y | | | Y | Y | | | | 18/09/07 |
| 79 | 532 | M | 8 | Downstream Jantra | RO | BG | Y | | | | | | | | Y | | | 18/09/07 |
| 79 | 539 | M | 8 | River km 539 - 532 | RO | BG | | | | Y | | | | | | | | 18/09/07 |
| 80 | 500 | L | 8 | Upstream Ruse | BG | RO | | | | Y | | | | | | | Y | 18/09/07 |
| 80 | 500 | R | 8 | Upstream Ruse | BG | RO | | | | Y | | | | Y | | | | 18/09/07 |
| 80 | 500 | M | 8 | Upstream Ruse | BG | RO | Y | | | | | | Y | | Y | | | 18/09/07 |
| 80 | 500 | M | 8 | River km 500 - 500 | BG | RO | | | | Y | | | | | | Y | | 18/09/07 |
| 81 | 498 | M | | /Russenski Lom | BG | | Y | | | | | | Y | | Y | | | 20/09/07 |
| 82 | 488 | L | 8 | Downstream Ruse/Giurgiu | BG | RO | | | | Y | | | | | | | | 20/09/07 |
| 82 | 488 | R | 8 | Downstream Ruse/Giurgiu | BG | RO | | | | | | | | | | | | 20/09/07 |
| 82 | 488 | M | 8 | Downstream Ruse/Giurgiu | BG | RO | Y | | | | | | Y | | Y | | | 20/09/07 |
| 82 | 488 | M | 8 | River km 488 - 488 | BG | RO | | | | Y | | | | | | | | 20/09/07 |
| 83 | 434 | L | 8 | Upstream Arges | RO | BG | | | | Y | | | | | | | Y | 21/09/07 |
| 83 | 434 | R | 8 | Upstream Arges | RO | BG | | | | Y | | | | | | | Y | 21/09/07 |
| 83 | 434 | M | 8 | Upstream Arges | RO | BG | Y | | | Y | | | Y | | Y | | | 21/09/07 |
| 83 | 458 | M | 8 | River km 458 - 435 | RO | BG | | | Y | | | | | | | Y | | 21/09/07 |
| 84 | 432 | M | | /Arges | RO | | Y | | | | | | Y | | Y | | | 21/09/07 |
| 85 | 429 | L | 8 | Downstream Arges, Oltenita | RO | BG | | | | Y | | | | | | | Y | 21/09/07 |
| 85 | 429 | R | 8 | Downstream Arges, Oltenita | RO | BG | | | | Y | | | | Y | | | Y | 21/09/07 |
| 85 | 429 | M | 8 | Downstream Arges, Oltenita | RO | BG | Y | | | | | | Y | | Y | | | 21/09/07 |
| 85 | 427 | M | 8 | River km 427 - 378 | RO | BG | | | Y | | | | | | | Y | | 21/09/07 |
| 86 | 378 | L | 8 | Chiciu/Silistra | RO | BG | | | | Y | | | Y | | | | Y | 21/09/07 |
| 86 | 378 | R | 8 | Chiciu/Silistra | RO | BG | | | | | | | Y | Y | | | | 21/09/07 |
| 86 | 378 | M | 8 | Chiciu/Silistra | RO | BG | Y | | | | | | | | Y | | | 21/09/07 |
| 86 | 375 | M | 8 | River km 375 - 322 | RO | BG | | | Y | | | | | | | Y | | 21/09/07 |
| 87 | 295 | L | 9 | Upstream Cernavoda | RO | | | | | Y | | | | | | | | 22/09/07 |
| 87 | 295 | R | 9 | Upstream Cernavoda | RO | | | | | | | | | | | | | 22/09/07 |
| 87 | 295 | M | 9 | Upstream Cernavoda | RO | | Y | | | | | | Y | | Y | | | 22/09/07 |
| 87 | 302 | M | 9 | River km 302 - 280 | RO | | | | | Y | | | | | | | | 22/09/07 |
| 88 | 235 | L | 9 | Giurgeni | RO | | | | | Y | | | | | | | | 23/09/07 |
| 88 | 235 | R | 9 | Giurgeni | RO | | | | | Y | | | | | | | Y | 23/09/07 |
| 88 | 235 | M | 9 | Giurgeni | RO | | Y | | | Y | | | Y | | Y | | | 23/09/07 |
| 88 | 255 | M | 9 | River km 255 - 251 | RO | | | | | Y | | | | | | | | 23/09/07 |

| JDS2 No. | River km | Location | Danube type | Sampling site name | Country code(s) | | Sample matrices | | | | | | | | | | | | | Date of sampling |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Water | SPM | Sediments | Biota - Mussels | Biota - Fish | Macrozoobenthos | Phytobenthos | Phytoplankton | Water - XAD cartridges | Sediment - Pore water | | | | |
| 89 | 167 | L | 9 | Braila | RO | | | | Y | | | Y | | | | Y | | | | 23/09/07 |
| 89 | 167 | R | 9 | Braila | RO | | | | Y | | | | | | | | | | | 23/09/07 |
| 89 | 167 | M | 9 | Braila | RO | | Y | | | | | | | Y | | | | | | 23/09/07 |
| 89 | 231 | M | 9 | River km 231 - 170 | RO | | | | Y | | | | | | Y | | | | | 23/09/07 |
| 90 | 154 | L | | /Siret (rkm 1.0) | RO | | | | | | | Y | | | | | | | | 23/09/07 |
| 90 | 154 | R | | /Siret (rkm 1.0) | RO | | | | | | | Y | | | | | | | | 23/09/07 |
| 90 | 154 / 157 | M | | /Siret (rkm 1.0) | RO | | Y | | Y | | Y | | | Y | | | | | | 23/09/07 |
| 90 | 154 | M | | /Siret - SPM River km 1 - 1 | RO | | | Y | | | | | | | | | | | | 23/09/07 |
| 91 | 135 | M | | /Prut (rkm 1.0) | RO | MD | Y | | Y | Y | Y | Y | | Y | | | | | | 23/09/07 |
| 91 | 135 | M | | /Prut - SPM River km 1 - 1 | RO | MD | | Y | | | | | | | | | | | | 23/09/07 |
| 92 | 130 | L | 9 | Reni | RO | UA | | | Y | | | Y | | | | Y | | | | 24/09/07 |
| 92 | 130 | R | 9 | Reni | RO | UA | | | Y | | | Y | | | | | | | | 24/09/07 |
| 92 | 130 | M | 9 | Reni | RO | UA | Y | | | | | | | Y | | | | | | 24/09/07 |
| 92 | 61 | M | 9 | River km 61 - 51 | RO | UA | | Y | | | | | | | Y | | | | | 24/09/07 |
| 93 | 18 | L | 10 | Vilkova - Chilia arm/Kilia arm | RO | UA | | | Y | | | | | | | | | | | 25/09/07 |
| 93 | 18 | R | 10 | Vilkova - Chilia arm/Kilia arm | RO | UA | | | Y | Y | | | | | | | | | | 25/09/07 |
| 93 | 18 | M | 10 | Vilkova - Chilia arm/Kilia arm | RO | UA | Y | | Y | Y | | Y | | Y | | | | | | 25/09/07 |
| 94 | 8 | L | 10 | /Bystroe canal | UA | | | | Y | | | Y | | | | | | | | 25/09/07 |
| 94 | 8 | R | 10 | /Bystroe canal | UA | | | | Y | | | Y | | | | | | | | 25/09/07 |
| 94 | 8 | M | 10 | /Bystroe canal | UA | | Y | | Y | | Y | | | Y | | | | | | 25/09/07 |
| 94 | 8 | M | 10 | /Chilia arm rkm 12 - Bystroe canal rkm 7 | UA | | | Y | | | | | | | | | | | | 25/09/07 |
| 95 | 0 | L | 10 | Sulina - Sulina arm | RO | | | | Y | | | | | | | Y | | | | 26/09/07 |
| 95 | 0 | R | 10 | Sulina - Sulina arm | RO | | | | | | | | Y | | | | | | | 26/09/07 |
| 95 | 0 | M | 10 | Sulina - Sulina arm | RO | | Y | | | | Y | Y | | Y | | | | | | 26/09/07 |
| 95 | 0 | M | 10 | Sulina arm River km 38 - 26 | RO | | | Y | | | | | | | Y | | | | | 26/09/07 |
| 96 | 0 | L | 10 | Sf.Gheorghe - Sf.Gheorghe arm | RO | | | | Y | | | | | | | | | | | 26/09/07 |
| 96 | 0 | R | 10 | Sf.Gheorghe - Sf.Gheorghe arm | RO | | | | Y | | | | | | | | | | | 26/09/07 |
| 96 | 0 | M | 10 | Sf.Gheorghe - Sf.Gheorghe arm | RO | | Y | | Y | | Y | Y | | Y | | | | | | 26/09/07 |
| 96 | 0 | M | 10 | Sf.Gheorghe arm River km 107 - 107 | RO | | | Y | | | | | | | | | | | | 26/09/07 |
| AR1 | 234 | M | | Upstream Pitesti | RO | | Y | | | | | | | Y | | | | | | 20/09/07 |
| AR2 | 121 | M | | Upstream Bucharest | RO | | Y | | | | | | | Y | | | | | | 20/09/07 |
| DR1 | 77 | M | | Drava D.Miholjac | HR | | Y | | | | | | | Y | | | | | | 02/09/07 |
| IS1 | 17.7 | M | | Orechovitza | BG | | Y | | | | | | | Y | | | | | | 15/09/07 |
| IS2 | 320 | M | | Before Reservoir Iskar | BG | | Y | | Y | | | | | Y | | | | | | 15/09/07 |

| JDS2 No. | River km | Location | Danube type | Sampling site name | Country code(s) | | Sample matrices | | | | | | | | | | | Date of sampling |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Water | SPM | Sediments | Biota - Mussels | Biota - Fish | Macrozoobenthos | Phytobenthos | Phytoplankton | Water - XAD cartridges | Sediment - Pore water | | |
| JA1 | 51.2 | M | | Karantzi | BG | | Y | | | | | | | Y | | | | 18/09/07 |
| JA2 | 131.7 | M | | Yabalka-Gabrovo | BG | | Y | | Y | | | | | Y | | | | 18/09/07 |
| MO1 | 79 | M | | Morava, Lanzhot (River km. 79-76). | CZ | | Y | | Y | | | | | | | | | 21/08/07 |
| MO2 | 17 | M | | Dyje, Pohansko (River km 17-14) | CZ | | Y | | Y | | | | | | | | | 21/08/07 |
| OL1 | 163 | M | | Upstream Rm. Valcea | RO | | Y | | | | | | | Y | | | | 16/09/07 |
| OL2 | 61 | M | | Downstream Slatina | RO | | Y | | | | | | | Y | | | | 16/09/07 |
| PR1 | 404 | M | | Ungheni | RO | MD | Y | | Y | | | | | Y | | | | 23/09/07 |
| PR2 | 220 | M | | Bumbata-Leova | RO | MD | Y | | | | | | | Y | | | | 23/09/07 |
| RL1 | 10 | M | | Basarbovo | BG | | Y | | | | | | | Y | | | | 20/09/07 |
| RL2 | 37 | M | | Beli Lom, Pisanetz | BG | | Y | | Y | | | | | Y | | | | 20/09/07 |
| SA1 | 254 | M | | Sava downstream Zupanja | RS | | Y | | | | | | | | | | | 20/09/07 |
| SA2 | 195 | M | | Jamena | RS | | Y | | Y | | | | | | | | | 07/09/07 |
| SA3 | 136.4 | M | | Sremska Mitrovica | RS | | Y | | Y | | | | | | | | | 07/09/07 |
| SA4 | 62 | M | | Uš e | RS | | Y | | Y | | | | | | | | | 07/09/07 |
| TI1 | 744 | M | | Tiszabecs | HU | | Y | | Y | | | | | | | | | 27/08/07 |
| TI2 | 334 | M | | Szolnok | HU | | Y | | Y | | | | | Y | | | | 31/08/07 |
| TI3 | 160 | M | | Szeged (HU border) | HU | RS | Y | | Y | | | | | Y | | | | 31/08/07 |
| TI4 | 152 | M | | Martonoš | RS | | Y | | Y | | | | | | | | | 04/09/07 |
| TI5 | 66 | M | | Novi Be ej | RS | | Y | | Y | | | | | | | | | 04/09/07 |
| TI6 | 9 | M | | Titel | RS | | Y | | Y | | | | | | | | | 04/09/07 |
| VM1 | 237.2 | M | | Bagrdan | RS | | Y | | Y | | | | | Y | | | | 08/09/07 |
| VM2 | 154.1 | M | | Varvarin | RS | | Y | | Y | | | | | Y | | | | 08/09/07 |
| VM3 | 34.8 | M | | Ljubi evski Most | RS | | Y | | Y | | | | | Y | | | | 08/09/07 |

The "top 23" sampling sites analysed by EC JRC Ispra are highlighted in blue. L: left; R: right; M: middle.

For the list of fish sampling sites, see Chapter 8.

## Table 2: List of JDS2 cooperating laboratories.

| No. | List of laboratories | Country |
|---|---|---|
| 1 | Austrian Research Centres GmbH – ARC, Environmental Sciences, Seibersdorf | AT |
| 2 | University of Natural Resources and Applied Life Sciences, Institute for Hydrobiology and Aquatic Ecosystem Management, Department Water – Atmosphere – Environment, Vienna | AT |
| 3 | University of Natural Resources and Applied Life Science, Low-Level Counting-Laboratory Arsenal, Vienna | AT |
| 4 | International Atomic Energy Agency, Isotope Hydrology Section, Vienna | AT |
| 5 | ICIM National Research and Development Institute for Environmental Protection, Bucharest | RO |
| 6 | Institute for Biological Research, Belgrade | RS |
| 7 | Austrian Academy of Sciences, Institute of Limnology, Mondsee | AT |
| 8 | European Commission - Joint Research Centre, Institute for Environment and Sustainability, Rural, Water and Ecosystem Resources Unit, Ispra | IT |
| 9 | Bavarian Environment Agency, Munich | DE |
| 10 | Medical University Vienna, Clinical Institute of Hygiene and Medical Microbiology, Water Hygiene and Zentrum für Anatomie und Zellbiologie, Arbeitsgruppe Mikrobiologie and Vienna University of Technology, Institute of Chemical Engineering | AT |
| 11 | DVGW-Technologiezentrum Wasser (TZW), Karlsruhe | DE |
| 12 | Umweltbundesamt GmbH, Vienna | AT |
| 13 | Ukraine Research Hydrometeorology Institute, Kiev | UA |
| 14 | University of Vienna, Centre for Earth Sciences, Department of Environmental Geosciences | AT |
| 15 | University of Vienna, Faculty of Life Sciences, Department of Limnology and Hydrobotany | AT |
| 16 | VITUKI - Environmental Protection and Water Management Research Institute, Budapest | HU |
| 17 | Water Research Institute TGM, Brno and Prague | CZ |
| 18 | RECETOX, Masaryk University of Brno | CZ |
| 19 | Water Research Institute, Bratislava | SK |

# 3 Hydromorphology

Ulrich Schwarz and Wolfgang Kraier

## 3.1 Introduction

Hydromorphological alterations are recognised by the ICPDR as one of four basin-wide significant water management issues. In a related issue paper on hydromorphological alterations, the most significant alterations were categorised into longitudinal continuity interruptions (dams, weirs); lateral connectivity interruptions (loss of floodplains, bank reinforcements) and hydrological alterations (water abstraction (residual water) and hydro-peaking). The main impacts of hydromorphological alterations on the riverine habitats result in:

- The decline of species biodiversity;
- The decline of species abundance;
- Altered population composition;
- Hindrance to species migration and a corresponding decline in naturally reproducing fish populations (e.g. sturgeon).

The lack of harmonised standard methods for the survey and assessment of hydromorphological features made it necessary to develop a methodology conform with CEN that would also fit into the JDS2 daily time plan. The SOP (Standard Operational Procedure) defines two different methods for the longitudinal overview survey and the detailed site survey. The first one will assess the hydromorphological situation of the rivers and water bodies while the latter one is mainly for the interpretation of biological result at a particular sampling site.

In the case of large rivers, the importance of remote sensing techniques (including navigation maps) as well as historical maps was considered. The overview survey cannot provide a substitute for national surveys, which will be done on a more detailed level and along shorter stretches. However, the JDS2 is the first time that hydromorphological parameters have been surveyed systematically with a uniform method for the entire navigable longitudinal Danube stretch (over 2415 rkm) and at all 96 JDS sampling sites (including the main tributaries at confluences with the Danube and the northern and southern Danube Delta branches).

The hydromorphological parameters support the assessment of biological elements for water bodies under the EU Water Framework Directive (WFD). The strongest link is given to the physical habitat description of fish, macrozoobenthos and macrophytes (Biological Quality Elements - BQE). But it is also given to the capability of connected floodplains and natural channels with their natural functions as nutrient sinks; as self-purification areas (including resilience function after accidents with hazardous substances) and as retention areas for flood protection. The continuous longitudinal survey was designed to be used as an update of the Danube Basin Analysis 2005, concerning the hydromorphology of the River Danube.

In general, the survey leads to a better understanding as to where river habitats are impacted by hydropower, navigation and flood protection. Based on a hydromorphological risk assessment, the Programmes of Measures under the WFD will be planned. To achieve the objectives of the WFD in time, it will also be necessary to set technical measures (such as restoring continuity for migratory species or improving habitat conditions). Stretches with intact hydromorphological features that are threatened by navigation and hydropower projects should be protected.

## 3.2 Methods

The description and evaluation of hydromorphological characteristics for large rivers is strongly dependent on various background data (such as historical, topographical and navigation maps, satellite images and hydrologic, morphometric and landuse data). For a project like JDS2, a standardised method for hydromorphological survey and assessment did not exist. Therefore a special methodology was developed and defined in the Standard Operational Procedure (SOP), based on existing methodologies for large rivers meeting the requirements of the CEN framework standard EN14614 (2004) for the assessment of hydromorphological features of rivers and also the WFD. All 96 JDS sites were surveyed by GPS.

The following two-step approach, as described in the SOP, was applied:

–   As the JDS2 sampling sites selected for the biological and chemical analysis were not always representative of the morphological situation of the whole water body, the hydromorphological condition of the Danube was divided into stretches to be assessed continuously.

–   For this continuous longitudinal survey, a huge amount of existing information and data were used to pre-subdivide the Danube into homogeneous stretches (approx. 50 km long) and to prepare the necessary data for the evaluation (such as the general planform and sinuosity; the main river engineering structures; longitudinal and lateral continuum interruptions as well as on the floodplain and adjacent landuse. The survey was used to update, approve and validate the pre-results, in particularly for the river banks. A five-class evaluation for 1. channel 2. banks and 3. floodplain is the basis for the total evaluation (mean values out of the three categories).

–   For the detailed site survey of the 96 JDS sampling sites, additional data was prepared, such as substrate, flow velocity, in-channel features and shoreline index, in order to support the fieldwork and as a basis for the interpretation of biological data. A detailed parameter list is available in the SOP; no evaluation as was done for the continuous survey was undertaken.

Additionally, "Fact sheets" for all JDS sites (including tributaries) were prepared before the survey was undertaken. These contained the basic JDS site description data such as: station code, rkm, catchment size upstream, width, depth, main hydrological values, river slope, WFD type, ecoregion, water body code, basic cross-section sketches for the Danube River and a zoom of the navigation map, as well as satellite images and historical maps.

An extensive set of continuous digital geocoded documentation photographs was prepared. Both evaluations, the continuous survey and the detailed site survey, are supported by a GIS and database application.

## 3.3 Results

The results are extracted from the JDS2-HYMO Access database, which is divided into: the continuous survey, the site survey, an inventory of dams and hydrographical data for the survey time. The results are presented in this order.

### 3.3.1 Continuous Longitudinal Survey

A total of 66 homogenous stretches along the Danube including the three delta branches (covering 2,610 rkm) were prepared. The mean length of each evaluation stretch is 40 rkm; the smallest is 8 rkm (strongly altered town stretch) and the longest is 132 rkm along the Lower Danube). On the whole, a length of 15 rkm was defined as the minimum length, but due to clear-cut situations at modified town stretches, the size was decreased in about ten sections. In general, the length of homogenous segments increases from the upper to the Lower Danube.

### 3.3.1.1   Channel

Channel patterns have been altered along almost the entire Danube as a result of navigation and hydropower. In particular, within the German and Austrian stretch, only a very few reaches still host

near-natural conditions - disregarding navigation and the modified sedimentation regime (due to storage of gravel and sand behind dams and regular dredging for navigation and flood protection purposes).

Totally modified ('class five') canalised Danube stretches are found along city stretches (such as in Vienna) as well as the Gabcikovo Canal, but most of the chain of hydropower plants in Germany and Austria fall into 'class four' (severely modified) with smaller sections (below the minimum stretch size) categorised as 'class five.'

Moderate conditions can be found over long stretches in Hungary, mainly due to the greatly reduced length of the river due to meander cut-off carried out since the 17th Century.

Good conditions remain in some breakthrough/gorge reaches (such as in the Wachau, Austria and Danube Bend, Hungary) and lowland stretches in upper Serbia (without influence from the Iron Gate backwaters) and, in particular, along the Romania-Bulgarian stretch. Extant meandering reaches are very rare and most meanders have been cut - even within the last few decades, as has occurred in the Sft. (southern) Gheorge branch in the Danube Delta.

None of the stretches can be assessed as 'class 1' indicating reference conditions, due to the existence of river regulations for navigation and flood protection as well as altered sediment balance (dams in the upper and middle course of the Danube and in many tributaries).
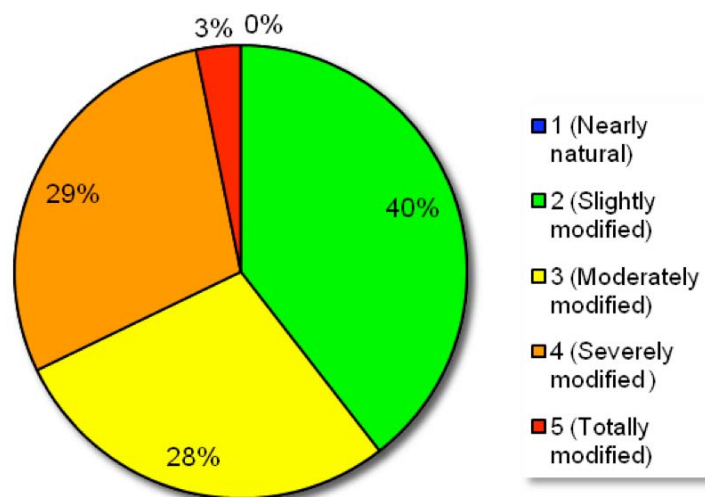


Figure 1: Channel assessment in five classes according to the SOP
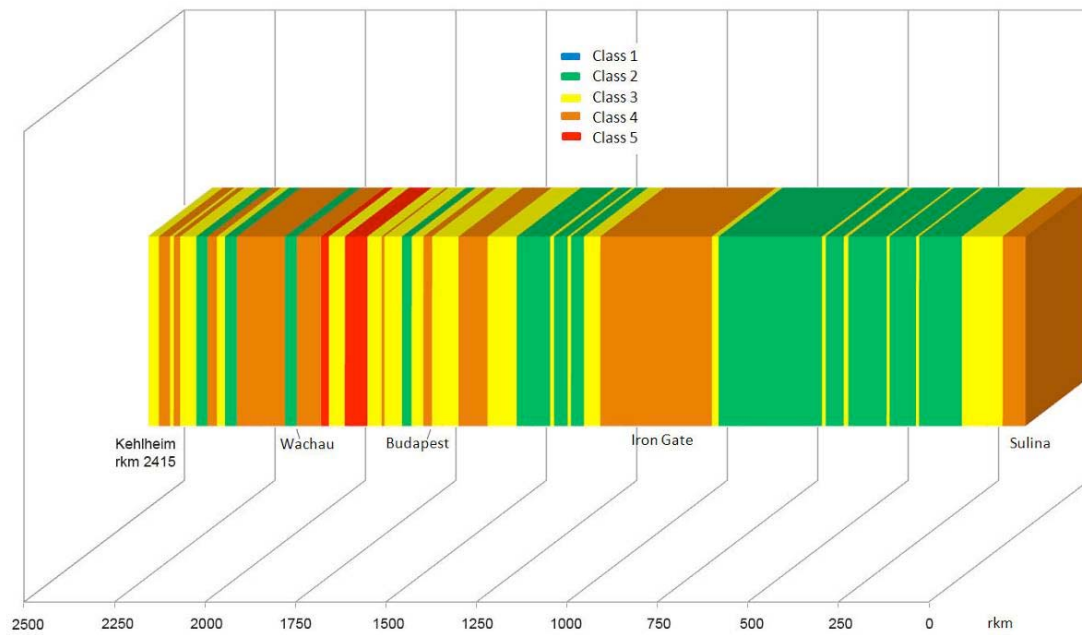
Figure 2: Channel assessment as longitudinal colour-ribbon visualisation
(compare with Figure 1)

### 3.3.1.2   Banks

The riverbanks are particularly reinforced in Austria and Germany, while further downstream the banks of the Danube are only totally reinforced in towns. In the Hungarian reach, the banks are reinforced in large sections ('class three'). Along the entire Lower Danube, bank reinforcement covers only a few percent of the total river course (see Figure 3 indicating 17% in 'class 1') but local erosion protection activities currently increase the length of reinforced banks.
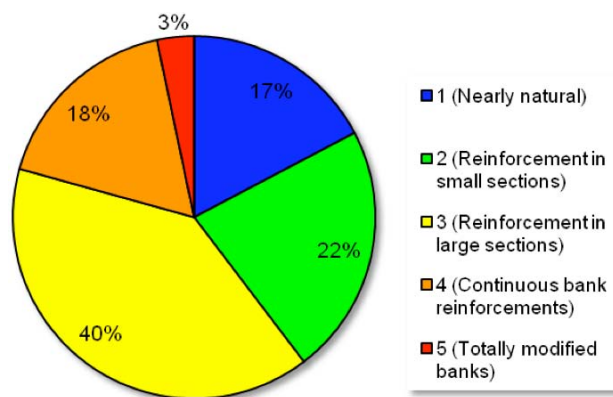


Figure 3: Bank assessment in five classes according to the SOP
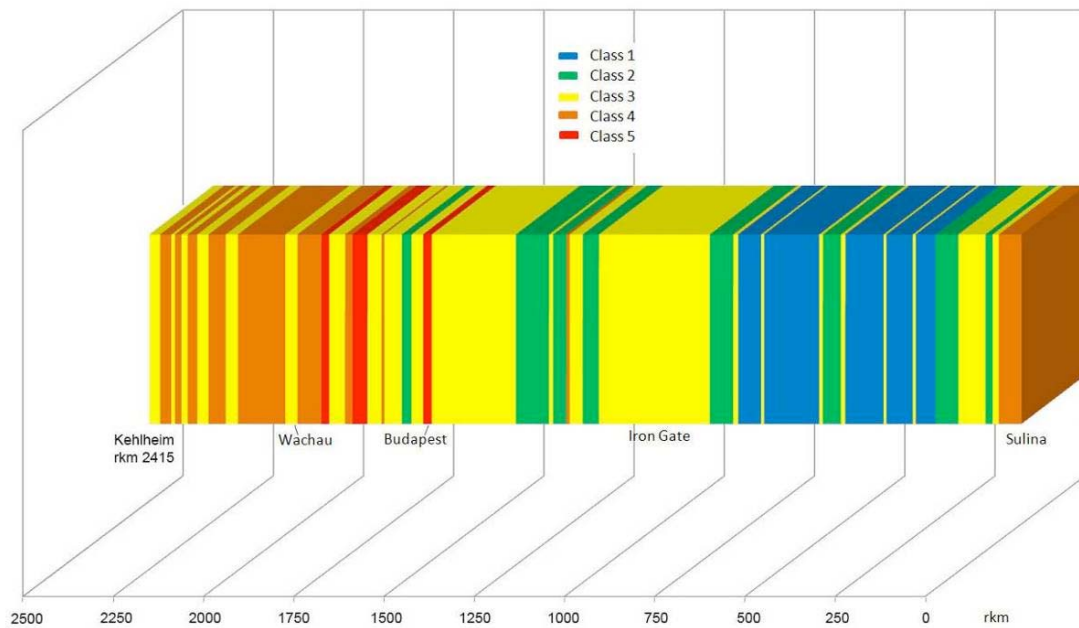
Figure 4: Bank assessment as longitudinal colour-ribbon visualisation (compare with Figure 3)

### 3.3.1.3   Floodplains

Only a few reaches along the Danube have nearly intact or intact floodplains (21% in total according to Figure 5 below). The largest, continuous, active floodplain areas remaining along the Danube are listed as follows:

–   Danube National Park (AT): 10,000 ha;

–   Danube-Drava National Park (HU): 28,000 ha (Danube section) ;

–   Kopački Rit and Gornje Podunavlje Nature Parks (HR/RS): ~40,000 ha ;

–   Floodplain forests of the Serbian Danube upstream of the Tisza confluence (RS): ~20,000 ha;

–   Small Braila Island Protected Area (RO): ~20,000 ha;

–   Danube Delta: ~500,000 ha.

Along the Hungarian Danube south of Budapest and along the entire Romanian-Bulgarian stretch, most of the floodplains are disconnected by narrow flood protection dikes.
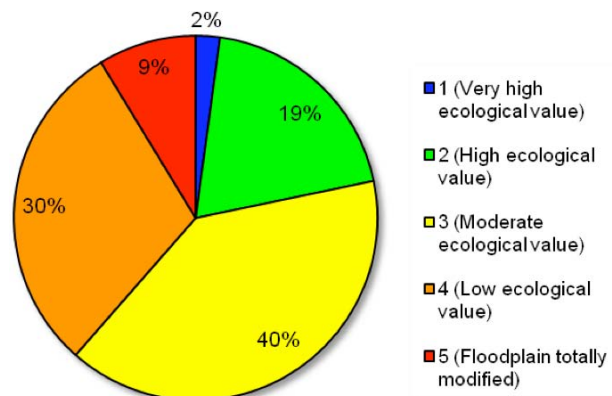


Figure 5: Floodplain assessment in five classes according to the SOP
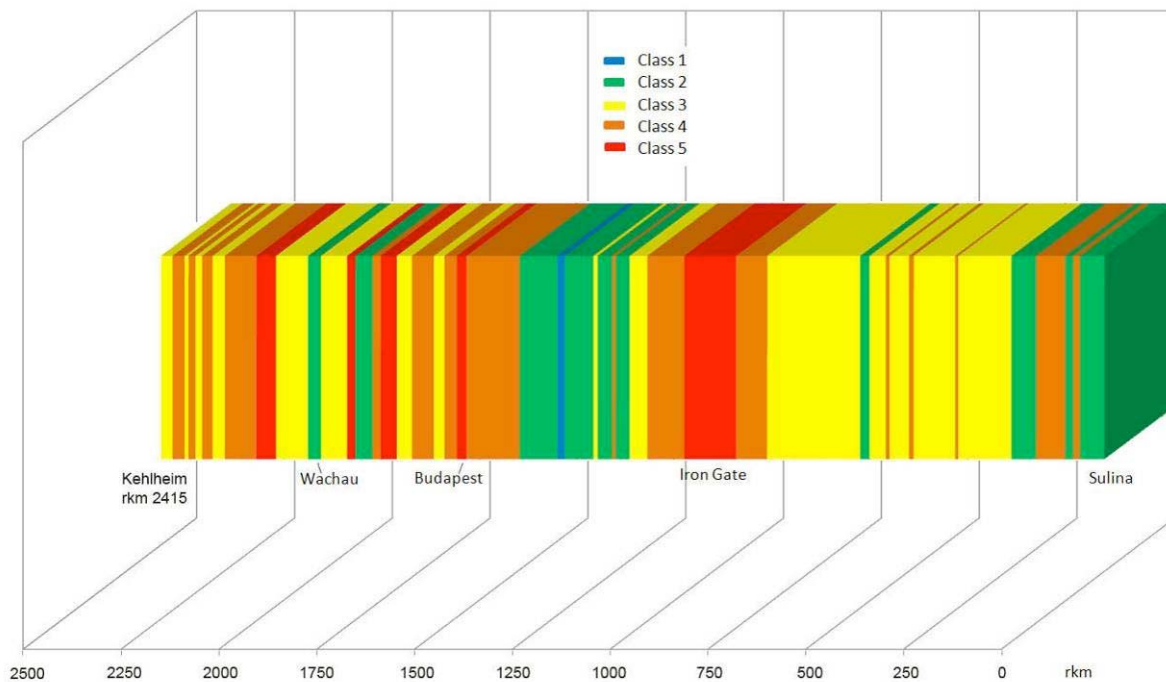
Figure 6: Floodplain assessment as longitudinal colour-ribbon visualisation
(compare with Figure 5)

### 3.3.1.4   Overall hydromorphological assessment

More than one third of the Danube from Kelheim to the Black Sea can be classified as still being 'class 2' i.e. having good hydromorphological conditions (see Figure 7).

However, another third of the Danube can be characterised as strongly altered.

The analysis for the Upper, Middle and Lower Danube (see Figure 8) indicates that the upper reach in Germany and Austria is the most affected by significant hydromorphological changes. There are only small free-flowing stretches such as Straubing-Vilshofen (Bavaria) or the Wachau and downstream of Vienna (Austria). On the other hand, the middle and lower courses of the Danube are interrupted by three large hydropower plants: the Gabcikovo dam and the two Iron Gate dams.
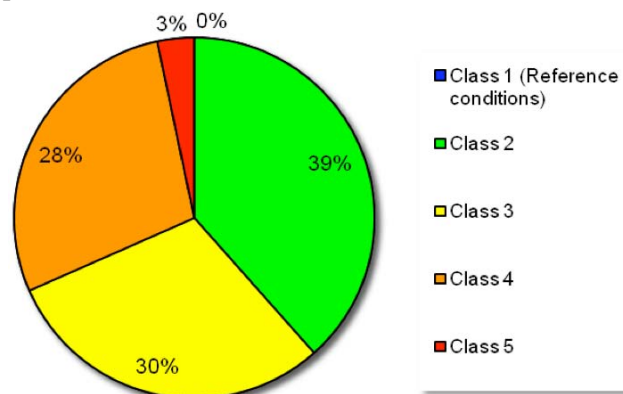


Figure 7: Overall total hydromorphological assessment in five classes according to the SOP
(mean of channel, banks and floodplain evaluation) coloured in WFD classification schema
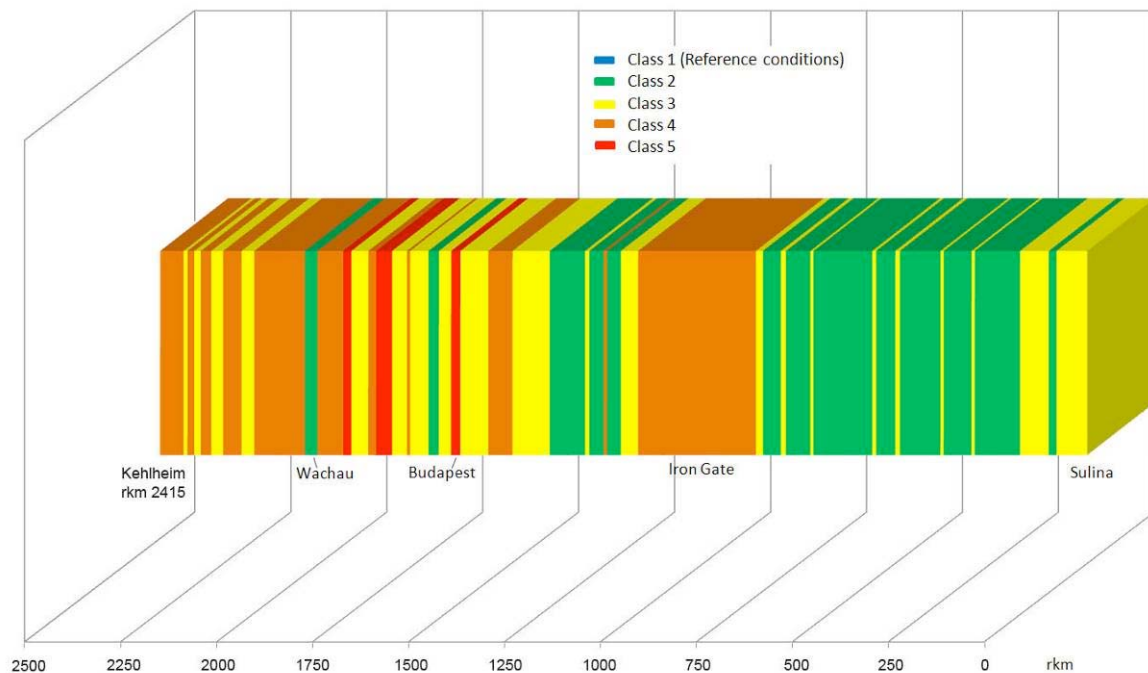
Figure 8: Overall total hydromorphological assessment in five classes as longitudinal colour-ribbon visualisation (compare with Figure 7)

In the case of the assessment of banks and floodplains, only very short stretches can be characterised as reference conditions ('class 1'). Concerning riverbanks, such stretches occurred along the steep banks of the Serbian, Bulgarian and Romanian Danube - longer stretches were observed in the Lower Danube. Regarding floodplains, stretches occurred along the protected sites of Kopački Rit and Gornje Podunavlje (HR, RS) and on the right bank at Small Braila Island (RO).

Restoration activities along the Danube are improving the ecological situation e.g. at the Bavarian Danube (upstream of Straubing) and the Austrian Danube (aside of the two passable dams at Melk and Vienna; the Danube National Park downstream of Vienna and the Wachau/tributary confluence - EC-Life projects). Nevertheless, so far they do not significantly change the results of the overall evaluation. Maps of the assessment stretches can be found on the CD-ROM.

### 3.3.2  Detailed site survey
The detailed site survey results serve as hydromorphological characterisation for use in the assessment of biological data and for synthetic analysis along with biological and chemical quality elements. The correlation between hydromorphology and the macrozoobenthic air-lift sampling data seem to match at best. More detailed information is available on the CD-ROM.

### 3.3.3  Comparison with JDS1 results
It is not possible to compare results for JDS1 and 2 as a continuous survey was not done in 2001. Only records about the general hydromorphological situation at the sampling sites can be compared, which has been prepared for macrozoobenthos and macrophytes data evaluation. A first comparison indicates that, as expected, there are no major changes (substrate, bank characteristics) since 2001.

### 3.3.4  Hydrological flow situation
During the JDS no significant high or low water situations reaching long-term annuality values for low or high water levels were recorded in the Upper Danube. However during the two weeks prior to the JDS2, Danube discharges at Regensburg almost reached a one year flood event.

The middle course of the Danube was characterised by discharges slightly below mean water. Whereas, the Lower Danube was subject to a continuous discharge increase from an annual low water situation (about 3,000 m³/s at Zimnicea, rkm 550) towards more than mean water (over 6,000 m³/s). The survey was caught up by the increasing water flow downstream of the Iron Gate and then rode the wave downstream to the delta.

### 3.3.5  Dams / migration barriers (disruption of longitudianl continuity)

A total of 18 dams can be listed for the entire navigable Danube from Kelheim to the Black Sea. Fish migration facilities (such as bypasses) exist and are functioning at only two dams (Melk and Wien-Freudenau). The resulting backwaters of impoundments directly depend on the height of dams and the slope of the river course. The longest backwater occurs at the Iron Gate reaching about 250 rkm; the shortest is found in Germany and Austria reaching about 5 rkm.

## 3.4  Conclusions

### 3.4.1  General conclusions

–   The hydromorphological survey was particularly successful as a basis for an updated pressure and impact analysis and risk assessment of the Danube River, as well as for the development of a programme of measures. The countries are encouraged to continue further with more detailed national investigations.

–   The overall hydromorphological assessment showed that the hydromorphological situation of the Danube varies from source to mouth. It is much better in the Lower Danube than in the upper part.

–   Deterioration of the status quo should be prevented. Specific programmes of measures should start to improve the situation for migratory species and support floodplain restoration as well as habitat restoration/improvement.

–   The JDS sites are not always representative of the Danube's hydromorphological features as many were selected to detect discharges from agglomerations (water quality aspects). Moreover, too many sites are located just upstream of dams in the impoundments, within city areas or transition stretches (e.g. from cities to more natural stretches, or just downstream of dams) and are not sufficiently representative of long Danube stretches.

–   In addition to the natural debris seen in some stretches (such as wood and organic material), large amounts of waste debris (such as plastic bottles and bulky waste) were observed as a negative phenomenon.

### 3.4.2  Technical conclusions for the next JDS

–   The cruise requirements allowed for the continuous analysis of the main navigable channel only; no side channels were surveyed.

–   The hydromorphological site characteristics should be made even more detailed for microhabitats in order to support the biological assessment.

–   For the assessment of Danube water bodies, the continuous longitudinal survey is more relevant and important than the site survey due to its missing representativeness as described in the previous section. It provides good baseline information for the future.

–   Downstream of Belgrade, the quality of navigation maps decreases and the data shown is generally older. Unfortunately the new digital navigation maps do not show all river regulation works, shallows, bars/islands and indicative values for the channel depth and flow velocity.

- To ensure high quality of data and reliability of results, it is crucial that the assessment of hydromorphological features is done by a single expert during the whole survey.

- The presence of journalists and press teams on-board should be limited as much as possible, preferably to some selected stations.

### 3.4.3  Recommendations for measures

- If stretches do not reach good ecological status, countries should put additional effort into restoring more stretches and preserving those that still retain good hydromorphological conditions. The preliminary risk assessments and HMWB designations should be revised.

- Prevention of further bank revetments and reinforcements (such as those along the Lower Danube carried out with EC funding) where better environmental options are possible.

- Continuation and improvement of restoration measures along the Upper Danube by further reconnecting floodplains and providing more space for channel development (considerably reducing the bank reinforcement to the absolute minimum necessary).

- Large-scale restoration of floodplains along the Lower Danube (beginning with the reconnection of islands with ring dikes).

- Environmental impact studies and long-term monitoring of hydromorphological features for relevant planning control (checklist of exemptions).

# 4 Macroinvertebrates

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## 4.1 Introduction

Benthic macroinvertebrates are commonly applied for the quality assessment of rivers (Birk & Hering, 2002). Our good knowledge of their environmental requirements and of species' response to various environmental factors has led to these organisms being widely used as (bio)indicators in water management and in applied ecology (see Davis & Simon, 1995; Rosenberg & Resh, 1995). Numerous commonly used biological assessment systems for rivers and streams are based on so-called "metrics" or – synonymously used – "measures" or "biological attributes". The requirement of the European Water Framework Directive (EC, 2000/60; WFD) for an integrated assessment methodology to evaluate the ecological status of water bodies is a big challenge for the applied limnological sciences. To assess the ecological status of a water body, selected attributes of the biological indicators have to be considered and compared to relevant target values under reference conditions. As a consequence, new assessment systems and evaluation techniques have had to be developed throughout Europe during the last few years. Among other approaches, the applicability of multi-metric techniques i.e. combinations of several metrics and indices addressing different stressors or different components of the biocoenosis, has been tested (Brabec et al., 2004; Buffagni et al., 2004; Lorenz et al., 2004; Ofenböck et al., 2004; Pinto et al., 2004; Sandin et al., 2004; Vlek et al., 2004).

The longitudinal distribution of benthic macroinvertebrates along the entire Danube River has been investigated in earlier studies i.e. prior to the implementation of the EU WFD (Russev, 1998; Equipe Cousteau, 1992; JDS 2001 Final Report; Slobodnik et al., 2005; Csányi & Paunovic, 2006).

For the Danube, appropriate sampling methods for the benthic invertebrate fauna are under discussion and basinwide WFD compliant assessment methods are still lacking. During the JDS2, benthic invertebrates were sampled using standardised methods and this data can be used to evaluate the ecological status of the Danube in a harmonised way. The following chapters describe the methods applied; the characteristics of the macroinvertebrate community along the Danube and show a possibility for the application of WFD compliant assessment methods for the Danube River.

## 4.2 Methods

### 4.2.1 Sampling and sorting

For the JDS2, the Air-lift and Multicorer techniques were used as standard methods; equipment mounted on the ship was used for both. Additionally, samples were taken at the bank zones with the Kick & Sweep technique. This was done for the purpose of comparison of sampling efficiency regarding diversity and abundance; usability in standard monitoring programmes and for comparability with results from previous surveys.

#### 4.2.1.1 Air-lift and Multicorer sampling technique

During JDS2, benthic macroinvertebrates were sampled from the river bottom (depths between 1.2m and 11.5m; average 4.9m) by the Air-lift sampling method (Prehofer, 1998) at 81 sites. On certain

sites, due to low water level or very fine sediments, the Multi Habitat sampling method (9 sites, mostly tributaries) and the Multicorer sampling method (6 sites) were applied respectively.
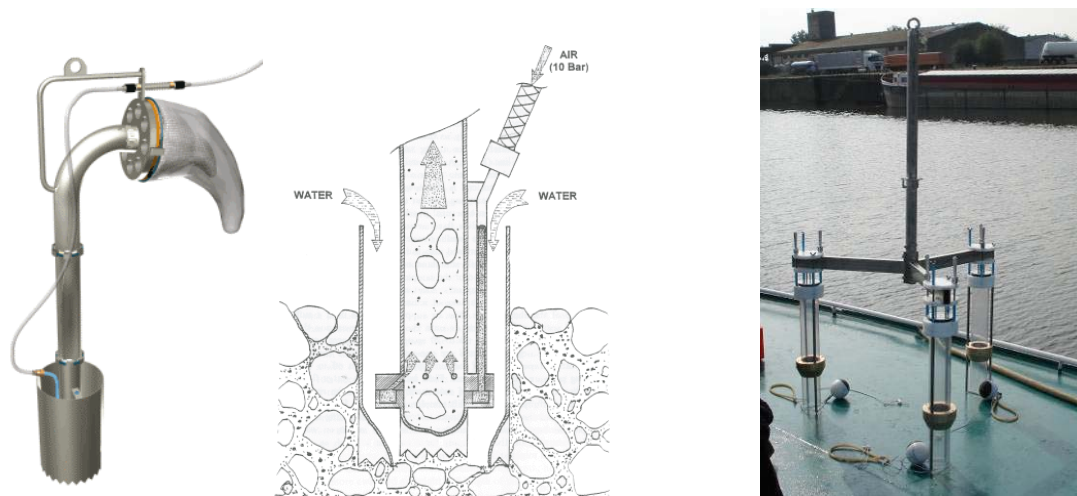


Figure 9: Left: Air-lift device (from Prehofer, 1998, modified), right: Multicorer (Photo: P. Leitner)

A total of 96 sites were sampled. At each site, six sampling units (three on the left and three on the right riverside) were taken and pooled to one sample. At sites where substrate composition or water velocity varied greatly between banks, the samples from the left and the right side were stored separately.

The collected material was rinsed through a net with a 100 µm mesh-size. Samples were pooled and the material was preserved in formaldehyde (4%) on-board and then stored for further determination in the laboratory at the BOKU in Vienna. After a curing time of at least two weeks, the material of each sample was fully sorted. The animals were counted, separated into their specific orders, weighed and identified by taxonomic experts to the highest degree possible.

To ensure harmonised data storage, the species per site information was filled into Access-based software, ECOPROF 3.0.1 (BMLFUW, 1995-2007), which is compatible with the ICPDR database. For the calculation of metrics and saprobic indices, only WFD compliant (semi-)quantitative and area related approaches (Air-lift and MHS) were used. Species list, diversity as well as cluster analyses for typological conclusions were based on all the data collected during JDS2, combining all methods.

### 4.2.1.2   Kick & sweep and dredge sampling

The kick & sweep (K&S) sampling technique (EN 27828:1994) was used in the shallow bank zone of the Danube (on 74 sites) and the tributaries (on 9 sites). A hand net with a 500 µm mesh size was used for sampling to a water depth of 1.5 m. The majority of habitat types at the bank zone were included in the sampling programme.

Due to elevated water levels downstream of the second Iron Gate reservoir (on the sites JDS65 to JDS96), dredging was used as an alternative sampling technique - altogether 32 cross sections (including 6 tributaries) were sampled with a triangular dredge (opening of 25 cm). All of the sub-samples (right, middle and left) were handled separately for later spatial analysis.

The material collected with K&S and dredging was rinsed through a net with a 500 µm mesh-size. The samples were preserved in formaldehyde (4%) on-board and later sorted at VITUKI, Budapest for further taxonomic determination by experts.

## 4.2.2  Analysis

### 4.2.2.1   Saprobic index and calculation of metrics

Regarding the stressor organic pollution, saprobic indices were calculated based on available national methods, using the software packages ECOPROF 3.0.1. and ASTERICS/PERLODES (www.fliessgewaesserbewertung.de). For the indication of water quality classes, the threshold values given in Table 3 were applied (Buijs, 2006).

### Table 3: Threshold values for the indication of water quality classes based on organic pollution

| Ecological status class | Saprobic reference condition (range of saprobic index) | | | |
|---|---|---|---|---|
| I – High | 1.65 – 1.80* | 1.75 - 1.85* | ≤ 1.75** | ≤ 2.00** |
| II – Good | 1.81 – 2.25 | 1.86-2.30 | 1.76 – 2.21 | 2.01 – 2.40 |
| III – Moderate | 2.26 – 2.85 | 2.31 – 2.90 | 2.22 – 2.68 | 2.41 – 2.80 |
| IV – Poor | 2.86 – 3.40 | 2.91 – 3.45 | 2.69 – 3.14 | 2.81 – 3.20 |
| V – Bad | >3.40 | >3.45 | >3.15 | >3.21 |

* Reference conditions and class boundaries in Germany – for national types 9.2 and 10.
** Reference conditions and class boundaries in Austria for the Danube.

Additionally, the software was also used for the calculation of more than 200 biological metrics. For further statistical analysis and graphic visualisation of metric values, the software package STATISTICA 7.1 (StatSoft. Inc., 2005) was used. All sampling sites were assigned to river reaches (according to Literáthy et al., 2002) and roughly pre-classified to free-flowing or impounded sites. Sites that exceeded the threshold value for good status concerning organic pollution were excluded from the metrics analysis. All metrics were analysed concerning their ability to distinguish between free-flowing sections and impounded sites.

Descriptive statistics (central tendency, range, distribution, outliers) were used to characterise metric performance. Metrics with insufficient data or too many zero values, as well as metrics that could not discriminate sufficiently among sites of different conditions, were eliminated. The remaining metrics were chosen as possible candidates for assessing the ecological status of the Danube River.

### 4.2.2.2   Multivariate analysis

For the examination of faunal similarities between sampling sites and for revising the existing Danube typology (Literáthy et al., 2002), multivariate statistical procedures were used (cluster analysis, PCA, SØRENSEN coefficient). For more detailed information see the Full Report of the JDS2 (on CD-ROM).

## 4.3 Results and discussion

### 4.3.1  Diversity, abundance and biomass

In total, 441 invertebrate taxa were documented by combining all methods used during the JDS2 (Air-lift, Multicorer, MHS, Kick & Sweep and Dredging). The most heterogeneous groups were Diptera (174 taxa) and Oligochaeta (53 taxa) followed by Ephemeroptera (42 taxa), Trichoptera (35 taxa) and Molluscs (Bivalvia 26 taxa, Gastropoda 27 taxa, respectively). Coleoptera (17 taxa), Amphipoda (13 taxa) and Hirudinea (11 taxa) are also noteworthy; other groups are important but less diverse. This overall characteristic in diversity does not change along the three reaches of the Danube, although the number of insects decreases considerably downstream (Figure 10).

Rare species found along the JDS2 are: the large burrowing Ephemeroptera, *Palingenia longicauda* (Prut River), and the Gastropoda species, *Theodoxus transversalis* (Lower Reach). Regarding

Amphipoda, the invasive species, *Corophium robustum,* was documented for the first time in Austria (Pöckl, in litt.); *Crangonyx pseudogracilis* is new to the fauna of the Danube (Bernerth & Stein, 2003; Berthold & Kaiser, 2005).
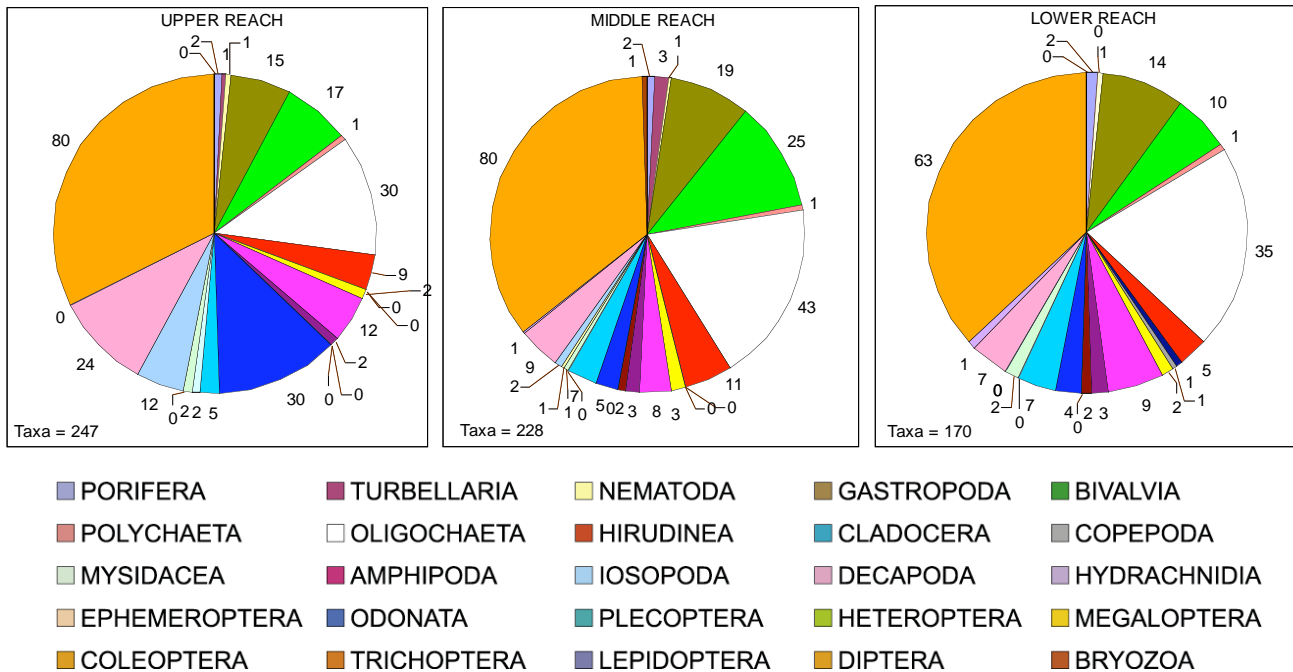


Figure 10: Number of taxa per taxa group along the different reaches of the Danube

Regarding abundance (ind./m²), Amphipoda are the dominant group in all Danube reaches and constitute up to 75 % while Isopoda (mainly *Iaera istri*) play an essential part in the Upper Reach and decrease downstream. Oligochaeta and Mollusca can be found in increasing numbers in the Lower Reach. EPT- Taxa (Ephemeroptera, Plecoptera and Trichoptera) were negligible – with the exception of the Upper Reach (sites 1 and 2). With regards to aquatic insects, only Chironomidae play a major role.

In terms of biomass, Mollusca are the most important organisms of the Danube and investigated tributaries. Due to their size Bivalvia make up more than 80% of the whole biomass, followed by Gastropoda (10% to 35%). Looking at the different reaches of the Danube (according to Literáthy et al., 2002), the increasing dominance of Mollusca from the Upper to the Lower Reach becomes evident. Although Crustacea are the most abundant group, they play only a minor role regarding biomass.

The considerable amount of data collected during JDS2 from the river bottom and bank zone illustrated the longitudinal distribution patterns of characteristic Danubian macroinvertebrate taxa that are important regarding biodiversity and the protection of species: e.g. the formerly widespread snail, *Theodoxus transversalis,* is now living in a very restricted section on the Lower Danube only (JDS sites 70-86). A detailed description is given in the Full Report on the CD-ROM.

## 4.3.2  Danube typology

On the basis of the combined data-set of all methods used during JDS2, the three main reaches proposed by Literáthy et al. (2002) were confirmed by the macrozoobenthic community. Hence, the typology was used as a prerequisite for the development of a type-specific assessment system. Only the most upper stretch (sites 1 and 2) showed totally different hydromorphological (e.g. stream size, substrate, depth) and biological characteristics (taxa richness, abundance, taxa composition) compared

to downstream sites, suggesting a separation from the rest of the Danube reaches. A more detailed multivariate analysis of the sampling sites and further comments and conclusions to the Danube typology can be found in the full report on a CD-ROM – including an additional revision of the Danube typology at a smaller scale resulting in five distinguished section types for the Danube River. This outcome may be of further relevance for future development of assessment methods that rely on one of the sampling methods of the JDS2 or on a combination of them, respectively.

### 4.3.3  Comparison of sampling methods

A comparison of methods used from the ship (Air-lift, Multicorer) with methods used at the bank zone (Kick & Sweep) is difficult because the two approaches are not only different in terms of technique but they are also sampling different spatial zones of the river.

The comparison of data-sets reveals an extraordinary predominance of Oligochaeta and Diptera regarding Air-lift and MHS. In addition, most of the other groups are more effectively caught with these methods. On the other hand, kick and sweep/dredging seems to be more effective in documenting Mollusca, Odonata, Mysidacea and Heteroptera taxa (Figure 11). It underlines that, regarding diversity, a combination of both Air-lift/MHS and kick and sweep/dredging is useful. In total, 362 taxa were collected by Air-lift/MHS and 202 taxa were found in the kick and sweep/dredging samples.

For the decision on methods for future WFD-compliant monitoring programmes, the objective for the use of the biological quality element macrozoobenthos has to be considered.

For assessing the total ecosystem with representative sampling of all habitats in the Danube at a given site (which is not easily manageable with any method), the Air-lift device seems to be appropriate to document the bottom fauna from higher depths in a standardised way. For an evaluation of bank zones and for a differentiation of conditions between right and left bank, the Kick & Sweep technique is recommended.
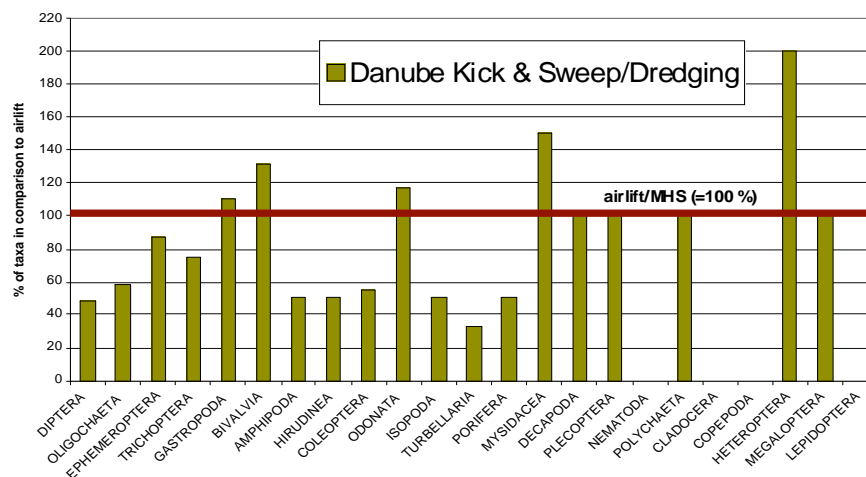


Figure 11: Sampling efficiency of the kick & sweep/dredging method compared to Air-lift/MHS sampling

However, a combination of both methods would have the best sensitivity for research purposes. For the use of such a combined method for ecological status assessment (according to the WFD), the difficult challenge is to overcome the fact that reference conditions and sampling efficiency differ between river bottom and river banks. At present, the adequate methodology for the investigation of large rivers is still under discussion.

### 4.3.4  Neozoa (invasive species)

The presence of Neozoa originating from the Ponto-Caspian area, Asia, Australia and North America is a crucial fact influencing the macrozoobenthic community of the Danube. The Danube is part of the Southern Invasive Corridor (Black Sea-Danube-Main/Danube Channel-Main-Rhine-North Sea waterway), one of the four most important European routes for invasive species (Galil et al., 2007). The river is exposed to intensive colonisation by aquatic invasive species with further spreading in both directions throughout the Danube Basin (north-west and south-east). With few exceptions, the Neozoa of the Danube belong to Crustacea and Mollusca.

Figure 12 shows the abundance and percentage of species of Neozoa along the three reaches of the Danube, indicating their essential importance for the ecosystem. Due to their tremendous abundance (up to 90% within the Upper Reach and even 100% within the Middle Reach as well as approximately 40% of all documented species in the Upper and Middle Reaches), their impact on each assessment system becomes evident.

Neozoa dominate the Danube not only locally but they are distributed along the entire stretch. The highest frequency along the Danube is shown by the mussel, *Corbicula fluminea*. It occurs in 93% of sites, followed by the crustaceans, *Corophium curvispinum* (90%) and *Dikerogammarus villosus* (69%).
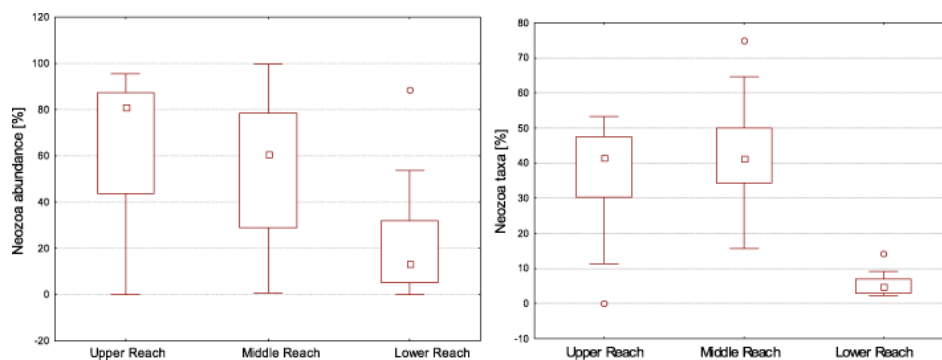


Figure 12: Box & whisker-plots of Neozoa abundance and taxa

As Neozoa dominate the fauna, their classification is a crucial factor in assessing ecological status. Most of them indicate ß-mesosaprobic water quality due to their national classification, which results in an overall good ecological status due to their dominance. Omitting Neozoa from the analysis leads to zero-values for the saprobic index (SI) in some cases. The approach for considering Neozoa (either as stressors or normal organisms) is still the subject of many discussions in EU member states. It is essential to deal with the issue of invasive species in the Danube Basin and to consider their influence from the point of view of management.

### 4.3.5  WFD-compliant criteria for assigning ecological status

Much information has already been compiled with respect to hydrobiological (reference) conditions in the Danube Basin (e.g. WFD Roof Report ANNEX 3: Typology of the Danube River and its reference conditions [ICPDR, 2005]). Yet, not all Danube countries have developed WFD-compliant methods up to date. In particular, commonly agreed assessments methods for the Danube River are missing. In the past, river quality was basically evaluated by assessing organic pollution. To achieve the demands of the WFD for an integrated biological assessment of macroinvertebrates and to assess the ecological status of a water body, further attributes of the species assemblage have to be considered and evaluated.

As already applied and proved in several EU member states, a modular assessment system is recommended for the biological quality indicator 'benthic invertebrates' based on:

– Assessment of **organic pollution** (saprobic condition); and

– Assessment of **general degradation** (hydromorphological and hydrological impact such as damming, impoundment etc.) e.g. using multimetric indices (MMI) or predictive models.

### 4.3.5.1   Organic pollution

For monitoring organic pollution, the saprobic system has a long tradition – the WFD-compliant implementation of this system is based on the deviation of the saprobic index from saprobic reference conditions (Stubauer & Moog, 2003, Ofenböck et al., 2007, Rolauffs et al., 2003). BMWP and ASPT are alternative indices that are widely used for assessment. It should be clearly pointed out that a WFD-compliant assessment of ecological status based exclusively on saprobic indices can only provide a rough indication of status, as several other pressures to the benthic invertebrate community are not evaluated with saprobic systems (see also Chapter 23: Comments on ecological status).

With the data gathered using the Air-lift and Multicorer method, all available national systems of saprobic indices were calculated and transferred to an indication of water quality class (which is given for each single site investigated during the survey). The relevant results for all Danube sites are summarised in Table 4.

The highest values of saprobic indices, indicating serious organic pollution, were detected in the Danube downstream of Pancevo and at Giurgeni. Regarding organic pollution, most sites (58) can be classified as having "indication of good ecological status" according to the WFD. For 8 sites, the SI shows an "indication of moderate ecological status"; for 3 sites: "poor ecological status" and for 9 sites: "high ecological status".

The saprobic indices of the tributaries (near confluences with the Danube) vary between 2.1 and 3.26 (Austrian SI). The rivers Sio, Jantra and Russenski Lom achieve SI values even higher than 3.0. The Arges River is excessively polluted and did not host any macroinvertebrate specimens at the confluence with the Danube. As no reference condition is agreed for the tributaries, a WFD-compliant assignment to ecological quality classes is not possible. In addition to the JDS2 sampling programme, the tributaries were sampled by national teams.

## Table 4: Saprobic indices and indication of water quality classes for all Danubian sampling sites.

| Sampling Site | Method | Saprobic reference condition | Classified taxa | Saprobic Index | Indication of Water Quality Class | Sampling Site | Method | Saprobic reference condition | Classified taxa | Saprobic Index | Indication of Water Quality Class |
|---|---|---|---|---|---|---|---|---|---|---|---|
| JDS1 Donaurieden (bei Ulm) | D-SI | 1,65 | 36 | 1,94 | II | JDS47 downstream Novisad | RO-SI | 2,0 | 14 | 2,15 | II |
| JDS2 Kehlheim | D-SI | 1,75 | 29 | 2,23 | II | JDS48 upstream Tisa | RO-SI | 2,0 | 7 | 2,16 | II |
| JDS3 uh. KW Geisling | D-SI | 1,75 | 8 | 2,20 | II | JDS50 downstream Tisa | RO-SI | 2,0 | 2 | 2,11 | II |
| JDS4 Deggendorf | D-SI | 1,75 | 11 | 2,18 | II | JDS52 upstream Pancevo | RO-SI | 2,0 | 14 | 2,22 | II |
| JDS5 Niederalteich | D-SI | 1,75 | 12 | 2,16 | II | JDS53 downstream Pancevo | RO-SI | 2,0 | 14 | 3,09 | IV |
| JDS7 Jochenstein | A-SI | 1,75 | 14 | 2,31 | III | JDS54 Grocka | RO-SI | 2,0 | 9 | 2,29 | II |
| JDS8 oh. KW Abwinden | A-SI | 1,75 | 12 | 2,12 | II | JDS55 Danube up. Velika Morava | RO-SI | 2,0 | 9 | 2,26 | II |
| JDS9 Ybbs/Persenbeug oh. KW | A-SI | 1,75 | 10 | 2,2 | II | JDS57 Danube down. Velika Morava | RO-SI | 2,0 | 10 | 2,27 | II |
| JDS10 Oberloiben | A-SI | 1,75 | 13 | 1,87 | II | JDS58 Starapalankaram | RO-SI | 2,0 | 15 | 2,43 | III |
| JDS11 Greifenstein | A-SI | 2,0 | 16 | 2,54 | III | JDS59 Banatska Balanka | RO-SI | 2,0 | 12 | 2,15 | II |
| JDS12 Klosterneuburg | A-SI | 2,0 | 11 | 1,84 | I | JDS60 Goluback/Koronin | RO-SI | 2,0 | 19 | 2,58 | III |
| JDS13 Wildungsmauer | A-SI | 2,0 | 10 | 1,83 | I | JDS61 Donij Milanovac | RO-SI | 2,0 | 11 | 2,69 | III |
| JDS14 Hainburg | A-SI | 2,0 | 9 | 1,95 | I | JDS62 Tekjia/Orsova | RO-SI | 2,0 | 8 | 2,44 | III |
| JDS16 Bratislava | SK-SI | 2,0 | 13 | 2,27 | II | JDS63 Vrbica/Simijan | RO-SI | 2,0 | 15 | 2,47 | III |
| JDS17 Gabcikovo | SK-SI | 2,0 | 9 | 2,30 | II | JDS64 Iron Gate II | RO-SI | 2,0 | 11 | 2,13 | II |
| JDS18 Medvedov | RO-SI | 2,0 | 9 | 2,09 | II | JDS65 upstream of Timok | RO-SI | 2,0 | 16 | 2,21 | II |
| JDS19 Moson Danube | RO-SI | 2,0 | 12 | 2,84 | IV | JDS67 pristol | RO-SI | 2,0 | 8 | 2,13 | II |
| JDS20 Komarno | RO-SI | 2,0 | 10 | 2,11 | II | JDS68 Calafat | RO-SI | 2,0 | 14 | 2,26 | II |
| JDS22 Iza | RO-SI | 2,0 | 8 | 2,09 | II | JDS69 downstream Kozloduy | RO-SI | 2,0 | 16 | 2,29 | II |
| JDS23 Esztergom | RO-SI | 2,0 | 12 | 2,12 | II | JDS70 upstream Iskar | RO-SI | 2,0 | 7 | 2,06 | II |
| JDS26 Szob | RO-SI | 2,0 | 9 | 2,11 | II | JDS72 downstream Iskar | RO-SI | 2,0 | 2 | 1,78 | I |
| JDS27 Szentendre Island Mainchannal | RO-SI | 2,0 | 11 | 2,11 | II | JDS73 upstream Olt | RO-SI | 2,0 | 12 | 2,14 | II |
| JDS28 Szentendre Island Arm | RO-SI | 2,0 | 6 | 2,15 | II | JDS75 downstream Olt | RO-SI | 2,0 | 5 | 1,90 | I |
| JDS29 Budapest upstream | RO-SI | 2,0 | 10 | 2,07 | II | JDS76 downstream Turnu Magurele | RO-SI | 2,0 | 9 | 1,93 | I |
| JDS30 Budapest upstream side arm | RO-SI | 2,0 | 9 | 2,09 | II | JDS77 downstream Zimnicea | RO-SI | 2,0 | 7 | 2,38 | II |
| JDS31 Rackeve-Soroksar sidearm | RO-SI | 2,0 | 11 | 2,31 | II | JDS79 downstream Jantra | RO-SI | 2,0 | 13 | 2,32 | II |
| JDS32 Budapest downstream | RO-SI | 2,0 | 11 | 1,94 | I | JDS80 upstream Ruse | RO-SI | 2,0 | 3 | 2,18 | II |
| JDS33 Adony/Lorev | RO-SI | 2,0 | 11 | 2,12 | II | JDS82 downstream Ruse/Giugiu | RO-SI | 2,0 | 4 | 1,48 | I |
| JDS34 Rockere-Sorokser Arm-end | RO-SI | 2,0 | 20 | 2,28 | II | JDS83 upstream Arges | RO-SI | 2,0 | 9 | 2,10 | II |
| JDS35 Dunaföldvar | RO-SI | 2,0 | 7 | 2,06 | II | JDS85 downstr. Arges | RO-SI | 2,0 | 4 | 1,81 | I |
| JDS36 Paks | RO-SI | 2,0 | 11 | 2,26 | II | JDS86 Silistra | RO-SI | 2,0 | 16 | 2,76 | III |

| Sampling Site | Method | Saprobic reference condition | Classified taxa | Saprobic Index | Indication of Water Quality Class | Sampling Site | Method | Saprobic reference condition | Classified taxa | Saprobic Index | Indication of Water Quality Class |
|---|---|---|---|---|---|---|---|---|---|---|---|
| JDS38 Baja | RO-SI | 2,0 | 13 | 2,35 | II | JDS87 downstream Crnawoda | RO-SI | 2,0 | 12 | 2,16 | II |
| JDS39 Hercegszanto | RO-SI | 2,0 | *5* | *2,23* | *II* | JDS88 Giurgeni | RO-SI | 2,0 | *5* | *3,15* | *IV* |
| JDS40 Batina | RO-SI | 2,0 | *7* | *2,13* | *II* | JDS89 Brailla | RO-SI | 2,0 | *7* | *2,23* | *II* |
| JDS41 upstream Drava | RO-SI | 2,0 | *2* | *2,20* | *II* | JDS92 Reni | RO-SI | 2,0 | *7* | *2,16* | *II* |
| JDS43 downstream Drava | RO-SI | 2,0 | 15 | 2,17 | II | JDS93 Vilkova | RO-SI | 2,0 | 19 | 2,24 | II |
| JDS44 Dalj | RO-SI | 2,0 | *5* | *2,20* | *II* | JDS94 Bystroye canal | RO-SI | 2,0 | *6* | *2,15* | *II* |
| JDS45 Ilok  Backa Palanka | RO-SI | 2,0 | *6* | *2,13* | *II* | JDS95 Sulina | RO-SI | 2,0 | 12 | 2,16 | II |
| JDS46 upstream Novisad | RO-SI | 2,0 | 11 | 2,25 | II | JDS96 St. George arm | RO-SI | 2,0 | *3* | *2,11* | *II* |

D-SI: German SI; A-SI: Austrian SI; SK-SI: Slovak SI; RO-SI: Romanian SI. Saprobic index values and indications of water quality based on less than 10 indicator taxa are scientifically questionable and written in italic.

These results give an impression of the saprobic conditions in the Danube - however they may not be appropriate for all sites as national adaptations to the assessment system would be needed in some countries. Thus the results should be taken as a proposal and demonstration of an assessment that requires further plausibility checks by national experts. Additionally, the calculations of SI are based on pooled samples taken from the river bottom representing the cross-section of the Danube. However, at the banks the conditions can be different and can even vary between right and left bank as was demonstrated by the analysis of the riverbanks (for further information see Full Report on the CD-ROM).

For the WFD-compliant standard monitoring of benthic invertebrates, several different indices could be used as long as a scientifically sound basis for the type-specific identification of reference conditions is available. However, according to our study, new or revised saprobic indicator values for species are needed for the Middle and Lower Danube.

### 4.3.5.2   General degradation
In addition to the assessment of organic pollution, an integrated biological assessment of benthic invertebrate fauna should also consider a number of other characteristics of the species assemblages (including taxonomic composition, abundance, ratio of disturbance sensitive taxa to insensitive taxa and diversity). These attributes need to be integrated and compared to respective target values under reference conditions. Out of more than 200 metrics calculated, about 20 metrics were finally selected as candidates for use in assessment systems in the Danube River. These metrics include functional measures, sensitivity/tolerance measures and richness measures, as well as composition/abundance measures. As the response of metrics to stressors differs between river sections, river type specific candidate metrics were selected. Higher numbers of suitable metrics were found for the Upper (15) and Middle Reaches (12) of the Danube, where the main stressor is impoundment. For the Lower Reach, where organic pollution becomes a more important stressor and only two sites are affected by impoundment, a lower number (7) of applicable metrics was found. Results for the indication of the ecological status are not given here as the application of the MMI-method requires agreement on reference conditions and boundary values. More details and the metrics are given in the full report on the CD-ROM – this information could be used to implement a multimetric index in a national assessment method or within the Danube intercalibration process.

### 4.3.5.3  Proposal for assigning the ecological quality class

The evaluation of the overall ecological quality should be based on the results of both modules (organic pollution and general degradation), preferably using the worst case as shown in Figure 13.
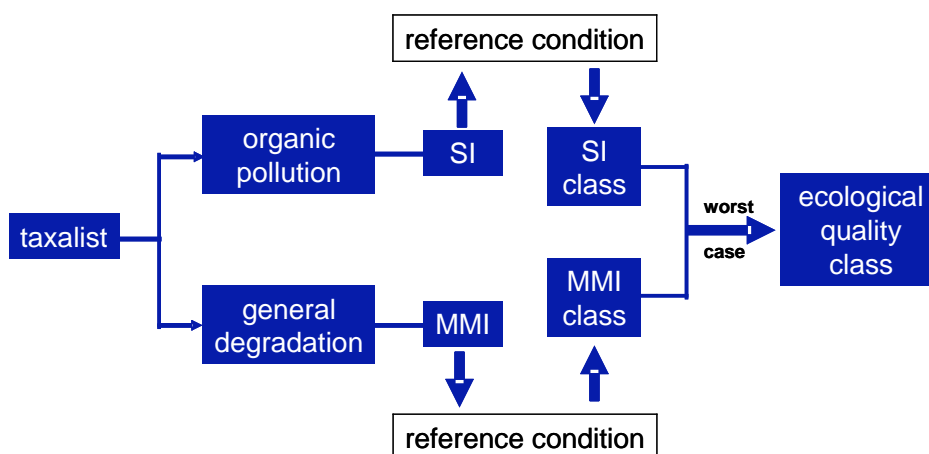


**Figure 13: Evaluation of the river quality using two modules, e.g. saprobic index and multimetric index (MMI)**

## 4.4 Conclusions

The macrozoobenthic community documented during JDS2 comprises 411 taxa. Regarding diversity, the most heterogeneous groups are Diptera and Oligochaeta. In terms of abundance, the fauna is dominated by Crustacea (Amphipoda and Isopoda) while Mollusca are the predominant group regarding biomass. However aquatic insects, especially EPT-taxa, play only a minor role in the Danube River.

The three established typological reaches of the Danube (Upper – Middle – Lower) can be confirmed with multivariate analysis of the macrozoobenthos data. Additionally the Danube typology could be revised at a smaller scale resulting in five distinguished section types of the Danube River.

Comparison between the methods Air-lift/MHS/Multicorer and Kick & Sweep/Dredging is difficult because the two approaches are not only different in terms of technique but are also sampling different spatial zones of the river. More taxa were collected with the Air-lift/MHS/Multicorer method as compared to the Kick & Sweep/Dredging method (362 and 202 respectively). In general, the Air-lift/MHS/Multicorer method seems to be more effective regarding a standardized documentation of benthic invertebrates as it is a quantitative method and covers the largest area of the river ecosystem. In order to select the most appropriate method for future WFD-compliant monitoring programmes, the objectives for the use of biological quality element macrozoobenthos have to be considered.

For assessment of the ecological status, a modular system is proposed consisting of an index for organic pollution and an index for general degradation. For the assessment of organic pollution, most Danube states are already using an index based system (e.g. saprobic index). To cover hydromorphological degradation, the development of a multi-metric approach is proposed.

Regarding organic pollution, the saprobic indices for the JDS2 sites in the Danube vary between 1.83 and 3.15. Most of the sites (58) can be classified as demonstrating an "indication of good ecological status" according to the WFD. For 8 sites the SI shows an "indication of moderate ecological status"; for 3 sites: "poor ecological status" and for 9 sites: "high ecological status". Comparison of the different national classification systems shows that the assessment systems need to be harmonised.

Tributaries (near the confluences with the Danube) show saprobic indices between 2.1 and 3.26 (Austrian SI). The rivers Sio, Jantra, and Russenski Lom achieve SI-values higher than 3.0. The Arges

River is excessively polluted and did not host any macroinvertebrate specimens at the confluence with the Danube. As no reference condition is agreed for the tributaries, a WFD-compliant assessment procedure is not possible.

The bottom fauna of the Upper and Middle Reach of the Danube is dominated by Ponto-Caspian Neozoa (mostly Crustacea and Mollusca). Their relative abundance averages between 60% and 80% and they represent up to 40% of the total number of taxa. Neozoa are not only locally abundant but cover the whole Danube stretch. As Neozoa dominate the fauna their classification is a crucial factor in assessing ecological status. Most of them indicate ß-mesosaprobic water quality due to their national classification, which results in an overall "good ecological status" due to their dominance. Omitting Neozoa from the analysis leads to zero-values of the saprobic index in some cases.

A more elaborate analysis of the JDS2 results including further comments and conclusions can be found in the full report.

## 4.5 References

ASTERICS/PERLODES Software (2008): Deutsches Bewertungssystem auf Grundlage des Makrozoobenthos. Version 3.1.0.:
http://www.fliessgewaesserbewertung.de/downloads/ASTERICS_Version3.10_Softwarehandbuch.pdf

BERNERTH, H. & S. STEIN (2003): *Crangonyx pseudogracilis* und *Corophium robustum* (Amphipoda), zwei neue Einwanderer im hessischen Main sowie Erstnachweis für Deutschland von *C. robustum*. Lauterbornia 48:45-57.

BERTHOLD, E. & I. KAISER (2005): Weitere Funde von *Crangonyx pseudogracilis* und *Chelicocorophium robustum* (Amphipoda) im Main. Lauterbornia 50: 15-17.

BIRK, S. & D. HERING (2002): Waterview web-database: a comprehensive review of European assessment methods for rivers. FBA News 20: 4.

BIRK, S. (2003): Overview of biological and hydromorphological assessment methods in the Danube River Basin. UNDP/GEF Danube Regional Project: Activity 1.1.7 Ecological status assessment and classification systems in the Danube River Basin: 7-28.

BRABEC, K., S. ZAHRADKOVA, D. NEMEJCOVA, P. PARIL, J. KOKES & J. JARKOVSKY (2004): Assessment of organic pollution effect considering differences between lotic and lentic stream habitats. Hydrobiologia 516: 331–346.

BUFFAGNI, A., S. ERBA, M. CAZZOLA & J. L. KEMP (2004): The AQEM multimetric system for the southern Italian Apennines: assessing the impact of water quality and habitat degradation on pool macroinvertebrates in Mediterraneanrivers. Hydrobiologia 516: 315–331.

BUNDESMINISTERIUM FÜR LAND- UND FORSTWIRTSCHAFT, UMWELT UND WASSERWIRTSCHAFT (1995-2007): Ecoprof Version 3.0.1. Software zur Archivierung und Auswertung gewässerrelevanter Daten. www.ecoprof.at. Bundesministerium für Land- und Forstwirtschaft, Wien.

CSÁNYI, B., PAUNOVIC, M. (2006). The Aquatic Macroinvertebrate Community of the River Danube between Klostenburg (1942 rkm) and Calafat – Vidin (795 rkm). Acta Biol. Debr. Oecol. Hung 14, 91-106.

DAVIS, W. S. & T. P. SIMON (eds) (1995): Biological Assessment and Criteria Tools for Water Resource Planning and Decision Making. Lewis Press, Boca Raton, Florida.

EN 27828:1994 Water quality - Methods for biological sampling – Guidance on hand-net sampling of benthic macro-invertebrates.

GALIL BS, NEHRING S, PANOV VE. 2007. Waterways as invasion highways – Impact of climate change and globalisation. In: Nentwig W: Biological Invasions. Ecological Studies Nr. 193, Springer, Berlin: 59–74.

ICPDR (2005): ANNEX 3 - Typology of the Danube River and its reference conditions. 9p.

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

LORENZ, A., D. HERING, C. FELD & P. ROLAUFFS (2004): A new method for assessing the impact of hydromorphological degradation on the macroinvertebrate fauna of five German stream types. Hydrobiologia 516: 107–127.

MCCUNE, B. & M.J. MEFFORD (1999): PC-ORD. Multivariate Analysis of Ecological Data, Version 4. MjM Software Design, Gleneden Beach, Oregon, USA. 237 pp.

MOOG, O. (1995): Fauna Aquatica Austriaca – Wasserwirtschaftskataster.

OFENBÖCK, T., O. MOOG, J. GERRITSEN & M. T. BARBOUR (2004): A stressor specific multimetric approach for monitoring running waters in Austria using benthic macro-invertebrates. Hydrobiologia 516: 253–270.

OFENBÖCK, T., MOOG, O., HARTMANN, A. & I. STUBAUER (2007): Leitfaden zur Erhebung der biologischen Qualitätselemente, Teil A2 – Makrozoobenthos. Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft, Wien: 214 p.

PAUNOVIC M., CSÁNYI B., SIMIC V., STOJANOVIC B. AND CAKIC P. 2006. Distribution of *Anodonta (Sinanodonta) woodiana* (Rea, 1834) in inland waters of Serbia. Aquatic Invasions 1: 154–160.

PEHOFER, H.  (1998): Large River Vol. 11 No 2 Arch. Hydrobiol. Suppl. 115/2, Uni Innsbruck: p. 213-232.

PINTO, P., J. ROSADO, M. MORAIS & I. ANTUNES (2004): Assessment methodology for southern siliceous basins in Portugal. In Hering D., P. F. M. Verdonschot, O. Moog & L. Sandin (eds), Integrated Assessment of Running Waters in Europe. Kluwer Academic Publishers, The Netherlands. Hydrobiologia 516: 191–214.

ROLAUFFS, P., HERING, D., SOMMERHÄUSER, M., JÄHNIG, S. & S. RÖDIGER (2003): Entwicklung eines leitbildorientierten Saprobienindexes für die biologische Fließgewässerbewertung. Umweltbundesamt

Texte 11/03. Forschungsbericht 200 24 227, 137 S.

ROSENBERG, D. M. & V. H. RESH (eds), 1995. Freshwater biomonitoring and benthic macroinvertebrates. Chapman & Hall, New York, London.

RUSSEV, B. (1998): Das Makrozoobenthos der Donau – Dynamik der Veränderungen durch antropogenen Einfluβ. In: Kuzel-Fetzman, E. – Naidenow, W. – Russev, B. (eds): Plankton und Benthos der Donau. – Ergebnisse der Donau-Forschung, Band 4. pp. 257-364.

SANDIN, L., J. DAHL & R. K. JOHNSON  (2004): Assessing acid stress in Swedish boreal and alpine streams using benthic macroinvertebrates. Hydrobiologia 516: 129–148.

SCHMEDTJE, U., BACHMANN, J., BEHRENDT, H., BIRK, S., BIZA, P., D'EUGENIO, J., V. GILS, J., GRATH, J., HAMCHEVICI, C., HANSEN, W., INTERWIES, E., KAMPA, E., LINDINGER, H., LISKA, I., POPESCU, L., POPOVICI, M., POTTGIESSER, T., SIGMUND, G., SOMMERHÄUSER, M., SPECK, S., STUBAUER, I., VOGEL, B., WELLER, P., WINKELMANN-OEI, G. & A. ZINKE (2005): Danube Basin Analysis (WFD Roof Report 2004). The Danube River Basin District - River basin characteristics, impacts of human activities and economic analysis required under Article 5, Annex II and Annex III, and inventory of protected areas required under Article 6, Annex IV of the EU Water Framework Directive (2000/60/EC) - Part A - Basin-wide overview. ICPDR, Vienna: 191 pp.

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.

SØRENSEN, T. (1948): A method of establishing groups of equal amplitude in plant sociology based on similarity of species content. Det. Kong. Danske Vidensk. Selsk. Biol. Skr. 5(4): 1-34.

STATSOFT, Inc. (2005): STATISTICA für Windows [Software-System für Datenanalyse] Version 7.1. www.statsoft.com

STUBAUER, I. & O. MOOG (2003): Integration of the Saprobic System into the WFD approach - A proposal for the Danube River. 2nd Surface Water Workshop, Zagreb, 4-5 September 2003. http://danubis.icpdr.org/undp-drp/

VLEK, H. E., P. F. M. VERDONSCHOT & R. C. NIJBOER (2004): Towards a multimetric index for the assessment of Dutch streams using benthic invertebrates. Hydrobiologia 516: 173–189.

ZELINKA, M. & P. MARVAN (1961): Zur Präsisierung der biologischen Klassifikation der Reinheit fließender Gewässer. Archiv Für Hydrobiologie 57: 389–407.

# 5 Phytobenthos

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## 5.1 Introduction

Algae are important primary producers in many surface waters of temperate regions. This makes this organism group especially suitable for use as a bioindicator to monitor long-term changes in aquatic ecosystems, especially related to eutrophication. Both phytoplankton and phytobenthos and macrophytes are identified as Biological Quality Elements under the European Water Framework Directive (2000/60/EC), and as such should be monitored to determine anthropogenic influences on aquatic ecosystems. Especially in rivers, phytobenthos are considered to be a suitable parameter to determine the impact of nutrient pollution because the organisms are generally sessile and therefore represent the status of realised nutrients at the sampled location.

According to the Water Framework Directive (WFD), a major requirement for the good ecological status of rivers and lakes is that "changes do not indicate any accelerated growth of [algae,] phytobenthos or higher forms of plant life resulting in undesirable disturbance to the water balance of organisms present in the water or to the physico-chemical quality of the water…" (Annex V 1.2.1/1.2.2). For the supporting physico-chemical quality elements, it is required to estimate the magnitude of all significant point and non-point source pollution (Annex II 1.4) including "substances that contribute to eutrophication (in particular nitrates and phosphates)" (Annex VIII). However, because the primary focus is on the biological effects resulting from elevated nutrient levels, high nutrient concentrations alone - without the corresponding biological impacts - will not result in the downgrading of the ecological status of the river. In order to meet the requirements of the WFD, information on both biodiversity and biomass for each biological quality element is required. In the Danube, eutrophication is an important anthropogenic pressure threatening the quality of the river water. Therefore, phytobenthos biomass and biodiversity were investigated in great detail during the JDS2.

## 5.2 Methods

### 5.2.1 Sampling

Sampling of phytobenthos for JDS2 was based on the combination of two standards ('EN 13946: Water quality - Guidance standard for the routine sampling and pre-treatment of benthic diatoms from rivers' and 'CEN/TC 230 N 0540: Water quality - Guidance standard for the surveying, sampling and laboratory analysis of phytobenthos in shallow running water'). In addition, fluorescence measurements for phytobenthos biomass determinations were performed.

A segment of the river that had substrate suitable for sampling was selected. For preference, epilithon was taken from at least five boulders, or more than five pebbles, at all sampling sites. Where hard substrata were absent, epiphyton was sampled following the afore-mentioned EN standards. Firstly, a measurement for chlorophyll-a was taken at the sampled stones. An area of a minimum of 10 cm$^2$ was then brushed thoroughly from each stone (for maximum concentration of the sample). The sample was then transferred to the sample container and labelled. All field information needed was recorded in the standardised field protocol.

Two types of phytobenthos samples were taken. Samples used for benthic diatoms analysis were preserved in formaldehyde (1 – 4% solution) for the long-term storage of the samples. The second sample was used for on-board analysis of living phytobenthos. These unpreserved samples were stored in the refrigerator until the analysis. When macroscopic algae (e.g. Cladophora or Hydrodictyon) were present, separate sample containers were used for easier determination. Samples of phytobenthos were taken from the euphotic zone, usually up to 1 m depth. At some locations, especially in the Lower Danube, there were problems in finding any suitable substrata, due to natural conditions as well as the increase in water level.

### 5.2.2  Living phytobenthos analysis

After sampling, the microscopic analysis of living phytobenthos was performed using light microscopy at 400x – 1000x magnification. All the important determination characteristics of the species were recorded using image analysis. The determination was carried out as comprehensively as possible using up-to-date determination keys for the individual algal groups. The quantity of the individual species was estimated using a scale from 1 to 9.

Based on phytobenthos sampling, together with the microscopic analysis, an estimation of the ratio of cyanobacteria, chlorophyta, diatoms and other algal groups was calculated.

### 5.2.3  Benthic diatom analysis

The preparation and quantification of benthic diatoms samples followed instructions set out in EN 14407 (2004). Diatoms were cleaned using 40% hydrogen peroxide ($H_2O_2$) and permanent slides were mounted using Naphrax. On average, 400 valves were counted on each slide in random transects using a lights microscope with DIC (Differential Interference Contrast) at 1000x magnification. The determination largely followed Krammer & Lange-Bertalot (1986–1991); Krammer (1997a,b, 2000, 2002, 2003); Lange-Bertalot (1996, 2001) and Lange-Bertalot & Krammer (1987).  Based on diatom inventories, 17 diatom indices were calculated using the software Omnidia 4.2. (Lecointe & et al., 1999).

### 5.2.4  Phytobenthos biomass

Fluorescence measurements for phytobenthos biomass determinations were performed using the Benthofluor® fluorometer (bbe Moldaenke, Kiel, Germany) according to Aberle et al. (2006).  On each of five or more stones, five sub-areas were measured. Each measurement was done 3-4 times to obtain a sufficient database on chlorophyll-a for statistical analysis. Three main algal groups were distinguished: diatoms, green algae and cyanobacteria. For each of these groups, and for the total benthic algal biomass, the chlorophyll-a level was determined in $\mu g/cm^2$.

## 5.3 Results

### 5.3.1  Living phytobenthos species diversity

166 samples were collected at 135 sites (124 from the River Danube and 11 from the tributaries). Some of the samples contained diatoms only. Together 52 taxa were identified (diatoms excluded).

Species diversity in living phytobenthos comprised diatom species (excluded here due to separate assessment), Cyanobacteria (Cyanophyta), Chlorophyta and Rhodophyta. Cyanobacteria were represented by the filamentous species *Heteroleibleinia fontana* (Hansgirg) Anagnostidis et Komarek; *H. kützingii* (Schmidle) Compere; *Homeothrix varians* Geitler; *Lyngbya martensiana* Meneghini ex Gomont; *Oscillatoria limosa* Agardg ex Gomont; *Phormidium retzii* (Agardh) Gomont ex Gomont and *Ph. tergestinum* (Kützing) Anagnostidis et Komarek, which occurred in more than 75 % of samples. Coccal cyanobacteria were often observed as well (mainly *Chroococcus*, *Chamaesiphon* and

*Pleurocapsa* species). Planktonic species such as *Pseudanabaena catenata* Lauterborn were also present.

Fewer species of Chlorophyta occurred at individual sampling stations but they were usually more abundant in the shallow pools of the river (e.g. *Cladophora glomerata* (L.) Kützing; *Hydrodictyon reticulatum* (L.) Lagerheim); *Spirogyra* sp.; *Stigeoclonium tenue* (Aghard) Kützing). *Cladophora glomerata* often accompanied water macrophytes. Samples of phytobenthos also contained planktonic species.

The red algae, *Hildebrandia rivularis* (Liebmann) Aghard, was found upstream of the Abwinden-Asten dam and then also upstream of the Greifenstein dam, together with *Bangia atropurpurea* (Roth) Aghard.

Based on phytobenthos sampling, together with microscopic analysis, an estimation of the ratio of cyanobacteria, green algae, diatoms and other algal groups was performed (Figure 14). Cyanobacteria and green algae prevailed (from the point of view of relative abundance) at most of the sampling stations. However, at eight JDS2 stations diatoms comprised the most abundant group.
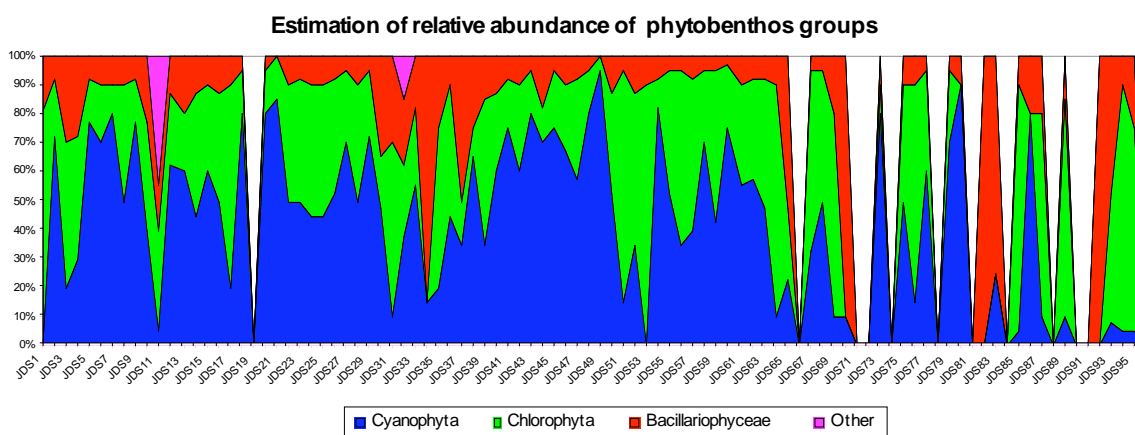


Figure 14: Ratio of individual phytobenthos groups of cyanobacteria (Cyanophyta), diatoms (Bacillariophycea), green algae (Chlorophyta) and 'others' (including Rhodophytes - JDS11 and bacteria - JDS32) in living samples at each sampling site

### 5.3.2 Phytobenthos biomass

In the downstream part of the Danube, it was not possible to find appropriate substrate for the measurement of phytobenthos biomass. Longitudinal trend lines of the biomass measurements at the left and right side of the river (including the mouth of tributaries) show a slight increase in chlorophyll-a along the Danube. The increase recorded on the right side of the river was slightly higher, but not statistically significant (Figure 15B).

Phytobenthos biomass also seems to increase downstream, when comparing the average biomass of the Upper, Middle and Lower Danube (Figure 15A).

A                                                        B
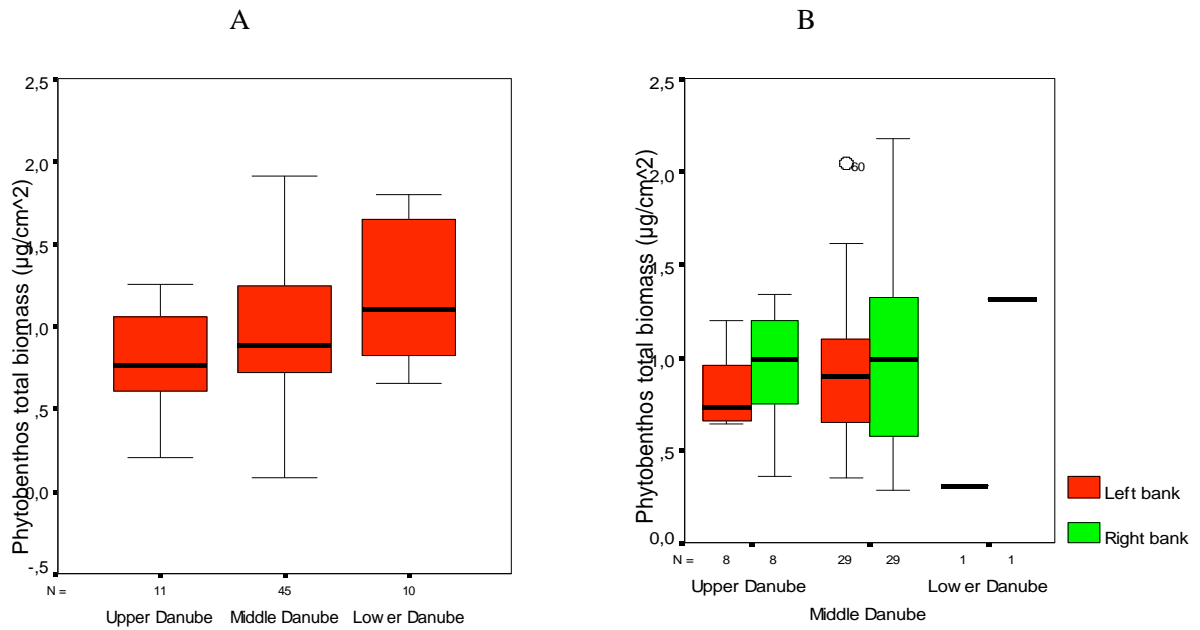


Figure 15: Phytobenthos biomass (A) and differences between left and right bank (B) in the Upper, Middle and Lower Danube

Within the individual Danube River Types (Figure 16), biomass shows the highest concentrations in Type 7 (Iron Gate reservoir).

But when comparing results of phytoplankton and phytobenthos biomass in the JDS2 stations, the Middle Danube shows the highest variability; Upper and Lower Danube show lower phytoplankton levels, resulting in better growing opportunities for phytobenthos (Figure 17). Chlorophyll-a from phytoplankton and phytobenthos in relation to total phosphorus indicates that lower total phosphorus levels seem to favour phytobenthos slightly.
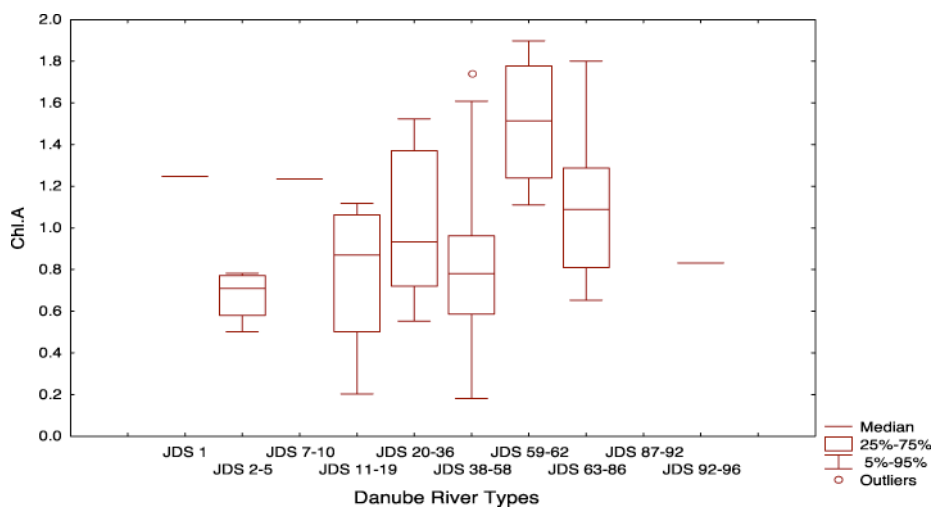


Figure 16: Phytobenthos biomass division within the individual Danube River Types
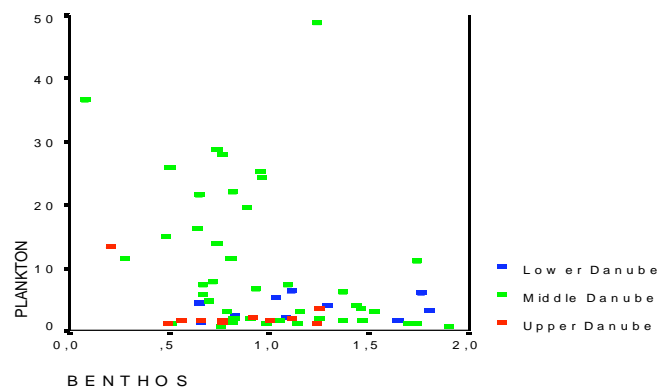
Figure 17: Phytoplankton and phytobenthos biomass (chlorophyll-a) in JDS2 sampling stations

### 5.3.3  Benthic diatoms

Altogether 166 benthic diatom samples were collected at 135 sites (124 from the River Danube and 11 from tributaries). In total, 391 diatom taxa were identified in 160 samples, 6 samples were not analysed due to prevailing detritus in the samples and an obviously unrepresentative diatom community. Figure 18 presents the results of a cluster analysis using species diversity and shows a significantly high similarity of sites. Although the species composition is highly similar within the sampling sites on the River Danube, the cluster analysis distinctly separated benthic diatom communities from the Upper Danube and the beginning of the Middle Danube (from Germany, the station upstream of Iller, to Slovakia, Bratislava station) from samples coming from the Middle and Lower Danube downstream of Bratislava. However, several sampling stations in Germany were distinctly differing in species composition (Geisling power plant, Niederalteich) indicating higher trophic and saprobic levels. In general, the species composition at the sampling sites changed gradually, depending on the confluence of tributaries (apart from other abiotic descriptors), while, in general, the largest differences in species structure were revealed between particular tributaries e.g. Drava, Morava, Tisza, Sava and Russenski Lom (Figure 18).
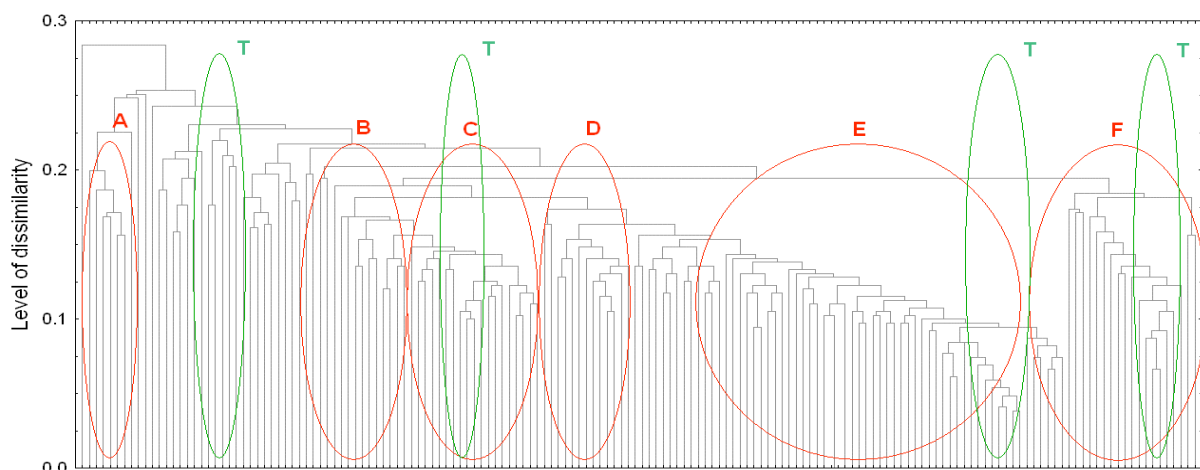


Figure 18: Dissimilarity of diatom samples

In spite of a large similarity of samples, there are significant differences between the River Danube (A-F) and some tributaries (T). Several groups of clusters can be separated, representing specific regions or river parts:
−   A: Iron Gate region (Iron Gate reservoir (Tekija/Orsova) JDS41 – Iron Gate II JDS44, Type 5,6);
−   B: Hungary/Croatia region (Paks JDS36 – Dalj JDS44, Type 5,6);
−   C: Serbia, Tisza-Sava region (Dalj JDS44 - Sava JDS51, Type 6);
−   D: Slovakia/Hungary region (Vah JDS21 - Paks JDS36, Type 5);
−   E: Downstream of Iron Gate region (Upstream Timok JDS65 – Russenski Lom JDS81, Type 8);
−   F: Upper/Middle Danube region (Upstream Iller JDS1 – Bratislava JDS17, Type 1,2,3,4).

Regarding the frequency of taxa, 200 diatom taxa appeared at more than one sampling location; 75 taxa were found with a frequency higher than 20% and only 13 diatom taxa showed a frequency of more than 50%. Comparing the relative abundance of the dominant taxa, only 21 species obtained the average relative abundance at all sites higher than 1%. Among them *Navicula recens* (Lange-Bertalot) and *Navicula tripunctata* (O.F.Müller) Bory were the most abundant and most frequent. Generally, species from the genus *Amphora*, *Cocconeis*, *Eolimna*, *Gyrosigma*, *Luticola*, *Navicula*, *Nitzschia*, *Rhoicosphenia* and *Reimeria* were among the most abundant and were usually dominant at the sampling sites. There were several taxa with unknown species identity – so far identified to the genera level – that reached a relative abundance higher than 5%. Identification of the taxa will be further verified in detail and the taxa will be examined by scanning electron microscope (SEM) in order to clarify the species identities.

Regarding the autecological preferences of the most frequent and dominant species, the sites were mostly dominated by eutrophic to hypertrophic species e.g. *Amphora pediculus* (Kutzing) Grunow; *Navicula tripunctata* (O.F.Müller) Bory*; Navicula viridula* (Kutz.) Ehr. var. *rostellata* (Kutz.) Cleve; *Luticola goeppertiana* (Bleisch in Rabenhorst) D.G. Mann; *Navicula recens* (Lange-Bertalot); *Navicula erifuga* Lange-Bertalot; *Nitzschia inconspicua* Grunow; *Nitzschia clausii* Hantzsch and *Nitzschia palea* (Kutzing) W.Smith, indicating beta mesosaprobic to polysaprobic conditions. Most of the taxa were alcaliphilous.

### 5.3.3.1 Diatom indices

Based on diatom inventories, 17 diatom indices were calculated using the software Omnidia 4.2. (Lecointe et al., 1999). Results of the calculations are shown in Table 5.

## Table 5: Summary of diatom indices values

|  | SLA | DES | L&M | SHE | WAT | TDI | GDI | CEE | IPS | IBD | IDAP | EPI-D | IDP | SID | TID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Minimum | 5.8 | 1.4 | 3.6 | 3.7 | 1.5 | 48.0 | 4.2 | 3.9 | 4.0 | 3.2 | 5.5 | 3.6 | 3.9 | 4.9 | 2.6 |
| Maximum | 13.9 | 19.9 | 15.3 | 17.6 | 18.9 | 99.5 | 14.9 | 18.3 | 18.6 | 18.3 | 17.4 | 16.1 | 15.8 | 15.5 | 14.6 |
| Average | 10.8 | 15.5 | 11.8 | 12.0 | 11.4 | 79.3 | 11.4 | 10.8 | 11.5 | 9.6 | 11.2 | 9.9 | 10.6 | 11.9 | 5,9 |
| Median | 10.8 | 16.2 | 12.0 | 12.1 | 11.5 | 80.0 | 12.0 | 11.1 | 11.3 | 9.4 | 11.6 | 9.6 | 10.7 | 11.9 | 5.7 |
| St. Deviation | 1.5 | 3.4 | 2.0 | 1.9 | 3.2 | 8.6 | 1.9 | 3.0 | 2.6 | 2.8 | 2.9 | 2.0 | 1.6 | 1.4 | 1.5 |

CEE (Descy & Coste 1990); DES (Descy 1979); EPI-D (Dell' Uomo et al. 1999); GDI (Rumeau & Coste 1988), (Prygiel & Coste 1996); IBD (Lenoir & Coste 1996); IPS (Coste in CEMAGREF 1982); L&M (Leclercq & Maquet 1987); SHE (Schiefele & Schreiner 1991); SID (Rott et al. 1999); SLA (Sláde ek 1986); TID (Rott et al. 1999); TDI (Kelly & Whitton 1995); WAT (Watanabe et al. 1986); IDAP (Prygiel & Coste 1996), IDP (Gomez & Licursi 2001).

Correlations of all calculated indices with environmental variables and pollutants were performed in order to select diatom indices that are most suitable to evaluate the level of pollution and degradation of water environment. Due to the broad applicability of the IPS index (Ács et al., 2004; Eloranta and Andersson, 1998; Goma et al., 2004; Hlubikova et al., 2007; Kelly et al., 1995; Kwandrans et al., 1998; Prygiel and Coste, 1993; Vilbaste, 2004), it was selected for a preliminary assessment of water quality.

The IPS ("Specific Pollution Sensitivity Index"– Coste in Cemagref, 1982) was developed in France as a national assessment index (Prygiel et al., 2002) for detection of total water pollution. The IPS was tested and selected as an appropriate tool for water quality evaluation in Poland (Kwandrans et al, 1998); in Finland (Vilbaste 2004); in Hungary (ECOSURV, 2005) and in Spain (Gomá et al. 2004). It is used for water quality evaluation in some regions of France (Prygiel & Coste 1993). The calculation of the IPS includes most of the species of the OMNIDIA database. Indicator taxa are divided into five classes according to their sensitivity to pollution and into three classes according to indicative weight.

The values of the IPS index seem to decrease downstream, indicating a longitudinal increase in pollution (organic, nutrient and general degradation). Comparing the IPS values in different parts of

the longitudinal profile, four groups of sites can be distinctly separated according to level of pollution (Figure 19):

−   Group 1: highest quality - at sites Upstream of Iller (Germany: JDS 1) to Greifenstein (Austria: JDS 11);

−   Group 2: displaying a change in water quality in terms of a higher level of pollution - from Klosterneuburg (Austria: JDS 12) to Batina (Croatia: JDS 40);

−   Group 3: displaying the worst level of pollution - at sites from upstream of the Drava (Croatia: JDS 41) to Starapalanka – Ram (Serbia: JDS 58);

−   Group 4: displaying a large variability of index values - at sites downstream of Banatska Palanka/Bazias (Serbia/Romania: JDS 59-95) probably due to multiple factors that, besides pollution, form the structure of the benthic diatom communities and thus significantly increase the uncertainty of diatom-based assessment.
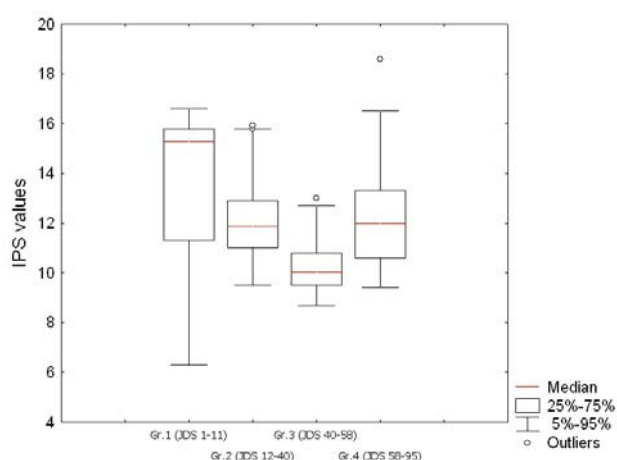


Figure 19: Comparison of IPS values in the Upper, Middle and Lower Danube

Different parts of the River Danube are grouped in 4 groups according to different intervals of IPS: Gp. 1: JDS 1-11; Gp. 2: JDS 12–40; Gp. 3: JDS 41–58, Gp. 4: JDS 59-95.

Water quality assessment of the Danube and tributaries, based on the IPS index five-class scale of sensitivity to pollution (Lecointe & et al., 1999; Figure 20), indicates quality IV in the Danube at Geisling (left side) and Ipeľ tributary.  Most other sites of the Danube and its tributaries were distributed within classes II – III.
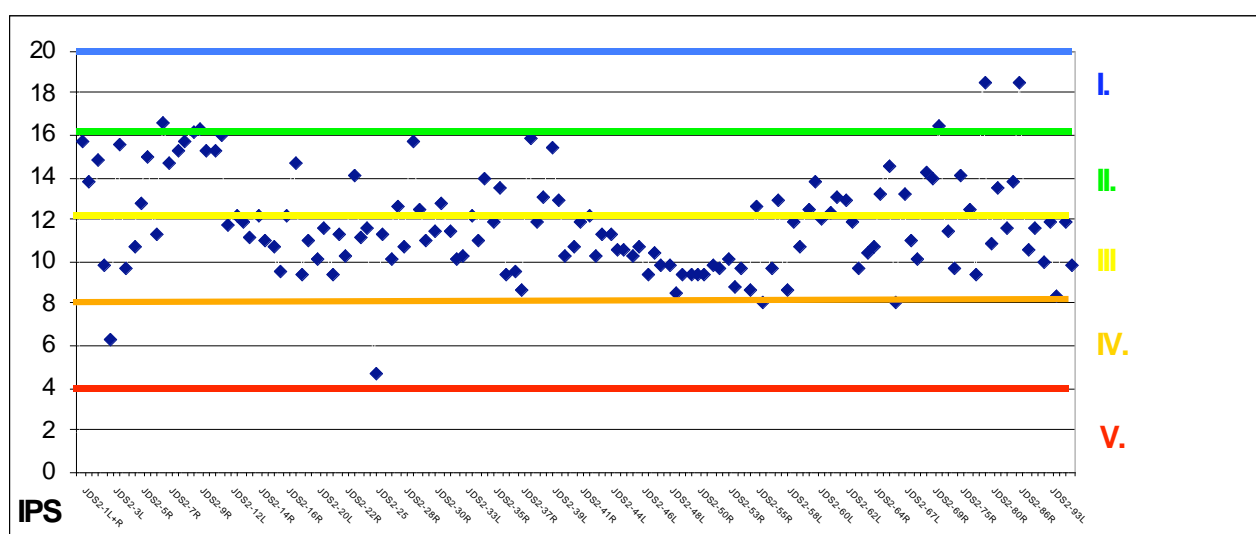


Figure 20: Assessment of water quality of the Danube and tributaries based on the IPS index five-class scale of sensitivity (OMNIDIA) to pollution

In order to indicate ecological status (according to the WFD) based on results from the JDS2 sampling sites, the Slovak classification system for phytobenthos-based assessment was used. This system (Hlubiková et al. 2007) is based on a multimetric index comprising three separate diatom indices: IPS ("Specific Pollution Sensitivity Index" - Coste in Cemagref, 1982); CEE ("Descy & Coste Index" - Descy & Coste, 1991) and EPI-D ("Diatom-based Eutrophication/Pollution Index" - Dell'Uomo et al., 1999). For Ecological Quality Ratio (EQR) calculation, average values of three diatom indices were used in order to compensate for differences in the applicability or sensivity of particular indices depending on the taxa included in the calculation or particular changes of the indication potential of species in different regions. It should be mentioned that this system was proposed for natural river types, including large rivers at altitudes below 200 m a.s.l. in Hungarian lowland. Reference values and class boundaries for this river type were derived by modelling. Values of EQR for the individual JDS2 stations (Figure 21) are diverse within all classes, although most results fall within classes II – IV.
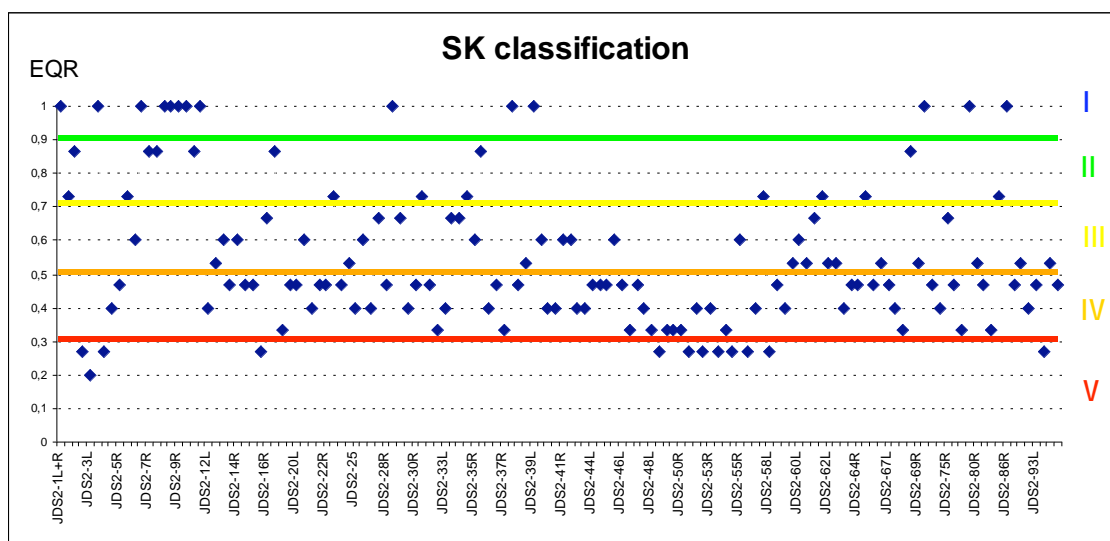


Figure 21: Assessment of the status of the Danube and the tributaries based on the Slovak classification system according to phytobenthos (benthic diatoms)

Comparing with JDS1 data, the species diversity of the phytobenthos (including diatoms) was higher in JDS2 at 438 (compared with 340 for the JDS1). This was caused by the fact that during JDS2 living samples were identified and also probably by the use of the semi-quantitative sampling method.

## 5.4  Conclusions

– Based on results of the JDS2, the species diversity of phytobenthos was high; in total, 443 taxa were identified.

– In spite of a large similarity of diatom samples, there were significant differences between the River Danube and some tributaries. 6 groups of clusters can be separated representing specific regions or river parts.

– During JDS2, phytobenthos biomass was measured for the first time along the whole course of the Danube. Therefore it is not possible to compare the data with previous results. There was evidently a slight increase of chlorophyll-a along the River Danube, but not statistically significant.

– Biomass of individual Danube River Types showed highest concentrations in Type 7 (Iron Gate reservoir).

– When comparing the results of phytoplankton and phytobenthos biomass, the Middle Danube shows the highest variability; the Upper and Lower Danube show lower phytoplankton levels, resulting in better growing opportunities for phytobenthos.

– With JDS2 data, 17 indices for potential use in monitoring programmes were calculated. In comparison, the evaluation of JDS1 data was based on the saprobic index only.

– Values of IPS (Specific Pollution Sensitivity Index) decrease downstream indicating a longitudinal increase in pollution (organic, nutrient).

## 5.5 References

ABERLE, N., M. BEUTLER, C. MOLDAENKE, AND K. H. WILTSHIRE (2006): 'Spectral fingerprinting' for specific algal groups on sediments in situ: a new sensor. *Archiv für Hydrobiologie* 167: 575-592.

LECOINTE, C., COSTE, M., PRYGIEL, J. & ECTOR, L. (1999): Le logiciel OMNIDIA version 2, une puissante base de donnés pour les inventaires des diatomées et pour le calcul des indices diatomiques européens. – Cryptog. Algol. 20 (2): 132-134.

ÁCS, E., SZABÓ, K., TÓTH, B. & KISS, K. T. (2004): Investigation of benthic algal communities, especially diatoms of some Hungarian streams in connection with reference conditions of the Water Framework Directives. – Acta Bot. Hung. 46 (3-4): 255-277.

ELORANTA, P. & ANDERSSON, K. (1998): Diatom indices in water quality monitoring of some south-Finnish rivers. – Verh. Int. Ver. Limnol. 26: 1213-1215.

KELLY, M. G., PENNY, C. J. & WHITTON, B. A. (1995): Comparative performance of benthic diatom indices used to assess river water quality. – Hydrobiologia 302: 179-188.

KRAMMER, K. (1997a): Die cymbelloiden Diatomeen. Eine Monographie der weltweit bekannten Taxa. Teil 1. Allgemeines und *Encyonema* part. Bibliotheca Diatomologica Band 36. J. Cramer, Stuttgart. 382 pp.

KRAMMER, K. (1997b) - Die cymbelloiden Diatomeen. Eine Monographie der weltweit bekannten Taxa. Teil 2. *Encyonema* part., *Encyonopsis* und *Cymbellopsis*. Bibliotheca Diatomologica Band 37. J. Cramer, Stuttgart. 469 pp.

KRAMMER, K. (2000): Diatoms of Europe. Diatoms of the European Inland Waters and Comparable Habitats. Vol. 1. The genus *Pinnularia*. A.R.G. Gantner Verlag K.G, Ruggell. 703pp.

KRAMMER, K. (2002): Diatoms of Europe. Diatoms of the European Inland Waters and Comparable Habitats. Vol. 3. *Cymbella*. A.R.G. Gantner Verlag K.G, Ruggell. 584pp.

KRAMMER, K. (2003): Diatoms of Europe. Diatoms of the European Inland Waters and Comparable Habitats. Vol. 4. *Cymbopleura, Delicata, Navicymbula, Gomphocymbellopsis*, Afrocymbella. A.R.G. Gantner Verlag K.G, Ruggell. 530pp.

KRAMMER, K. & LANGE-BERTALOT, H. (1986): Bacillariophyceae, 1. Teil: Naviculaceae. – In: ETTL, H., GERLOFF, J., HEYNIG, H. & MOLLENHAUER, D. (Eds): Süsswasserflora von Mitteleuropa 2/1. – G.Fischer-Verlag, Stuttgart, 876 pp.

KRAMMER, K. & LANGE-BERTALOT, H. (1988): Bacillariophyceae, 2. Teil: Bacillariaceae, Epithemiaceae, Surirellaceae. – In: ETTL, H., GERLOFF, J., Heynig, H. & Mollenhauer, D. (Eds): Süsswasserflora von Mitteleuropa 2/2. – G. Fischer-Verlag, Stuttgart, 596 pp.

KRAMMER, K. & LANGE-BERTALOT, H. (1991a): Bacillariophyceae, 3. Teil: Centrales, Fragilariaceae, Eunotiaceae. – In: ETTL, H., GERLOFF, J., HEYNIG, H. & MOLLENHAUER, D. (Eds), Süsswasserflora von Mitteleuropa 2/3. – G. Fischer-Verlag, Stuttgart, 576 pp.

KRAMMER, K. & LANGE-BERTALOT, H. (1991b): Bacillariophyceae, 4. Teil: Achnanthaceae. Kritische Ergänzungen zu *Navicula* (Lineolatae) und *Gomphonema*, Gesamtliteraturverzeichnis. – In: ETTL, H., GERLOFF, J., HEYNIG, H. & MOLLENHAUER, D. (eds), Süsswasserflora von Mitteleuropa 2/4. – Gustav Fischer-Verlag, Stuttgart, 437 pp.

LANGE-BERTALOT, H. (1993): 85 Neue Taxa und über 100 weitere neu definierte Taxa ergänzend zur Süsswasserflora von Mitteleuropa Vol. 2/1-4. Bibliotheca Diatomologica 27. J. Cramer, Stuttgart. 393 pp.

LANGE-BERTALOT, H. (1996): Iconographia Diatomologica. Annotated Diatom Micrographs Vol. 2. Indicators of Oligotrophy. 800 taxa representative of three ecologically distinct lake types. Koeltz Scientific Books. 390 pp.

LANGE-BERTALOT, H. (2001): Diatoms of Europe. Diatoms of the European Inland Waters and Comparable Habitats. Vol. 2. *Navicula* sensu stricto. 10 Genera Separated from *Navicula* sensu lato. *Frustulia*. A.R.G. Gantner Verlag K.G, Ruggell. 526pp.

Lange-Bertalot, H. & Krammer, K. (1987): *Bacillariaceae, Epithemiaceae, Surirellaceae*. Bibliotheca Diatomologica 15. J. Cramer, Stuttgart. 289 pp.

Prygiel, J. & Coste, M. (1993): The assessment of water quality in the Artois Picardie water basin (France) by Lange-Bertalot, H. & Krammer, K. (1989) - *Achnanthes* eine Monographie der Gattungen. Bibliotheca Diatomologica 18. J. Cramer, Stuttgart. 393 pp.the use of diatom indices. – Hydrobiologia 269/270: 343-349.

Prygiel J., Carpentier P., Almeida S., Coste M., Druart J.-C., Ector L., Guillard D., Honoré M.-A., Iserentant R., Ledgeanck P., Lalanne-Cassou C., Lesniak C., Mercier I., Moncaut P., Nazart M., Nouchet N., Peres F., Peeters V., Rimet F., Rumeau A., Sabater S., Straub F., Torrisi M., Tudesque L., v.d. Vijver B., Vidal H., Vizinet J., Zydek N. (2002): Determination of the biological diatom index (IBD NF T 90-354): results of an intercalibration exercise. Journal of Applied Phycology, 14, 19-26.

Prygiel, J. & Coste, M. (1996): Recent trends in monitoring French rivers using algae, especially diatoms. – In: Whitton, B.A. & Rott, E. (eds): Use of algae for monitoring rivers II: 87-96.

Kwandrans, J., Eloranta, P., Kawecka, B. & Woitan, K. (1998): Use of benthic diatom communities to evaluate water quality in rivers of Southern Poland. – J. Appl. Phycol. 10: 193-201.

Rott, E., Pipp, E., Pfister, P., Van Dam, H., Ortler, K., Binder, N. & Pall, K. (1999): Indikationslisten für Aufwuchsalgen in österreichischen Fliessgewässern. Teil 2: Trophienindikation (sowie geochemische Präferenzen, taxonomische und toxikologische Anmerkungen).- Wasserwirtschaftskataster herausgegeben vom Bundesministerium f. Land-u. Forstwirtschaft, Wien 248 pp.

Vilbaste, S. (2004): Application of diatom indices in the evaluation of the water quality in Estonian streams. – Proc. Estonian Acad. Sci. Biol. Ecol. 53/1: 37-51.

Van Dam, H., J. Padisák & C. Kovács (2005): BQE Report Phytobenthos, ECOSURV, Ministry of Enviroment and Water, Hungary, EuropeAid/114951/D/SV/2002-000-180-04-01-02-02, 54 pp.

Gomà, J, Ortiz, R., Cambra, J & Ector, L. (2004): Water quality evaluation in Catalonian Mediterranean rivers using epilithic diatoms as bioindicators. – Vie & Milieu, Sér. A., Biol. Mar. 54 (2-3): 81-90.

Hlúbiková, D. Hindáková, A., Haviar, M., Miettinen, J. (2007): Application of diatom water quality indices in influenced and non-influenced sites of Slovak rivers (Central Europe). Large Rivers Vol. 17, No. 3-4. Arch. Hydrobiol. Suppl. 161/3-4, p. 443-464.

Literáthy, P. Koller – Kreimel, V. (eds.) (2002): Joint Danube Survey. Technical Report of the International Commission for the Protection of the Danube River: September 2002. Vienna: International Commission for the Protection of the Danube River, 1-261.

# 6 Macrophytes

Georg A. Janauer, Brigitte Schmidt and Anita Greiter

## 6.1 Introduction

The biological quality element "Macrophytes and Phytobenthos" is composed of information on the status of macroscopic and microscopic aquatic vegetation (according to the EU Water Framework Directive - WFD). In this chapter of the JDS2 Report, the macrophyte compartment is described. Macrophytes indicate trophic, water flow, substrate and morphology related qualities, and provide an essential structural basis for other biological quality elements e.g. macroinvertebrates and young fish. In the Danube River Basin, their occurrence relates to different River Section Types of the main river channel and secondary water bodies (e.g. side channels, floodplain lakes) and tributaries. In the micro- and meso-scale, they exert several ecosystem functions; retention of nutrients and suspended solids can be mentioned as the most important in this context.

## 6.2 Methods

The JDS2 field survey of aquatic plant species (characean algae, bryophytes, ferns and angiosperms) and their abundance and growth form followed the European Standard EN 14184 (2003) in compliance with (i) the EU WFD (Annex V, 1.3.6., p.57), (ii) the JDS1 (2001), (iii) the International Danube Macrophyte Survey ( www.midcc.at) and (iv) the basic methodology compiled by Kohler & Janauer (five-level estimator scale, 1995). At each JDS site, six survey units of 1 km length were assessed (separate left/right bank, slow continuous passage, minimum number of stops at 200 m and 700 m for collection of species by hand or rake). Survey unit length was determined by GPS. Species were documented by digital photography. Habitat parameters included bank structure, sediment type (choriotope), connectivity type, flow class, land-use type (CORINE) and transparency.

Most species were determined on the spot. Those species needing microscopic determination were collected in labelled plastic-bags and kept in a cool-box for transport in excess of 2 hours. Bryophytes were stored dry in labelled paper bags. Voucher specimens (except bryophytes) were stored in labelled glasses filled with 70% methylated spirit.

The indication of ecological status assessment complies with the (v) Austrian Directive for Running Waters – Macrophytes (2007), Leitfaden zur Erhebung der biologischen Qualitätselemente, Teil A4 (2008; www.lebensministerium.at). The reference conditions were adapted to the conditions of the Danube River in the different Section Type reaches. However, no sufficient and scientifically sound historical quantitative data or modelling approaches are available to produce a solid database for the macrophyte reference conditions for the whole length of the Danube River. Various sources were used to make reference conditions needed for indicating ecological status at the JDS2 sites: in particular, results from side channels, historical maps, saprobiotic maps of the Danube and JDS1 and JDS2 data on chemical components, as well as data from the whole-length-macrophyte survey in the midcc project and available literature on the ecological characterisation of species. The complete database of reference condition data will be published at a later date.

## 6.3 Results

### 6.3.1 Completeness of the JDS2 macrophyte survey

96 sites with an accumulated length of 556.5 km were sampled during the JDS2 survey, which is approx. three times longer than for the JDS1. Based on previous statistical calculations (source: midcc-project), this expansion was a minimum requirement for acquiring a complete picture of the aquatic macrophyte vegetation, which was present in 486 Survey Units (87% of all sampled river-kilometres).

### 6.3.2 Similarity of Danube Section Types

**Table 6: Statistical comparison of Section Types: A-Values of the Multi Response Permutation Procedure (MRPP) between Section Types**

| Section | ST-1 | ST-2 | ST-3 | ST-4 | ST-5 | ST-6 | ST-7 | ST-8 | ST-9 | ST-10 |
|---------|------|------|------|------|------|------|------|------|------|-------|
| ST-1 | - | | | | | | | | | |
| ST-2 | 0,0978 | - | | | | | | | | |
| ST-3 | 0,0888 | 0,1340 | - | | | | | | | |
| ST-4 | 0,0300 | 0,0379 | 0,0633 | - | | | | | | |
| ST-5 | 0,0396 | 0,1500 | 0,1185 | 0,0781 | - | | | | | |
| ST-6 | 0,0296 | 0,1234 | 0,1000 | 0,0743 | 0,0376 | - | | | | |
| ST-7 | 0,1688 | 0,2816 | 0,2377 | 0,1121 | 0,0849 | 0,0424 | - | | | |
| ST-8 | 0,0269 | 0,1109 | 0,0919 | 0,0676 | 0,0429 | 0,0315 | 0,0399 | - | | |
| ST-9 | 0,0718 | 0,1482 | 0,1161 | 0,0467 | 0,0479 | 0,0413 | 0,1385 | 0,0282 | - | |
| ST-10 | 0,1311 | 0,1944 | 0,1364 | 0,0598 | 0,0741 | 0,0434 | 0,1455 | 0,0351 | 0,0671 | - |

Significant A-values shown in bold, P ≤ 0,001.

The results show that the different sections of the Danube River are habitat for different macrophyte species compositions and underline the ecological richness of this second largest river in Europe.

### 6.3.3 Dominance of plant groups – Relative Plant Mass

The development of macrophyte families and some higher taxa are a mirror of species group behaviour along the course of the Danube and show changes on a greater scale. Figure 22 presents relative plant mass values (RPM), accumulated for higher taxa in each river section, and the total number of species recorded in the same reach (categories relating to Figure 22 are printed in *italics*):

– *Bryophytes* (mosses and liverworts) prefer large stones and running water, where $CO_2$ is easily available. This group is dominant in the uppermost three sections of the Danube.

– The aquatic *ferns, Salvinia natans* and *Azolla filiculoides,* show their highest abundance in Section 6, characterised by the confluence with the Drava, Tisza und Sava. This section is also marked by the highest species richness – a total of 32 species – recorded in the main river channel.

– Many *Ranunculaceae* species were limited to fast flowing reaches in the upper part of rivers, where clear cool water was present.

– *Lemnidae* were exceptionally abundant over the whole Danube in 2007. Surprisingly, peak abundances were recorded in survey units with faster flow than would be expected, but their occurrence in almost still waters like the Iron Gate or the Danube Delta was sparse.

– *Potamogetonaceae* were rather evenly distributed across Sections 3 to 10. This is mainly due to the wide ecological amplitude of the species *P. pectinatus*, which is tolerant to a wide range of habitat parameter properties e.g. nutrient load and flow velocity.

- *Ceratophyllaceae* and *Haloraghaceae*, represented by *Ceratophyllum demersum* and *Myriophyllum spicatum*, play an important role in the dominance relationships of aquatic plant species, as they increase in importance downstream.
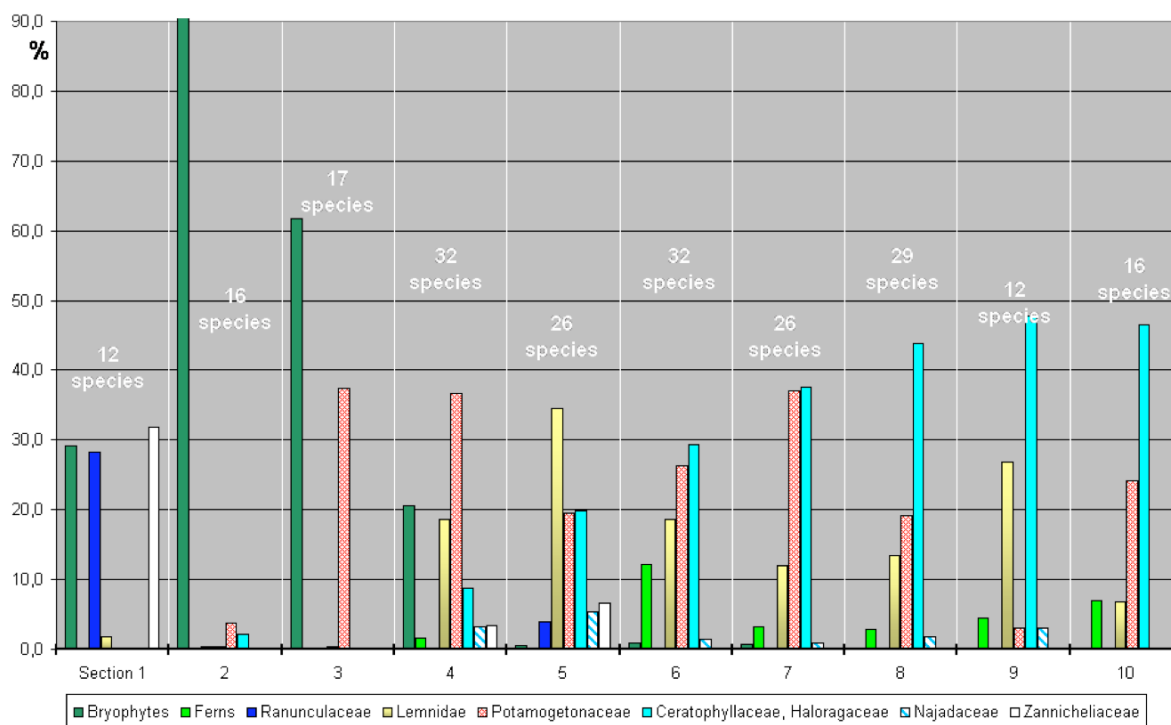


Figure 22: Relative Plant Mass Values (RPM) and number of species recorded in each Danube reach

### 6.3.4  Comparison with outcomes from JDS1

The basic difference between the JDS2 and JDS1 is seen in the number of aquatic macrophyte species detected during the two surveys:

|  | Species number | Species number |
|---|---|---|
|  | Algae, bryophytes, vascular species | Helophytes |
| JDS2 | 69 | 60 |
| JDS1 | 44 | 4 |

A considerable difference in species numbers is apparent when comparing the two surveys, but this can be easily explained:

- Aquatic macrophyte species number and abundance may differ between successive years as flood regime, over-wintering conditions and spring temperature regime define species dominance in the following vegetation period.

- The JDS2 macrophyte survey was considerably optimised when compared to JDS1 as a separate boat was available for the macrophyte study and a total of six river-kilometres were assessed at the sampling sites compared to considerably shorter survey units during JDS1. Thus the detected species numbers increased by 57%.

In general, the aquatic species spectrum was considerably richer in JDS2 than that in JDS1. The helophyte group included not only reeds but also many species of ecological value and relevance as this group is indicative to some extent for EC Habitat Directive issues.

With regard to conservation activities in European Community countries, JDS-2 revealed differential information on species rareness and neophytic species, which should be incorporated into national conservation approaches.

### 6.3.5 Aspects regarding the indication of an ecological status

The Danube is not only the most international river in Europe (regarding the number of countries sharing its catchment), it is also a river of its own character as no other flowing water equals its length, its size and catchment area in Western and Central Europe. Therefore expert judgement had to be used to develop reference conditions (of course based at least in part on the rich database of the midcc-project).

For JDS2 sites located in hydro-electric power plant reservoirs, it was quite evident that most of them probably failed to meet the conditions needed for the indication of a good ecological status. In the regulated, but free-flowing stretches, the conditions were different and many JDS2 sites were considered to meet the conditions for the indication of a good ecological status, and in some cases they might be close to natural conditions. However, some tributary mouth stretches in the lower reaches of the Danube River alter this general situation considerably, as is the case for the Sio, Timok, Russenski Lom, Arges, Siret and Prut. The missing or very low number of macrophytes reflects the indication of poor ecological status. This is to be noted as the downstream sampling sites in the Danube indicate good status in the cases where they are situated a considerable distance from the mouths of these tributaries (e.g. downstream of Russenski Lom: 10 km; Timok, Drava, Morava, Sio >11 km; Arges: 3 km; *Braila*: 13 km). The mixing of water from the tributaries with the great discharge of the Danube River causes dilution of the loads of nutrients and pollutants.

Downstream of such impacts on water quality, the respective side of the Danube river channel is directly influenced and can fail to meet the indication of good status, while the other side of the river stays in the indication of good status (e.g. Braila).

The methodological approaches used for macrophytes in JDS2 specify some limits for applying the above-mentioned method when a too low number or abundance of species is recorded. In this case no calculation of the indication of ecological status should be done. Following this recommendation, several survey sites in the Lower Danube River reach could not be classified. Yet, this recommendation was not strictly followed as, especially in the Middle and Lower Danube, conditions are completely different from the Upper Reach. A "supplement" calculation was performed and the results are indicated in the respective table by numbers shown in *italics*.

When no macrophytes at all are present, other authors regard the indication of ecological status in these rare cases as "bad". Yet, in difference to Western European countries, the total absence of macrophytes in the Central and Eastern European countries does not necessarily indicate total devastation caused by human impact and a resulting indication of moderate, poor or bad ecological status. Therefore, in such a situation the reasons for macrophytes absence must be thoroughly sought.

### 6.4 Conclusions

Lessons learned from the JDS2 macrophyte survey:

– The survey length used in JDS2 was 3 km on each side of the river. According to our calculations this seems to be the absolute minimum accumulated survey length to cover the variability of macrophyte species occurrence and abundance. Further macrophyte studies in the Middle and Lower Danube River shall not be restricted to shorter river length. In the uppermost part of the river (Germany) this minimum length seems to be sufficient at least for power plant reservoirs, but not for free-running reaches.

– In JDS2, as well as in JDS1, an emphasis was laid on survey sites in power plant reservoirs. It would be highly interesting to additionally sample the parts of the Danube where neither water depth nor flow conditions are influenced by reservoir-typical conditions.

- For calculating the impacts of the discharge of tributaries and municipal wastewater, the "downstream sampling sites" should be located closer to the point of impact and those distances should take into account the magnitude of the impact e.g. smaller tributaries with low water quality are more quickly mixed with the high discharge in the main Danube channel.

- In Sections 1-4, mosses occurred in a nearly continuous belt along the banks. Their preferred habitats are natural stones and rocks in reaches with high water flow velocity and on the riprap in the splashing water zone, which is found in impounded stretches, as well as in not impounded but regulated reaches. Where natural rock or stones occur, the mosses were considered to meet the indication for natural conditions. In the lowland river, the water-mosses hardly find corresponding substrates and were classified worse. Plants indicating slow-flowing or still water, or a higher nutritional status and occurring in the Middle and Lower Reach of the Danube, were classified better than in the Upper Reach.

- Section Type 4 was not homogeneous with respect to the macrophyte assemblage and it is recommended to reassess the indication of status of this section and to possibly divide it close to Bratislava City.

- Section 7, the Iron Gate, holds a special position as an ancient cataract stretch between the Middle and Lower Danube. The constrained width of the Danube in several gorge stretches results in rocky banks and rapids, whereas in the wider parts of the Iron Gate stretch, calm waters and finer substrates form suitable habitat conditions for many different macrophytes. Despite the typical impoundment conditions in the Iron Gate, we can find both aspects - mosses on rocks and quite a high diversity of other macrophytes outside of the distinct gorge stretches. Based on these facts, this stretch shows an indication of good conditions, alternating between constrained and wider parts of the Iron Gate river course.

- The high number of 69 species recorded is an outcome of the enhanced length of the survey units used for the JDS2. The unexpected spread of duckweeds in the main river channel was probably triggered by the warm winter period. The fern, *Salvinia natans*, has been found in an oxbow system near Vienna over the last 3 years and could be an indication of climate change induced migration of thermophilic species up the Danube River. Another migrating species described in the literature is the helophyte, *Chamaesyce glyptosperma*, which was found near Novi Sad. The submerged invasive species, *Elodea nuttallii*, migrated from Western Europe down the river into the delta area and is in the process of replacing *Elodea canadensis*.

- Rare species found in the JDS2 were: *Azolla filiculoides, Lemna turionifera, Potamogeton zizii* and *P. trichoides, Riccia fluitans, Utricularia vulgaris, Wolffia arrhiza. Trapa natans* and *Stratiotes aloides* were very scarce, and the occurrence of *Eichhornia crassipes* must be considered a 'human impact'.

- Following the discussions before the start of the JDS2, it seems advantageous to have supporting surveys by national teams, which should cover longer river lengths than 3 + 3 km, which could be carried out at the JDS sites. In the next JDS this could considerably extend the data basis for an enhanced macrophyte assessment. Theoretical support for this requirement could be a detailed statistical interpretation of certain important river stretches collated in the midcc-project database.

- Following the recommendations of the Water Framework Directive, a three-year repetition period for future JDS surveys would be highly appreciated to enable coverage of inter-annual variations of macrophyte species composition and abundance not caused by human impact.

# 7 Phytoplankton

Martin T. Dokulil and Christina Kaiblinger

## 7.1 Introduction

Autotrophic phytoplankton is an essential quality element in lakes and large rivers. Primary producers are important in the carbon cycle and the oxygen budget through photosynthetic processes. The accumulated biomass serves as food for other trophic levels. As elements of water quality, phyto-plankton composition and biomass primarily indicate eutrophication. These metrics required by the EU Water Framework Directive (WFD) shall therefore evaluate the trophic status. Similarly as for the trophic concept used in lakes, additional parameters relevant to assess the trophic level are necessary. Total phosphorus (TP) is assumed to be the most relevant nutrient for phytoplankton growth. Nitrogen concentration is needed to judge any deficiency relative to phosphorus and chlorophyll-a is used as an additional measure of biomass. Development of diatoms can be estimated from the concentration of dissolved silica. Availability of under-water light, important for photosynthesis, can be calculated from suspended solids.

Phytoplankton can also be used to estimate impacts from chloride concentration or to evaluate changes in hydromorphology that affect phytoplankton assemblages. Regulated stretches decrease retention time resulting in reduced biomass development. Impounded or artificially deepened river sections are more similar to lakes, indicated by an increase in species more common in standing waters and a reduction in the contribution from benthic taxa usually common in free-flowing rivers.

Within the Danube River Basin, phytoplankton assessment is particularly relevant because the River Danube, as well as several of the larger tributaries, have a great potential to produce large amounts of phytoplankton biomass. Some stretches may even carry 'true' river plankton (potamoplankton). Monitoring of phytoplankton diversity will help to assess changes in nutrient input and pollution control. The development of the nutrient levels and the associated phytoplankton biomass in the Danube River Basin ultimately has a large impact on the Black Sea.

## 7.2 Methods

Samples were taken from the surface in the middle of the river with a black bucket (8 l) and used for all further analysis. A qualitative sample was taken with a plankton net (10 µm); Secchi-depth and incident PAR-radiation were measured. On-board analysis included the immediate measurement of 'active' chlorophyll-a by delayed fluorescence (DF), estimation of algal groups (DF) and photosynthetic parameters (FRRF). Suspended solids (SS) were filtered (1 l) on pre-combusted and pre-weighed glass filters (GF/F) for gravimetric evaluation of total suspended solids (TSS, 105°C, 4h) and loss on ignition (ISS, 550°C, 4h) later in the laboratory. Samples were filtered onto GF/C filters for total chlorophyll-a analysis, stored at -35°C and extracted and analysed according to ISO (10 260) later in the laboratory. Quantitative samples (100 ml) for phytoplankton counting and sizing were fixed with Utermöhl's acetic acid Lugol solution, preserved with a few drops of formalin in brown screw cap glass bottles and stored in a cool dry place. These samples were counted in the laboratory applying the sedimentation technique (NIKON, Opticount V 08/2001, Lucia V 3.51). Algae were largely determined on-board using the unpreserved concentrated net-samples. Sub-samples preserved with formalin were evaluated for diatom species by the acid-combustion technique.

## 7.3 Results

During the investigation period, samples were taken from 96 locations (78 from the Danube and 18 from tributaries). In addition, 12 samples from upstream tributaries brought in by the national teams were analysed. Results from the main variables measured are displayed in Figure 23. Total suspended solids (TSS) are low (4.6-7.0 mg/l) in the German stretch. After the confluence with the River Inn (TSS 31.7 mg/l ), turbidity increases and remains moderately high throughout the Austrian part. A first peak of 30.5 mg/l is recorded at Bratislava (SK) after the confluence with the River Morava (114.8 mg/l). The high values there are a reflection of the high discharge in Morava during the observation period. TSS remains low or moderate (3.9-18.0 mg/l ) thereafter until Novi Sad (RS) where a second peak of around 20 mg/l occurs. The rivers Tisza and Sava further dilute TSS, which remain low to moderate (average 9.7 mg/l) until the rivers Iskar and Olt bring in loads of suspended solids. From km 602 onwards, TSS steadily increase towards the outflow to the Black Sea (87.6 mg/l). The increase is mainly due to inorganic suspended solids washed in from the large tributaries. Organic suspended solids, derived from loss on ignition, are low with an average of 4.5 mg/l (=25% of TSS) and a peak of 12.4 mg/l at Novi Sad (RS). High contribution from organic substances is found in the tributaries, particularly Arges (83%) and Timok (76%) but also downstream of Novi Sad (61%). The concentration of the suspended solids is reflected in Secchi depth visibility (Figure 23, top panel). These two variables are significantly correlated ($r^2 = 0.75$, n = 96).

Both chlorophyll-a and phytoplankton biomass concentration remain at low levels in the upstream and downstream section of the Danube (Figure 23 panel 2 & 4). Values higher than 10 µg/l chl-a or 2 mg/l algal biomass occur between Baja (HU, km 1481) and Grocka (RS, km 1132). These concentrations thresholds are reached or slightly exceeded at certain points further downstream e.g. upstream of Cernavoda, near Braila and in the Sulina Canal, all in RO. Highest concentrations of around 28 µg/l chl-a or 6.5mg/l biomass are reached in the Novi Sad/Tisza confluence region between km 1262 and km 1200. Photosynthetic activity of the phytoplankton (indicated by Fv/Fm in panel 2 of Figure 23) follows a similar pattern reaching high rates when biomass is sufficiently large, discharge moderate and underwater light conditions acceptable. Phytoplankton fresh-weight biomass is significantly correlated with chlorophyll-a ($r^2 = 0.95$, n = 100; see insert in panel 4 of Figure 23).

Biomass and chlorophyll-a input by the larger tributaries to the River Danube is highly variable from very low values in e.g. the Inn and Jantra and high contribution from e.g. the Morava, Velika Morava or Timok (Figure 23, panels 2 & 4).

The phytoplankton of the River Danube is dominated by diatoms (Bacillariophyceae) and green algae (Chlorophyceae) with significant contribution from Cryptophyceae (Figure 23, third panel down). Their average contribution is 55.8, 28.5 and 14.6% respectively. Cyanobacteria are of no importance in the river and contribute too little to total biomass to appear in Figure 23. In the region of greatest phytoplankton development, diatoms and green algae together contribute about 90% to total biomass. Within the Bacillariophyceae, centric diatoms are most abundant and quantitatively most important. More than 100 benthic diatom species were identified but their contribution to total biomass is negligible. A wide variety of green algal species from the order Chlorococcales quantitatively contribute to phytoplankton biomass. Cyanobacteria are of greater importance in several of the tributaries such as the Morava (6.8%) and Jantra (14.5%). In the River Arges, 80% of the biomass originates from the Cyanobacterial species *Microcystis aeruginosa*.
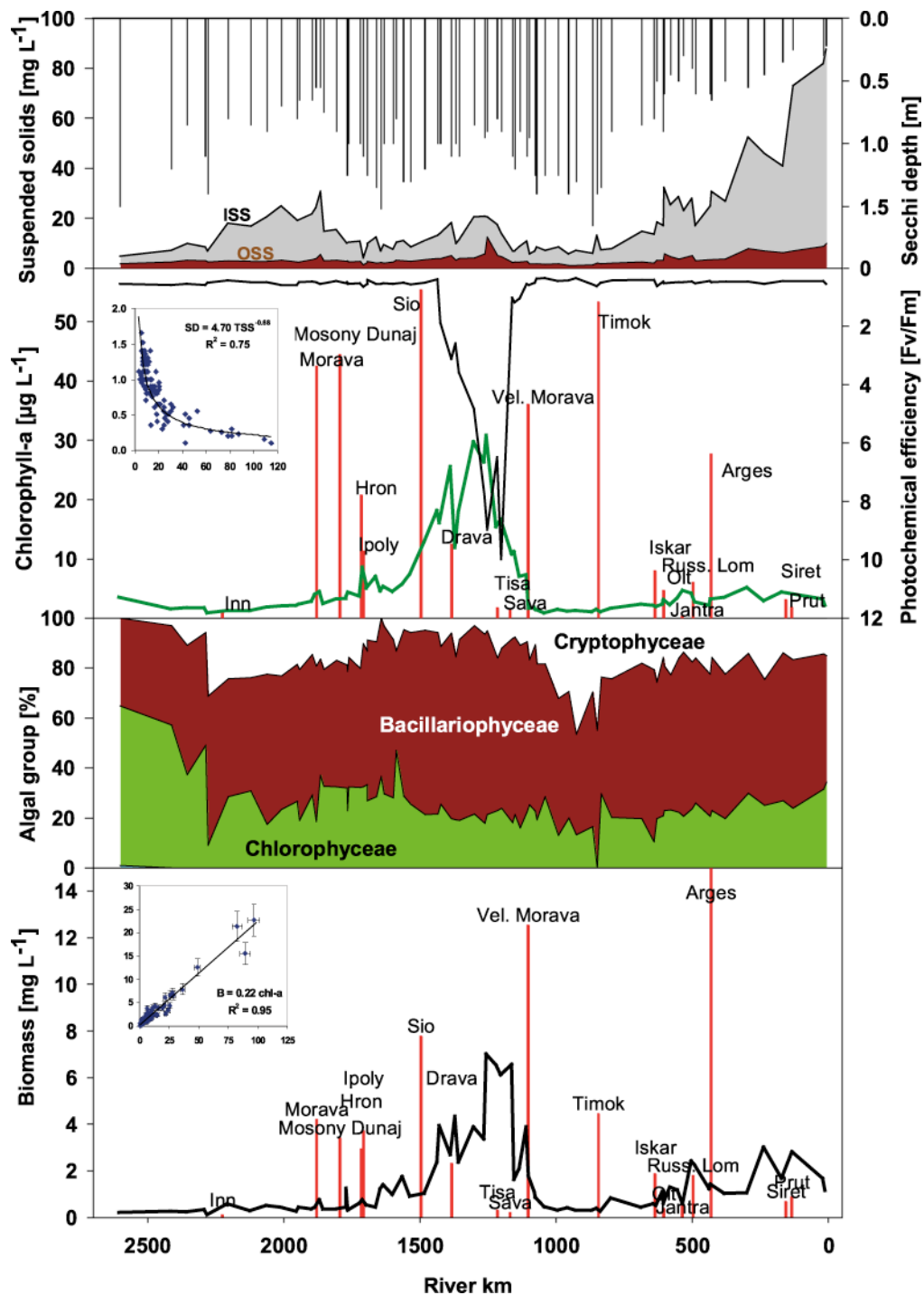
**Figure 23: Longitudinal transect of the River Danube from river km 2600 to the Black Sea obtained during JDS2 (August/September 2007)**

Variables from top to bottom: Secchi depth (SD), total suspended solids (TSS) as inorganic and organic part (ISS and OSS respectively); Chlorophyll-a in the river (green solid line) and in the mouth of the tributaries (red bars), photosynthetic activity (inverted thin line), the insert is the relation of SD to TSS (r²=0.75); Contribution of the main algal groups (%); phytoplankton biomass in the river (black solid line) and in the mouth of the tributaries (red bars), the insert shows the relation of biomass to chl-a (r²=0.95). Units are indicated on the axes.

## 7.4 Conclusions

From the distribution of phytoplankton chlorophyll-a and biomass along the river corridor, three sections can be defined: an upstream section from upstream Iller to Baja with chlorophyll-a values below 10 µg/l and biomass concentration below 2 mg/l (km 2600 – 1481); a middle section where values exceed this threshold from downstream Baja to Grocka (km 1481 – 1132) and a downstream section with generally low values again. Maximum values of both parameters have decreased by about 5x compared to results from JDS1. Chlorophyll content of phytoplankton biomass varies from 0.1 – 0.84% with an average of 0.45%.

Assessment of water quality according to the Water Framework Directive (WFD) requires at least four sampling dates per year. One measurement is therefore insufficient and non-conclusive for classification using phytoplankton. According to the TNMN quality classification however, most chlorophyll-a values fall into water quality class I (<25 µg/l). Moderate values of quality class II are observed at km 1384, upstream of the Drava and in the Novi Sad region and downstream of the Tisza (km 1262-1200). From the tributaries, 15 streams are below 25 µg/l chl-a. Rivers Sio and Velika Morava (36.6 and 48.9 µg/l respectively) qualify in quality class II while the Arges (89.3 µg/l) must be ascribed to quality class III. River phytoplankton is largely characterised by centric diatoms while tributaries are very rich in species diversity.

In addition, the type-specific WFD criteria for the assessment of the ecological status in large rivers, established in Germany by Mischke et al. (2005), were applied. Using the metric chl-a for the pre-degradation assessment according to trophy, chlorophyll-a in the upper reach of the Danube (section type 1-5) is indicating high to good status, the middle reach (part of section 6 until rkm 1200) is indicated as good to moderate, and the lower reach (remaining part of section 6 and 7 -10) is indicated as high to good status. From the investigated tributaries, chl-a in the Arges has an indication of bad status, rivers Velika Morava and Sio are indicated as poor, and for all others chl-a indicates high or good status.

Compared to JDS1 both the concentrations of chlorophyll-a and the phytoplankton biomass have declined especially in the middle part of the River Danube, which can be seen as an indication of quality improvement. Longitudinal variations in both parameters, species composition and diversity, are similar to observations during JDS1.

It must be emphasized however, that direct comparison of chemical and biological concentrations of the two investigation periods might be inconclusive because of the different hydrological discharge situations. The smaller concentrations during JDS2 can partly be a reflection of dilution due to higher run-off. Comparison could certainly be improved by calculation of total load from discharge data for both periods.

## 7.5 References

MISCHKE, U. & OPITZ, D. (2005): Überarbeiteter Endbericht zum LAWA-Vorhaben: Entwicklung eines Bewertungsverfahrens für Fließgewässer mittels Phytoplankton zur Umsetzung der EU-Wasserrahmenrichtlinie. IGB-Berlin, 100 S.

# 8 Fish

Niels Jepsen, Christian Wiesner and Nikolaus Schotzko

## 8.1 Introduction

More than 70 species of freshwater fish inhabit the Danube along its entire course, covering various ecological and functional guilds. Yet, the ecology of many species is still poorly known. Although fish stocks have declined and species became endangered or even extinct in the last decades, fish are still of economic importance. Besides this importance, the fish population is potentially a good indicator of human pressures, in particular in the form of hydromorphological alterations. Specifically the loss of connectivity due to man-made barriers can be reflected in the character of the fish community.

### 8.1.1 Links to the Water Framework Directive (WFD)

The status of the fish population in the river is one of the biological quality elements necessary for an assessment of the ecological status according to the EU Water Framework Directive (WFD). According to the requirements of the Directive, species composition, abundance and population structure are the key elements to be analysed. However, fish sampling in large rivers is challenging and not a routine task and both sampling methodology and assessment tools are still under development. So, apart from the creation of a standardised data-set (being representative for the entire Danube course), methodological harmonisation and improvements across different countries, some even outside the EU, was a key task of this first fish survey of the entire Danube River.

There are several obvious links between the JDS2 fish sampling and ongoing work to develop and intercalibrate metrics for using fish as indicators of ecological status of rivers. Much effort has been exerted to develop WFD-compliant methods to evaluate the ecological quality (EQ) of rivers and streams by monitoring the fish populations. This work is rather advanced at the EU level (several national methods are available, a common metrics method is under development) but a major shortcoming has been the lack of fish data from large rivers. To enable inclusion of larger rivers in the national methods and the common metrics method, data from standardized sampling is needed. We hope that the data from the JDS2 will contribute significantly to testing a common approach for sampling and evaluating fish populations in large rivers. Due to the special nature of fish sampling, the fish survey was performed under a separate strategy than was applied for JDS2 but it was kept as a parallel activity using an independent vessel (Vienna 115) with an electrofishing boat. The whole exercise was managed by an international Core Team of fish experts (3 persons on board) in cooperation with national fish teams and harmonised with the JDS2 programme.

In order to decide upon the best practise and sampling method, a field workshop was arranged in Göd, Hungary, four months before the survey. Representatives of each national team and the Core Team met to test and evaluate the suggested methods and agree upon a sampling strategy.

## 8.2 Methods

### 8.2.1 Sampling

The minimum sampling design was a joint effort of the Core Team and the national teams and included the possibility of exchanging team members during the survey to achieve methodological harmonisation and experience exchange.

The sampling was based on the EU WFD and the European Standard "Water Analysis – Fishing with Electricity" (EN 14011; CEN, 2003) for wadeable and non-wadeable rivers.

The following methods were used to investigate the composition of the fish fauna on the different sites along the Danube:

– Electric fishing;

– Drift netting with trammel nets.


Electric fishing 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, which recover very fast. However, especially with juvenile fish, casualties can happen, but in general electric fishing is considered as a non-lethal sampling method. The method is also CEN-standardized and is recommended for use as the basic sampling method for WFD purposes.

Electric fishing is mostly effective in shallow waters (0 – 2.0 m); to sample fish from the main channel of the Danube, additional methods must be used. We decided to use trammel nets that we let drift approx. 1000 m downstream and thus catch some of the benthic species from deeper mid-channel areas. A trammel net is a combination of three nets, two outer ones with very large mesh size and an inner one with a small mesh size. The fish is then trapped in the "pockets" of a fine mesh net and so are not killed unlike with normal "gill-nets".

At each site, a set of at least 10 single electrofishing sub-samples (strips of 200-400 m in length according to riparian habitat availability, 8 during day, 2 during night) using different electrofishing gear devices (handheld and fixed boom anode) and, wherever possible, 3-4 drift net samples, were collected. This should optimise capture efficiency for different species and age classes. The electrofishing was undertaken by national teams and the drift net fishing was carried out by the Core Team.

However, given the available time, sampling gear, weather conditions and water level, the sampling design had to be adjusted individually for each site. The Core Team, in particular, was often restricted to limited time frames for sampling and had to spend most of its time carrying out electrofishing as several national teams could not fulfil the proposed programme in time and sometimes even lacked the required equipment. As Slovakia and Bulgaria could not provide a national team during the time of the survey, the Core Team provided all Danube main channel data in these countries, whereas national sampling from some tributary sites was performed later and included in the database. Due to these constraints, drift net fishing could only be performed occasionally. In all, data from 45 main river sampling sites and 21 sites in the tributaries could be collected.

All fish were identified, measured (total length) and briefly examined for external injuries, signs of disease or parasites.

The sampling effort varied greatly between the sites, but at most sites a sufficient number of species and individuals were caught to provide a representative picture of the site.
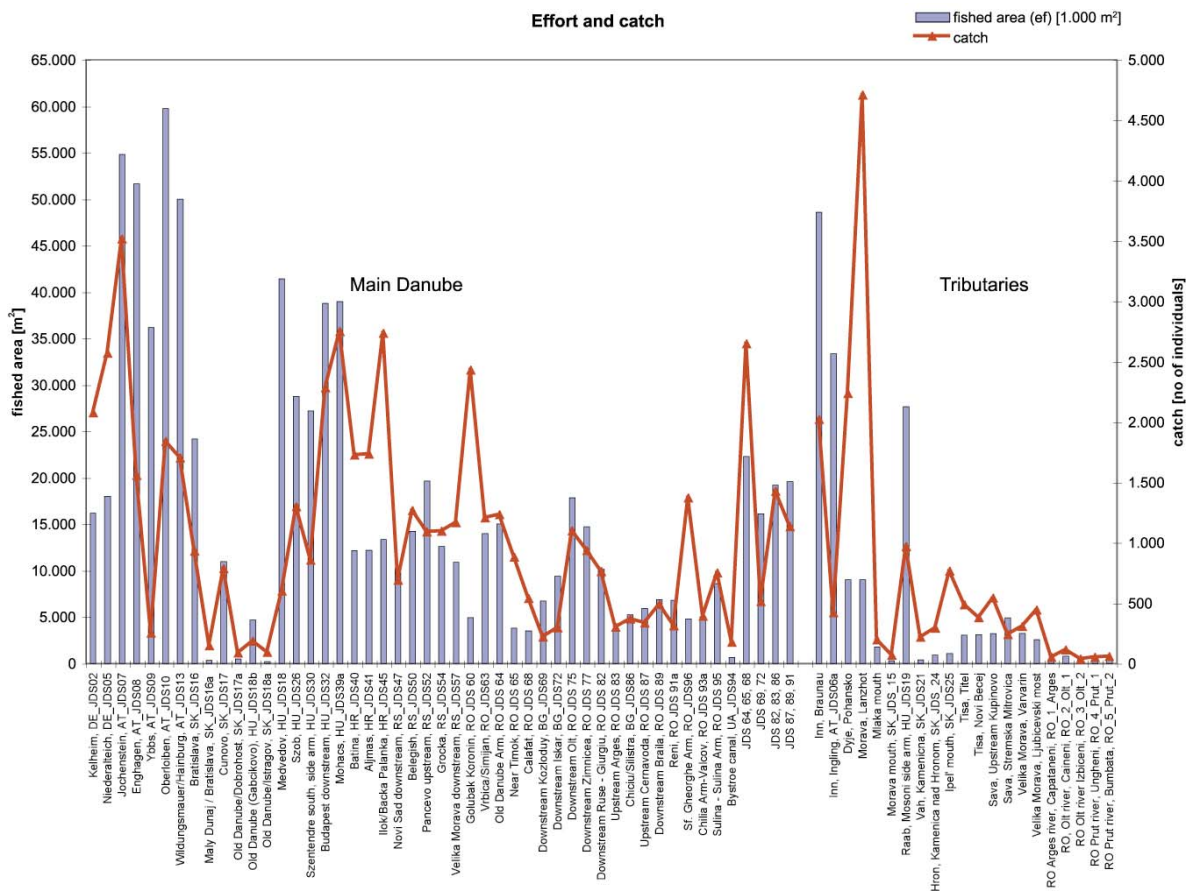
**Figure 24: Sampling effort and number of individual fish caught at each site by electro-fishing (including tributary sites)**

### 8.2.2 Ecological Quality Assessment, FISH-INDEX

According to the requirements of the WFD, all EU-member states must establish a monitoring network and use assessment methods for all four biological quality elements in all natural water bodies. Despite these clear requirements and much effort by most member states, it has proven difficult to develop solid, pressure sensitive indicators (metrics) based on fish communities. Thus, the current status is that there are 8-9 national methods available in the EU countries (officially approved at the national level) for assessing the ecological quality of *small and medium sized rivers* based on electrofishing sampling of the fish fauna. None of these national methods have yet been intercalibrated and officially accepted at the EU level as a tool for setting boundaries between the ecological quality classes. In the case of the Danube, there is the problem that no method has been developed for large rivers, and among the Danube countries, only Germany and Austria have developed their national methods. Thus, using the JDS2 fish results to evaluate the ecological quality along the Danube is a challenging issue.

It was decided to calculate the necessary scores from the Austrian Fish Index (FIA) and from the European Fish Index (EFI) developed by the EU-supported FAME project. The EFI has been developed by testing a large number of candidate metrics using a large pan-European fish database and testing their reaction to various pressures. The final EFI constitutes 10 metrics and produces a score between 0 and 1, which is then directly translated into an EQ-class. In the process of testing and intercalibration, it appeared that the EFI (as expected) had several shortcomings and limitations. It is known that the EFI is sensitive to water quality pressures, but not very good at showing hydromorphological pressures like canalisation and presence of obstacles (dams). In contrast the FIA

(with 9 metrics) was developed to be able to detect hydromorphological pressures in Austria so it is obvious that the two indices will show significant differences in scores at any site. Bearing this in mind, the assessment presented in Table 7 is at best a qualified guess of the "true state" of each site and represents an indication of the ecological status of a sampling site. Depending on the quality and quantity of data available per site, the FIA can be calculated individually or a grouping of sites is needed (pooled data from several sites). Furthermore, due to missing information/references, the reference fish coenosis for Romania could only be roughly sketched and needs critical revision later. The present results are currently used for developing an improved version of EFI, the EFI+ index, which has to be adjusted to also cover the Danube species.

## 8.3 Results

### 8.3.1 Sampling

The final data-set includes 45 Danube sites and 21 tributary sites. 40 Danube sites were sampled throughout the survey by the Core Team and national teams. Additional 5 sites (JDS9, 16a, 17a, 18a, 18b) were contributed by the national teams after the survey's completion. However, due to reduced sampling effort and comparability, only one of these additional sites (JDS9) can be used for a complete analysis. Data from the other sites were used only for assessing species occurrence and relative abundance.

Within the monitored sites, a rather impressive number of 71 different fish species was caught. A total of **49,039** fish of **66** species were sampled in the Danube and **14,564** fish of **58** species were sampled in the tributaries. This shows a very high species diversity for any river and the Danube probably still remains the European river with the greatest number of fish species (e.g. the Rhine has a total of about 60 species) despite the disappearance of several native species. Bleak (*Alburnus alburnus*) was by far the most abundant fish in the catch and made up almost 50% of the number of fish captured. Bleak was abundant throughout the whole river and the only species caught in all Danube sampling sites.

Only very few sick, deformed, injured or parasite infested fish were caught, so in general the fish population seemed healthy. In contrast to that, it is common to encounter a high proportion of deformed/damaged/ diseased fish in many Mediterranean reservoirs.

#### 8.3.1.1   Migratory species
The only native migratory species found were 2 specimen of *Acipenser stellatus* caught in the delta. The only migratory species caught in good numbers was eel. As the eels were only caught in the very upper river, and are considered non-native in the Danube Basin, it must be concluded that these are exclusively results of stocking of juvenile eel, mainly done in the German part of the Basin.

#### 8.3.1.2   Invasive Neogobius species
Several goby species (*Neogobius* spp.) were found in high or even dominating abundances along the rip-rap protected and regulated banks, an artificial habitat common along the upper and middle course of the Danube that is not used by other species to a similar extent. In contrast to that, downstream of the Iron Gate, where these species are native and the hydromorphological impact on the river (not considering the dams and impoundments of the Iron Gate) is much lower, their abundance is low.

#### 8.3.1.3   Exotic species
Several non-native fish species have established populations in the Danube Basin, mainly due to human activities such as stocking, angling and a general movement of fish. During the JDS2 some of these were even abundant and widely distributed.
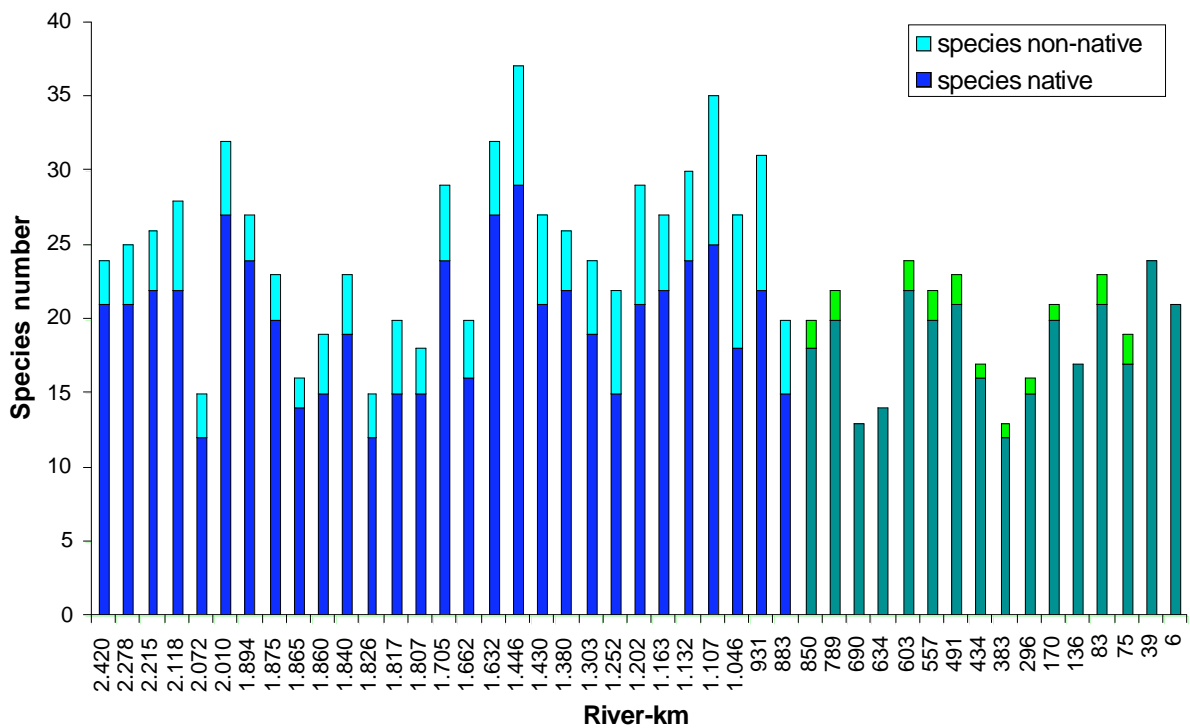
Figure 25: Native and exotic species along the Danube

Blue bars: upstream of Iron Gate II; green bars: downstream of Iron Gate II.

The presence of exotic species is not necessarily an indication of pressure, but an introduction of new species can change the fish composition and thus influence the status assessment. This may be the case in the Danube but because the species that might be affected most (small benthic species) are difficult to sample and thus are generally under-represented in the samples, such effects are hard to document. One example may be *Romanogibio vladykovi*, which seemed to have declined in the Upper Danube in the last decades, a time when these invasive goby species expanded their range. Unfortunately, no directly comparable data exist from 10-20 years ago.

However, the EFI does not depend on occurrence or abundance of individual species but uses grouping of species in functional or habitat guilds or as tolerant or sensitive species. This means that for both methods, EFI and FIA, the occurrence of exotic species can be important, but it should not affect the ecological status evaluation.
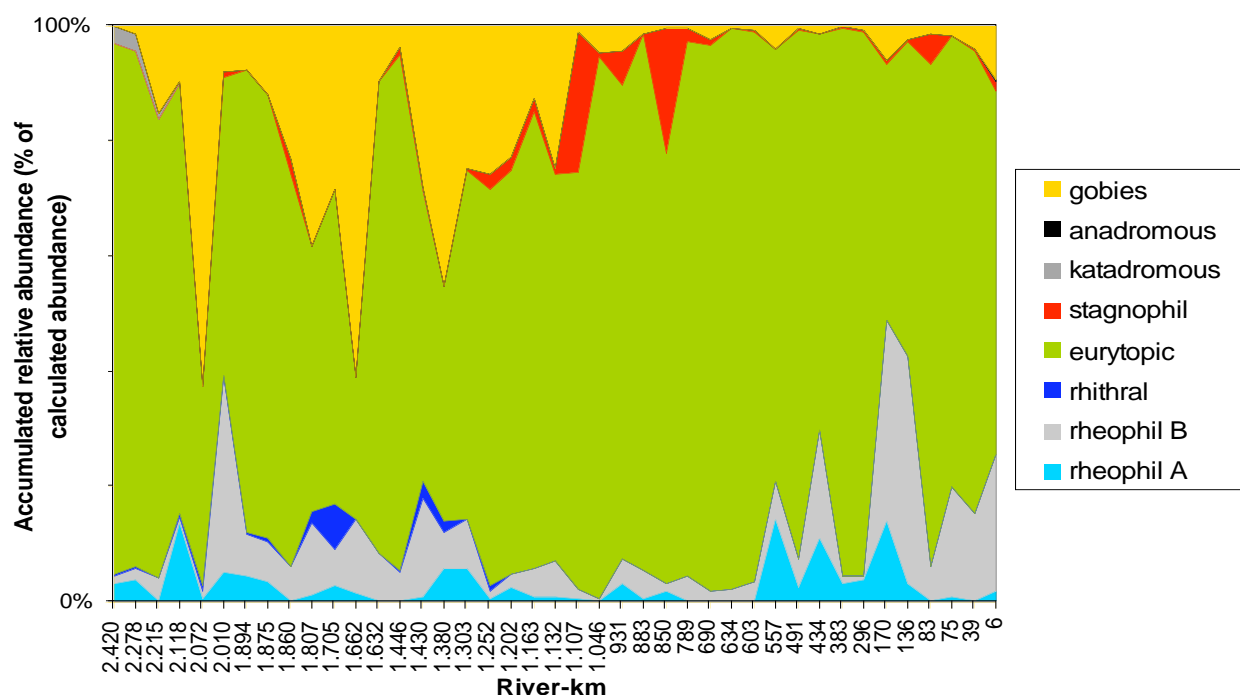
Figure 26: Relative abundance of the ecological guilds along the river course (based on calculated abundance; JDS16a, 17a, 18a, 18b excluded)

The occurrence of such non-native elements is clearly reflected in the ecological guild distribution (Figure 26). For practical reasons and because their biology and habitat preference is different from almost all other fish species in the Danube, goby species were put into a separate guild. Except the most upstream fish site, JDS2 Kelheim, the Upper Danube is heavily infested by the non-native goby species (*Neogobius* spp.). Further downstream, even in their native range (km 850-6), their importance is drastically lower and only slowly increases towards the delta, where *N. eurycephalus* contributes mainly to their abundance.

In the German sampling sites JDS2 and JDS5, the non-native eel (*Anguilla anguilla*), a katadromous species, is abundant due to stocking but soon vanishes from the catches throughout the Austrian section.

Gobies and eel benefit from the high extent of rip-rap habitat in these areas. On the opposite end, the record of *Acipenser stellatus* (anadromous native species) can faintly be seen at km 6 and shows the influence of the nearby Black Sea.

In the upper part, until km 1303, rheophil-A species are represented primarily by barbel (*Barbus barbus*) and nase (*Chondrostoma nasus*), whereas downstream of Iron Gate II this guild is mainly comprised of *Romanogobio vladykovi* and *Gymnocephalus schraetser*.

The rhithral guild, quite abundant in the Slovakian/Hungarian section is mainly represented by burbot *(Lota lota)* and thus, like the gobies, closely related to rip-rap bank protection.

The stagnophilous guild is represented primarily by bitterling (*Rhodeus amarus*) but also by the non-native pumpkinseed (*Lepomis gibbosus*). Typical backwater species were rarely caught as the sampling efforts focused on the main channel.

Indifferent species are present throughout the entire river course but their relative proportion is higher in the lower half of the river, especially because of increased abundance of Prussian carp (*Carassius gibelio*).

## 8.3.2  Ecological status

A true classification of the Danube, or even the sample sites, cannot be made by using data from just one day's sampling. Even if there had been well developed assessment methods available, much more information (e.g.  the pooled results from three temporally separated samples) would be needed to make a WFD-compliant assessment of the ecological status. However, based on the classification by EFI and FIA, rough indication of the status of the Danube River can be made. It seems clear that the uppermost sites have good water quality and that the fish fauna there is not negatively impacted by water quality pressure. However, the high abundance of the *Neogobius* species in parts of the Upper Danube is an indication of altered habitats. These species have not been historically present in the upper reach, but as they are associated with a certain type of littoral habitat, rocky substrate, they have greatly benefited from the hydromorphological changes carried out to enforce the banks, creating the characteristic rip-rap habitat.

There seems to be an effect from navigation in the upper river. The passing of ships introduces large waves, which can negatively impact juvenile fish living in the very shallow littoral habitats. Thus, there is some effect on reproductive success (population structure) especially of sentinel species like barbel and nase: e.g. there are clear differences between the population structure of barbel and nase at Kelheim (JDS2) without navigation and Jochenstein (JDS7) with a narrow channel and navigation. The newly built structures (such as sheltered side arms (eupotamon) in Enghagen (JDS8) and Oberloiben (JDS10)) are the habitats where the young of the year of these species were caught in higher numbers than in the main channel where they are affected by navigation effects.

Further downstream these effects seem to be less clear, probably due to the Danube getting wider. As there is always a combination of pressures on such a large river, a quick "survey" will not deliver clear results. However other studies have demonstrated this effect. One of the most recent was conducted in Weltenburg, upstream of Kelheim by Zauner et al. (2007), where a vessel travelled at different speeds in a reach of the Danube without commercial navigation and both larval drift and beaching of fingerlings was shown.

**Table 7: The EFI and FIA values calculated from each site and corresponding indication of ecological classification**

| River | Site name | r-km | EFI | StatusEFI | FIA | Status FIA |
|-------|-----------|------|-----|-----------|-----|------------|
| Danube | Kelheim, DE - JDS02 | 2 420 | 0.52 | Good | 2.21 | Good |
| Danube | Niederalteich, DE - JDS05 | 2 278 | 0.49 | Good | 2.26 | Good |
| Danube | Jochenstein, AT - JDS07 | 2 215 | 0.50 | Good | 4.00 | Poor |
| Danube | Enghagen, AT - JDS08 | 2 118 | 0.65 | Good | 4.00 | Poor |
| Danube | Ybbs, AT - JDS09 | 2 072 | 0.35 | Moderate | 5.00 | Bad |
| Danube | Oberloiben, AT - JDS10 | 2 010 | 0.49 | Good | 4.00 | Poor |
| Danube | Wildungsmauer - Hainburg, AT - JDS13 | 1 894 | 0.45 | Good | 2.33 | Good |
| Danube | Bratislava, SK - JDS16 | 1 875 | 0.40 | Moderate | 3.04 | Moderate |
| Danube | Maly Dunaj / Bratislava, SK - JDS16a | 1 865 | 0.43 | Moderate | 3.07 | Moderate |
| Danube | Cunovo, SK - JDS17 | 1 852 | 0.20 | Poor | 5.00 | Bad |
| Danube | Old Danube/Dobrohost, SK - JDS17a | 1 840 | 0.35 | Moderate | | * |
| Danube | Old Danube (Gabcikovo region), HU - JDS18b | 1 826 | 0.50 | Good | | * |
| Danube | Old Danube/Istragov, SK - JDS18a | 1 817 | 0.38 | Moderate | | * |
| Danube | Medvedov, HU - JDS18 | 1 807 | 0.47 | Good | 5.00 | Bad |
| Danube | Szob, HU - JDS26 | 1 705 | 0.47 | Good | 2.52 | Moderate |
| Danube | Szentendre south, side arm, HU - JDS30 | 1 662 | 0.49 | Good | 4.00 | Poor |
| Danube | Budapest downstream, HU - JDS32 | 1 632 | 0.48 | Good | 2.10 | Good |
| Danube | Mohacs, HU - JDS39a | 1 446 | 0.51 | Good | 1.96 | Good |

| River | Site name | r-km | EFI | StatusEFI | FIA | Status FIA |
|---|---|---|---|---|---|---|
| Danube | Batina, HR - JDS40 | 1 430 | 0.48 | Good | 2.45 | Moderate |
| Danube | Aljmas, HR - JDS41 | 1 380 | 0.43 | Moderate | 2.56 | Moderate |
| Danube | Ilok/Backa Palanka, HR - JDS45 | 1 303 | 0.37 | Moderate | 2.61 | Moderate |
| Danube | Novi Sad downstream, RS - JDS47 | 1 252 | 0.44 | Moderate | 3.34 | Moderate |
| Danube | Belegish, RS - JDS50 | 1 202 | 0.32 | Moderate | 2.67 | Moderate |
| Danube | Pancevo upstream, RS - JDS52 | 1 163 | 0.34 | Moderate | 2.81 | Moderate |
| Danube | Grocka, RS - JDS54 | 1 132 | 0.36 | Moderate | 2.62 | Moderate |
| Danube | Velika Morava downstream, RS - JDS57 | 1 107 | 0.31 | Moderate | 2.42 | Good |
| Danube | Golubak Koronin, RO - JDS60 | 1 046 | 0.15 | Bad | 2.89 | Moderate |
| Danube | Vrbica/Simijan, RO - JDS63 | 931 | 0.31 | Moderate | 2.20 | Good |
| Danube | Old Danube Arm, RO - JDS64 | 883 | 0.24 | Poor | | * |
| Danube | Near Timok, RO - JDS65 | 850 | 0.29 | Moderate | | * |
| Danube | Calafat, RO - JDS68 | 789 | 0.26 | Poor | | * |
| Danube | Downstream Kozloduy, BG - JDS69 | 690 | 0.23 | Poor | | * |
| Danube | Downstream Iskar, BG - JDS72 | 634 | 0.21 | Poor | | * |
| Danube | Downstream Olt, RO - JDS75 (+ Olt river confluence) | 603 | 0.30 | Moderate | 4.00 | Poor |
| Danube | Downstream Zimnicea/Svishtov, RO - JDS77 | 557 | 0.27 | Poor | 5.00 | Bad |
| Danube | Downstream Ruse - Giurgiu, RO - JDS82 | 491 | 0.29 | Moderate | | * |
| Danube | Upstream Arges, RO - JDS83 | 434 | 0.21 | Poor | | * |
| Danube | Chiciu/Silistra, BG - JDS86 | 383 | 0.25 | Poor | | * |
| Danube | Upstream Cernavoda, RO - JDS87 | 296 | 0.35 | Moderate | | * |
| Danube | Downstream Braila, RO - JDS89 | 170 | 0.32 | Moderate | | * |
| Danube | Reni, RO - JDS91a | 136 | 0.29 | Moderate | | * |
| Danube | Sf. Gheorghe Arm, RO - JDS96 | 85 | 0.26 | Poor | | * |
| Danube | Chilia Arm-Valcov, RO - JDS93a | 60 | 0.29 | Moderate | | * |
| Danube | Sulina - Sulina Arm, RO - JDS95 | 21 | 0.37 | Moderate | | * |
| Danube | Bystroe canal, UA - JDS94 | 8 | 0.60 | Good | | * |
| Danube | JDS64, 65, 68 (pooled sites) | 883-789 | 0.30 | Moderate | 1.98 | Good |
| Danube | JDS69, 72 (pooled sites) | 690-634 | 0.24 | Poor | 3.15 | Moderate |
| Danube | JDS82, 83, 86 (pooled sites) | 491-383 | 0.31 | Moderate | 2.12 | Good |
| Danube | JDS87, 89, 91a (pooled sites) | 296-136 | 0.34 | Moderate | 2 | Good |
| TRIBUTARIES | | | | | | |
| Inn | Inn, Braunau | 49 | 0.61 | Good | 4.00 | Poor |
| Inn | Inn, Ingling, AT - JDS06a | 5 | 0.41 | Moderate | 5.00 | Bad |
| Dyje | Dyje, Pohansko | 22 | 0.51 | Good | 2.08 | Good |
| Morava | Morava, Lanzhot | 79 | 0.54 | Good | 1.79 | Good |
| Mláka | Mlaka mouth | 0 | 0.33 | Moderate | 2.58 | Moderate |
| Morava | Morava mouth, SK - JDS15 | | 0.45 | Good | | |
| Mosoni Side Arm | Raab, Mosoni side arm, HU - JDS19 | 6 | 0.45 | Moderate | | |
| Váh | Vah, Kamenicna, SK - JDS21 | 1 | 0.46 | Good | | |
| Hron | Hron, Kamenica nad Hronom, SK - JDS24 | 1 | 0.57 | Good | | |
| Ipel' | Ipel' mouth, SK - JDS25 | 1 | 0.44 | Moderate | | |
| Tisa | Tisa, Titel | | 0.35 | Moderate | | |
| Tisa | Tisa, Novi Becej | | 0.40 | Moderate | | |

| River | Site name | r-km | EFI | StatusEFI | FIA | Status FIA |
|-------|-----------|------|-----|-----------|-----|------------|
| Sava | Sava, Upstream Kupinovo | | 0.36 | Moderate | | |
| Sava | Sava, Stremska Mitrovica | | 0.32 | Moderate | | |
| Velika Morava | Velika Morava, Varvarin | | 0.44 | Moderate | | |
| Velika Morava | Velika Morava, Ljubicevski most | | 0.37 | Moderate | | |
| Arges | RO Arges river, Capataneni, RO-1- Arges | | 0.40 | Moderate | | |
| Olt | RO, Olt river, Caineni, RO-2 - Olt-1 | | 0.30 | Moderate | | |
| Olt | RO Olt river Izbiceni, RO-3 - Olt-2 | | 0.26 | Poor | | |
| Prut | RO Prut river, Ungheni, RO-4 - Prut-1 | | 0.53 | Good | | |
| Prut | RO Prut river, Bumbata, RO-5 - Prut-2 | | 0.36 | Moderate | | |

* insufficient data-set.

Table 7 lists the EQ-values of the EFI and FIA calculations. Due to the limited sample sizes in the lower parts of the Danube (high water level, limited time) some sites were grouped together and pooled data used for the calculation of a more accurate FIA. However, the EFI values also show an improvement when pooling these sites. Thus, on individual site level, in the middle and lower part of the Danube, the sites are generally at a lower quality, showing Medium, Low or even Bad status. This is probably due to water quality problems in this part of the river but may also be affected by the reduced sampling effort. However, the EFI and the FIA results differ up to even two classes, so the overall picture is not clear.

Probably, FIA reflects the hydromorphological changes in the upper river whereas the EFI mainly reflects the water quality problems in the middle-lower river. As mentioned before, due to the limitations of sampling throughout the survey, these values may only be seen as a first indication of the status and not as a final and firm reference. Some of the added sites from national sampling programmes also did not provide sufficient data for FIA calculation (JDS17a, 18a, 18b) and thus no FIA is presented. The same is true for the individual sites within the Danube Delta (JDS93a, 94, 95, 96), but here a grouping is not recommended due to different hydromorphological status.

## 8.4 Conclusions

Considering the available resources and the harsh field conditions, the fish sampling went very well. There were some deviations from the planned sampling schedule and thus the database is slightly smaller than originally planned. It can be emphasized that sampling fish in a comparable way in a large river is rather complicated. Even during ideal conditions, with perfect planning and much experience, there will always be a high degree of variation in the sampling efficiency.

The results of the JDS2 give cause for some optimism regarding the possibilities for sampling fish in large rivers for the evaluation of the ecological status. It seems clear that with a standardised sampling programme, based on electrofishing in the shallow (littoral) areas, it is possible to gather detailed information about the fish community in a cost efficient and manageable way. With frequent sampling at sites in the tributaries and main river, it is possible to collect sufficient information to make meaningful assessment of the ecological quality of the whole basin in compliance with the WFD.

– In total, over 64,000 fish of 71 species were sampled, mainly by electrofishing.

– The results indicate that even a large river like the Danube can be sufficiently sampled by using simple electrofishing from a boat. It turned out that sampling at night in a large river can be highly effective. Therefore, these results should be taken as a reason for updating the CEN-Standard for electrofishing.

– The composition of the fish fauna indicates the importance of different pressures. During the JDS2, the results show that hydromorphological alteration is the main pressure in the upper

section, while the water quality is a prevailing pressure in the middle and lower sections. Navigation also seems to have a negative impact on fish populations, especially in the upper part of the Danube River.

– Several invasive/exotic fish species are present in the Danube and the tributaries, and there are some indications that this has caused some changes in the composition of the fish fauna, but due to the lack of data on long time series this cannot be verified.

– The site-to-site variation in the fish fauna composition, also expressed by the index scores, was very high and apparently the fish community shows a clear response to local conditions.

– The general lack of migratory species indicates a serious loss of connectivity, but the timing of sampling as well as the limitations in sampling mid-river, makes this a more speculative conclusion.

– The indication of ecological status of the sampling sites in the Danube and the tributaries range from good to bad. Most of the sites evaluated, fall into the category: **Indication of Moderate Ecological Status**.

# 9 Zooplankton

Katalin Zsuga

## 9.1 Introduction

Zooplankton is the main link between small phytoplankton and larger carnivores, primarily young fish. Several surveys have been **organised in the Danube River so far, w**hich investigated selected sections of the river. Rotatoria dominance and the similarly high proportion of nauplius and copepodit larvae among Crustacea were proved by Bothár (1974), Naidenow and Schewzowa (1990), Naidenow et al. (1991) and Gulyás (1994, 1995). The most frequent occurrence has been observed for species typical for still or slow-flowing eutrophic waters. According to the survey results, the dominant species of the river are: *Brachionus calyciflorus, Keratella, Synchaeta spp*., *Bosmina longirostris, Acanthocyclops robustus.* Bothár (1973) pointed out that the joining of the Drava and Tisza did not have an effect on Crustacea plankton.

Naidenow (1998) summarized the qualitative and quantitative aspects of Danube zooplankton in a comprehensive work, based on the results of 164 studies. Gulyás (2002) reported on the zooplankton survey made on the section between Neu-Ulm and Tulcea in summer 2001 (JDS1); 79 Rotatoria, 27 Cladocera and 14 Copepoda species were found at that time. The results showed that the stocks' individual numbers were the smallest on German, Austrian, Romanian and Bulgarian sections of the river, the highest in the section below Budapest and in the former Yugoslavia. Low rates of individual number were also observed at Neu-Ulm-Tass, as well as in the section between the Iron Gate reservoir and Danube Delta. The primary reasons for those results are the high water flow velocity in the upper section and high loads in the lower section.

## 9.2 Methods

During JDS2, 96 samples were collected from the Danube and its major tributaries for the zooplankton analysis. Three main characteristic groups, Rotatoria, Cladocera and Copepoda were investigated in detail. For the analysis of zooplankton, 50 litres of water were filtered through a plankton net of 50 μm mesh. The samples were preserved in the field by 4-5 % formaldehyde. The quantitative and qualitative composition of zooplankton was determined in a laboratory using a light- and stereomicroscope. For the exact identification of some Rotifera species their trophy were prepared using sodium hypoclorite solution. The abundance is given in ind/m$^3$ units. The ratio of Rotatoria, Cladocera, Copepoda and the characteristic, dominant species or taxa in the different reaches of the Danube was investigated.

## 9.3 Results

During JDS2, 126 zooplankton taxa were found, out of which, 87 Rotatoria, 30 Cladocera and 9 Copepoda have been registered. The majority of the species maintain planktonic living, however, in some areas tychoplanktonic elements, which penetrate the plankton from aquatic plant environment or from the surface of the sediment through mud-mixing, were found.

In the Upper Danube at the German, Austrian, Slovak and Hungarian section, the density of zooplankton is very low; the ratio of rotifers is five-tenfold of the individual number of microcrustaceans. From the Rotifera, *Brachionus angularis, Br. calyciflorus, Keratella cochlearis tecta* and *Synchaeta* spp. are dominant; their abundance ratio varies. In the rapid streaming of the Upper Danube, the copepodits of Harpacticoida group were also found.
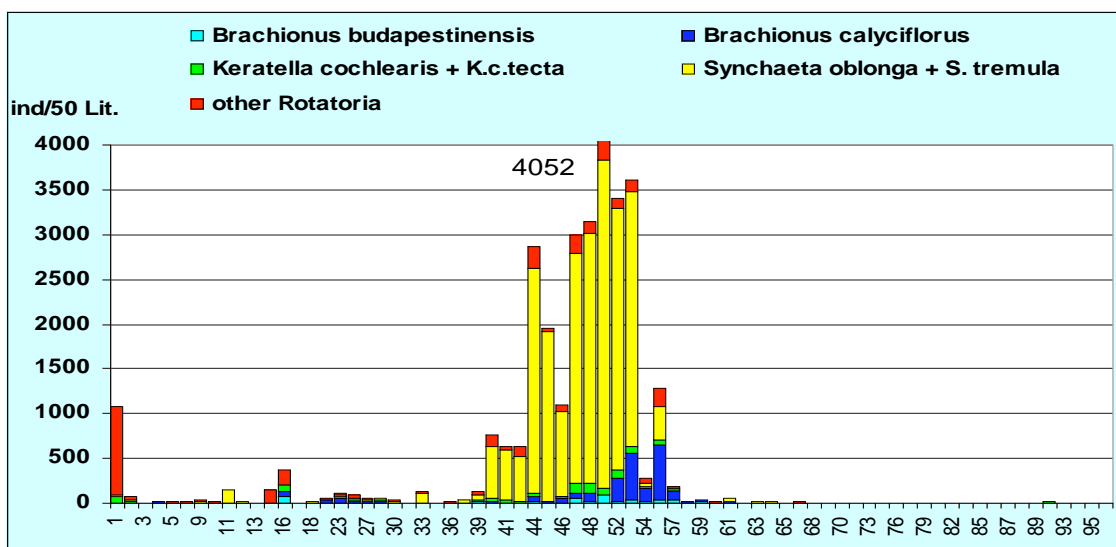


Figure 27:  Composition of the Rotatoria community in the main branch of the Danube

An increase in abundance was observed in the Serbian reach between JDS40-JDS53, with maxima at sampling points 50 (Belegis) and 53 (Figure 27). Among the three main zooplankton groups, that of Rotatoria is dominant; the ratio of microcrustaceans is below 10 %. In the rotifers community, the ratio of *Synchaeta tremula* and *S. oblonga* being 80-95 % indicates an **eutrophic-polytrophic** state of the river.

The density of cladocerans was highest at JDS53 (downstream of Pancevo). *Disparalona rostrata* became dominant at that site replacing *Bosmina longirostris* (Figure 28), indicating a changed character of that reach. In the zooplankton biomass, the wet biomass of rotifers is about 2.5-fold compared to cladocerans biomass, and circa 1.5-fold compared to copepods. This ratio is smaller in other reaches.
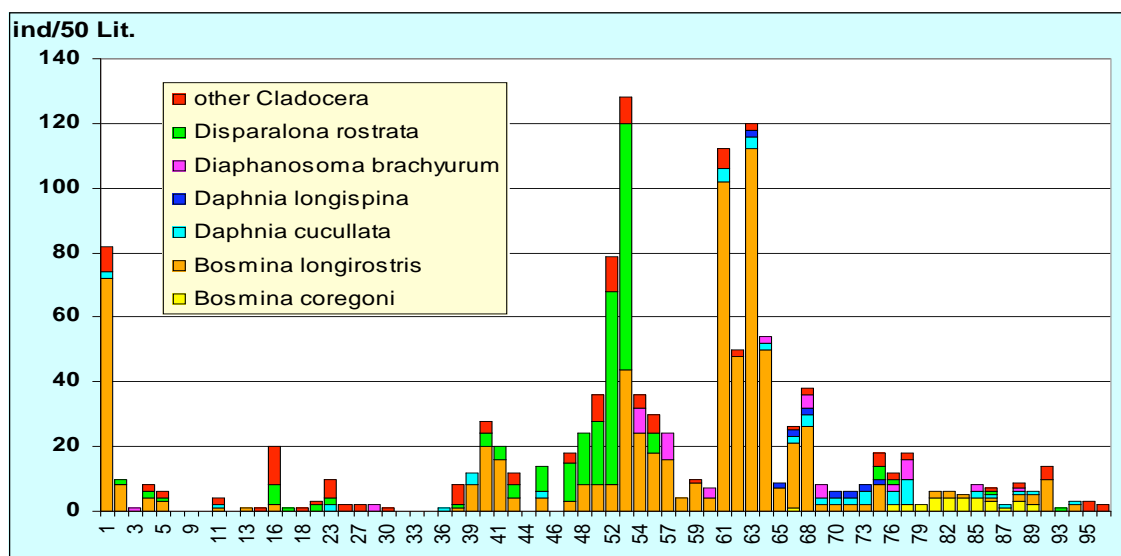
Figure 28: Composition of the Cladocera community in the main branch of the Danube

In the area of the Iron Gate reservoir (JDS60), abundance was low and a change of species composition was observed. The ratio of *Synchaeta oblonga* and *S. tremula* was reduced, *Brachionus calyciflorus and Keratella cochlearis tecta* becoming dominant species in the Rotatoria community. A change in cladocerans was observed as well when the tychoplanktonic *Disparalona rostrata* disappeared, but the high proportion of *Bosmina longirostris* remained. At the same time the ratio of euplanktonic *Daphnia cucullata* and *Diaphanosoma brachyurum* increased.

In the Lower Danube downstream to the delta the abundance of all zooplankton groups was low due to the effect of a flood wave. The individual number of rotifers was very small; none could be registered in the filtered water at several sampling sites. In this region the quantity of copepods was the highest. *Daphnia cucullata* and *Diaphanosoma brachyurum* were dominant elements of cladocerans and between JDS76 and JDS89 the *Bosmina coregoni* indicated the **eutrophic** environment. Among copepods *Thermocyclops crassus* and *T. oithonoides* were characteristic species and the *Eurytemora velox* was found in some sections as well.

Characteristic *veligera larvae* were found in **high proportion** in several sections of the Danube, but their species identification was not done. Further investigation of the *veligera larvae* in zooplankton of the Danube is suggested because of the importance of spreading invasive mussel species (i.e. Corbicula) along the Danubian waterway.


## 9.4 Conclusions

In the Danube River the individual species composition of zooplankton varied remarkably. Water velocity and the river load both had significant effects on the zooplankton density. High numbers were observed in the slow-flowing middle reach. Different sections were observed along the river, where zooplankton abundances differed considerably. There were also quantitative and qualitative variations within a single section.

During JDS2, 126 zooplankton taxa were found, out of which 87 Rotatoria, 30 Cladocera and 9 Copepoda have been registered. There were tychoplanktonic elements among the planktonic community, coming from aquatic plant stocks or from the sediment. **The dominant species indicated an eutrophic and polytrophic environment** (*Brachionus calyciflorus, Keratella cochlearis tecta,*

*Synchaeta oblonga, S. tremula, Bosmina longirostris, Daphnia cucullata, Diaphanosoma brachyurum, Thermocyclops crassus and T. oithonoides*).

There was no increased abundance or species number observed in reservoir sections. Only the effect of the Morava on the Danube zooplankton composition was registered; other tributaries did not influence the community of the main Danube River.

The ratios of dominant species were the same as in previous researches. The density of zooplankton was below that during the JDS1 in 2001; the maximum individual number could be registered in the Serbian reach, where the most eutrophic-polytrophic environment was found. There was no further increase of abundance in the Danube Delta.

A further investigation of veligera larvae in zooplankton along the River Danube is suggested, due to the importance of the invasion of alien species.

## 9.5 References

BOTHÁR (1974): Horizontale Planktonuntersuchungen an der Donau von Rajka bis Turnu Severin. (Stormkm 1850-930). (Danubialia Hungarica LXVIII). Annal. Unic. Sci. Budapest. 16: 157-162

GULYÁS, P. (1994): Studies on the Rotatoria and Crustacean Plankton in the Hungarian section of the Danube between 1848.4 and 1659.0 rkm. – In:KINZELBACH, R. Biologie der Donau. Limnologie Aktuell 2:49-61. Jena, New York

GULYÁS, P. (1994b): Veranderungen des Rotatoria and Crustacea Plankton in der Donaustrecke zwischen Rajka und Komárom infolge der in der Slovakei stattgefundenen Umteilung des Stromes. 30. Arbeistatung der IAD. Wissenschaftliche Kurzreferate. Zucz-Schweitz. 49-52

GULYÁS, P. (1995): Rotatoria and Crustacea plankton of the River Danube between Bratislava-Budapest. Miscnea Zool.hung. 10: 7-19.

GULYÁS (2002): A Rotatoria és Crustacea plankton minőségi és mennyiségi vizsgálata a Dunán. Vízügyi Közlemények. LXXXIV. Évf.2002. évi 4. füzet. Budapest. 601-620.

NAIDENOW W.- L. W. Schewzowa (1990): Die Verteilung des Metazooplanktons der Donau von Str-Km 20 bis Str-km 1928 im Marz 1988. Ergebnisse der Internat. Donauexpedition 1988. Wien

NAIDENOW et al. (1991): Genesis und Festaltung des Donauplanktons. Hydrobiology 37. Sofia.

NAIDENOW, (1998): Das Zooplankton der Donau. In: Kusel-Feztmann R. W. Naidenow, B. Russev. Plankton und Benthos der Donau – IAD Ergebnisse der Donau-Forschung. Band 4.

# 10  Microbiological water quality and DNA-based quantitative microbial source tracking

Alexander K.T. Kirschner, Gerhard G. Kavka, Branko Velimirov, Georg H. Reischer, Robert L. Mach and Andreas H. Farnleitner

## 10.1    Introduction

*Escherichia coli* and intestinal enterococci are used worldwide as sensitive indicators for the assessment of faecal pollution in the aquatic environment. Faecal indicators are excreted by humans and warm blooded animals in high concentrations and survive for a certain time in aquatic systems. Faecal pollution can be caused by point sources like discharges of sewage from human sources or livestock enterprises and by non-point sources like pasture, urban and agricultural run-off or water fowl (Kirschner et al., 2004). Faeces frequently contain pathogenic microorganisms like bacteria, viruses and parasites. Therefore intestinal indicator bacteria like *E. coli* and enterococci indicate the potential presence of pathogens and are especially well suited to indicating faecal pollution in surface waters.

Due to the hazard to humans caused by aquatic faecal pollution, strict quality regulations exist for water intended for irrigation, water-contact recreation (e.g. bathing), aquaculture and water for human consumption (e.g. EU Bathing Water Quality Directive, 2006/7/EC). According to the EU Water Framework Directive (WFD, 2000) protected bodies of water, such as recreational waters, including areas nominated as bathing waters, can be designated. Bathing waters have to fulfil the requirements of the Bathing Water Quality Directive.

Faecal pollution and microbiological contamination from anthropogenic sources have been shown to be a crucial problem throughout the Danube River Basin (Kavka and Poetsch, 2002). The river and its tributaries receive incompletely treated waste water e.g. from urban areas, animal farms and pasture leading to serious debasement of water quality. Thus detailed knowledge on the extent and origin of microbiological faecal pollution is crucial for watershed management activities in order to maintain safe waters according to their quality targets (Farnleitner et al., 2007). However, standard faecal indicators, such as *E. coli*, do not give information about the source of faecal input (Farnleitner et al., 2006). In this respect, the DNA-based quantitative microbial faecal source tracking (QMST) technique has shown great potential for specifically quantifying human faecal pollution and will thus allow more target oriented measures in the Danube catchment area (Reischer et al., 2008).

### 10.1.1 Aims and goals

Microbiological data were collected along the longitudinal stretch of the River Danube from the upper section (km 2600) to the delta (km 0), with the following goals:

–    Analysis of the extent and variation of faecal pollution by microbiological standard indicators along the longitudinal stretch of the River Danube, in side arms and main tributaries;

–    Identifying hot spots of faecal pollution of the Danube River Basin;

–    Assessment and description of the microbiological water quality in the sections of the river and tributaries and generation of an updated microbiological water quality map;

–    Comparison of data collected on-board with data from three reference laboratories;

–    Comparison of the data collected during the JDS2 with data from the JDS1 (2001);

–   Application of a DNA-based quantitative microbial source tracking (QMST) method for all tributaries and side arms to detect and quantify sources of human impact.


## 10.2    Methods

### 10.2.1 Sampling and storage
Water samples were collected from the ship in sterile 1 L Schott-flasks from all JDS2 sampling stations with a sampling rod at a water depth of approx. 20 to 30 cm. Samples were immediately processed in the on-board laboratory. Parallel samples for three reference laboratories were taken simultaneously and stored at 4°C until use.

### 10.2.2 *Escherichia coli*
*E. coli* was detected with Colilert 18 (IDEXX, Ludwigsburg, Germany), a most probable number technique, using three dilutions (100 ml, 1 ml, 0.01 ml). Samples were incubated at $35 \pm 0.5$°C for 18 - 22 hours and analysed in a UV-cabinet.

### 10.2.3 Intestinal Enterococci
Enterococci were analysed with MU/SF microtiter plates (BIORAD, Vienna, Austria), a most probable number technique, using four dilutions (200µl of 1:2, 1:20, 1:200; 1:2000 sample dilutions, ISO 7899-1, 1988). Samples were incubated at $43 \pm 1$°C for 32 - 40 hours and analysed in a UV-cabinet.

### 10.2.4 Classification system
To facilitate the interpretation of data, faecal indicators were classified by a system of five microbiological water quality categories according to Kavka et al. (2006) (Table 8).

The EU Bathing Water Quality Directive 2006 was taken into consideration in this scheme in that that faecal pollution levels of quality class I and II are below, but quality classes III, IV and V exceed, the faecal pollution threshold level for good bathing water quality.


**Table 8: Microbiologically based classification system of water quality according to faecal pollution.**

| Classification of faecal pollution | | Class | | | | |
|---|---|---|---|---|---|---|
| | | I | II | III | IV | V |
| Parameter | Faecal pollution | low | moderate | critical | strong | excessive |
| *Escherichia coli* EC | in 100ml water | ≤ 100 | > 100 - 1 000 | > 1 000 - 10 000 | > 10 000 - 100 000 | > 100 000 |
| Intestinal enterococci ENT | in 100ml water | ≤ 40 | > 40 - 400 | > 400 - 4 000 | > 4 000 - 40 000 | > 40 000 |
| Total coliforms TC | in 100ml water | ≤ 500 | > 500 -10 000 | > 10 000 - 100 000 | > 100 000 - 1 000 000 | >1 000 000 |

Indicator concentrations are given in colony forming units (CFU) or most probable numbers (MPN) per 100ml.


### 10.2.5  Quantitative microbial source tracking (QMST)
For QMST analysis, 22 tributary / side arm samples were taken from 19 official JDS sites and 3 additional tributaries 10 to 70 km upstream of their confluent site with the Danube (Drava, Tisza and

Sava). A volume between 125 and 250 ml was filtered through a 0.2µm polycarbonate filter. Filters were stored at -20°C during the rest of the cruise, transferred to the laboratory and stored at -80°C. DNA was extracted from the filters and BacH quantitative real-time PCR (qPCR) was performed on DNA extracts (Reischer et al., 2007). BacH is a Bacteroidetes (i.e. intestinal bacterial populations) based marker specific for human faecal origin and has previously been tested for tributary samples from the JDS1, as well as for a variety of samples from a karstic alpine region in Austria (Reischer et al., 2008).

## 10.3    Results

### 10.3.1 Variation of faecal indicator bacteria

*E. coli* numbers ranged over 6 orders of magnitude from undetectable to $1.5 \times 10^6$ MPN 100 ml$^{-1}$ (Figure 29). Critical to excessive faecal pollution (>1000 MPN 100ml$^{-1}$) was found in 33% of all sampling sites. The most polluted sites were identified as the tributaries or side arms of the Arges ($1.5 \times 10^6$), Russenski Lom ($2.4 \times 10^5$), Rackeve-Sorocksar Arm ($9.0 \times 10^4$) and Moson Danube ($9.0 \times 10^4$). 25% of all stations showed excellent water quality according to *E. coli* determination (low pollution <100 MPN 100 ml$^{-1}$).

Enterococci gave a similar picture of faecal pollution as *E. coli*. Values ranged from undetectable (detection limit: 38 MPN 100 ml$^{-1}$) to $3.5 \times 10^5$ MPN 100 ml$^{-1}$ (data not shown). 11% of all sites showed critical faecal pollution according to Table 8 (>400 MPN 100 ml$^{-1}$). The most polluted sites were Arges ($3.5 \times 10^5$) and Russenski Lom ($1.1 \times 10^5$). 50% of all stations showed excellent water quality (<40 MPN 100 ml$^{-1}$).
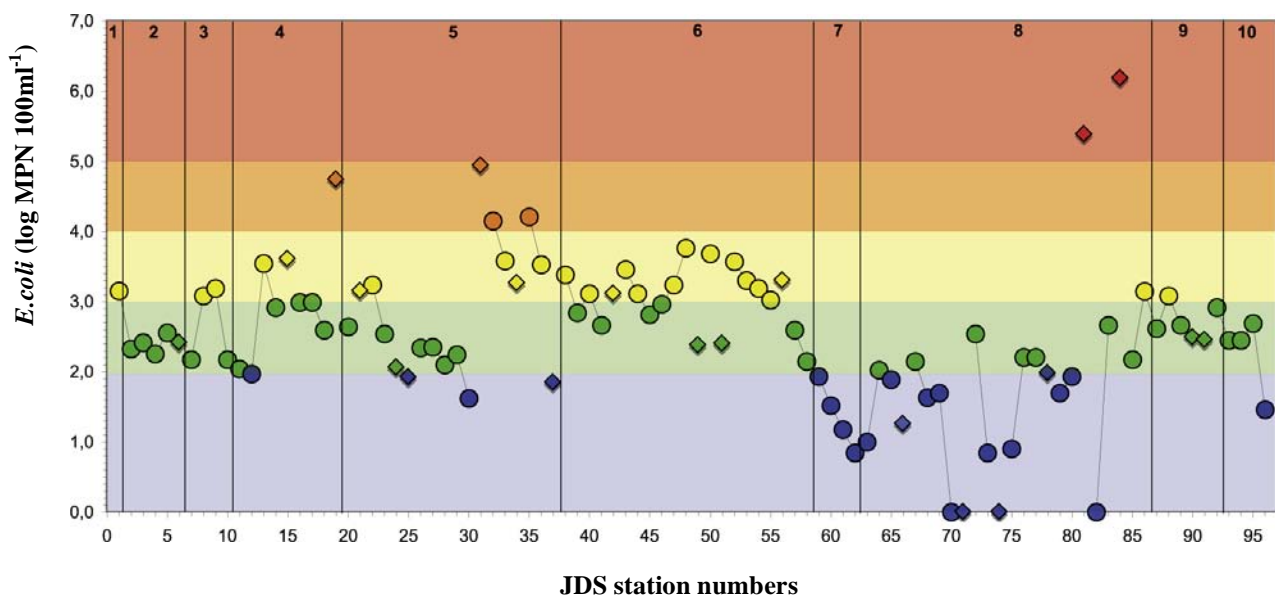


Figure 29: Variation of *E. coli* [log (MPN 100ml$^{-1}$)] along the longitudinal course of the Danube River (circles) and its main tributaries and side arms (diamonds)

Numbers designate the 10 different Danube Section Types, colours refer to the five water quality classes (Table 8).

## 10.3.2 Assessment of microbiological water quality for the sections of the Danube and tributaries

**Section Type 1 (Upper course of the Danube: rkm 2786 – rkm 2581):**
There was only a single sampling point within this uppermost stretch of the Danube, which showed moderate faecal pollution in the case of Enterococci (class II), while in case of *E. coli,* values were above the limit for class II. The influence of the high water situation during the first days of JDS2 may have led to elevated levels of faecal pollution in this stretch.

**Section Type 2 (Western Alpine foothills: rkm 2581- rkm 2225):**
The second section with five sampling stations was characterised by low to moderate faecal pollution (class I and II). The sample taken from the major tributary, the Inn, at the end of this stretch also showed moderate pollution values.

**Section Type 3 (Eastern Alpine foothills: rkm 2225 - rkm 2001):**
Within the third section, two stations downstream of Linz and Ybbs revealed critical levels of faecal pollution (class III) according to *E. coli* measurements. Enterococci showed moderate faecal pollution.

**Section Type 4 (Lower Alpine foothills: rkm 2001 - rkm 1789.5):**
A heterogenous situation was observed for section 4 of the Danube. Starting with low to moderate pollution levels (class I and II), the concentration of faecal indicators increased downstream of Vienna, where treated wastewater from the Viennese waste water treatment plant was received. Both parameters indicated critical faecal pollution (class III). After a decrease at Hainburg, the Morawa tributary again showed critical levels of faecal contamination. From Bratislava throughout the Gabcikovo channel, faecal pollution was low to moderate (class I and II). Moson Danube however, turned out to be one of the most polluted side arms/tributaries with strong faecal pollution levels (class IV), most probably caused by the city Györ.

**Section Type 5 (Hungarian Danube Bend: rkm 1789.5 - rkm 1497):**
This section was characterised by two completely contrasting stretches. Upstream of Budapest, mostly low to moderate levels of faecal pollution (class I and II) were observed. Also the tributaries, Hron and Ipoly, showed a good microbiological water quality. Downstream of the capital of Hungary, critical to strong faecal contamination (class III and IV) was faced, especially in the Rackeve-Sorocksar side arm. The Sio tributary showed only low faecal contamination.

**Section Type 6 (Pannonian Plain: rkm 1497 - rkm 1075):**
In section 6 most stations had critical levels of faecal contamination according to *E. coli* determination. Downstream of station 48 (upstream of Tisza) Danube faecal pollution levels started to decline gradually over approximately 100 km, the trend continuing in section 7. Enterococci were often below detection limit. Belgrade did not lead to a significant increase in faecal indicators, maybe because samples were taken only from the middle of the river. The tributaries Sava and Tisza were much less polluted than the Danube with excellent water quality (class I). Drava and Velika Morava exhibited critical pollution.

**Section Type 7 (Iron Gate: rkm 1075 - rkm 943):**
The whole section showed the best water quality of all sections. All *E. coli* and Enterococci values were within class I.

**Section Type 8 (Western Pontic Danube: rkm 943 - rkm 375.5):**
The longest Danube section was characterised by a mostly excellent to good water quality (class I and II). Low faecal contamination was also observed in the main tributaries of Timok, Iskar, Olt and Jantra. On the other hand, the most polluted side-arm (Russensky Lom) and tributary (Argus) showed with $2.4 \times 10^5$ and $1.5 \times 10^6$ MPN 100 ml$^{-1}$ *E. coli* levels which were 2500 – 3500 times higher compared to the corresponding upstream sampling points of the Danube.

**Section Type 9 (Eastern Wallachian Danube: rkm 375.5 - rkm 100):**

With the exception of one *E. coli* value (at sampling point 88), all stations within Danube section 9 were moderately polluted with *E. coli*, and Enterococci. Also the Siret and Prut tributaries showed good water quality (class II).

**Section Type 10 (Danube Delta: rkm 100 - rkm 7):**

In the Danube Delta, faecal pollution was low to moderate (class I and II) in all branches of the river.

### 10.3.3 Water quality map according to faecal pollution levels

Microbiological water quality classification based on *E. coli* and Enterococci faecal pollution levels was carried out using a "one-out-all-out-principle" as presented in Figure 30. However, it should be kept in mind, that a single measurement per site, as performed during the JDS2, is not sufficient to make a definitive and final assessment of compliance with the EU Bathing Water Directive 2006.
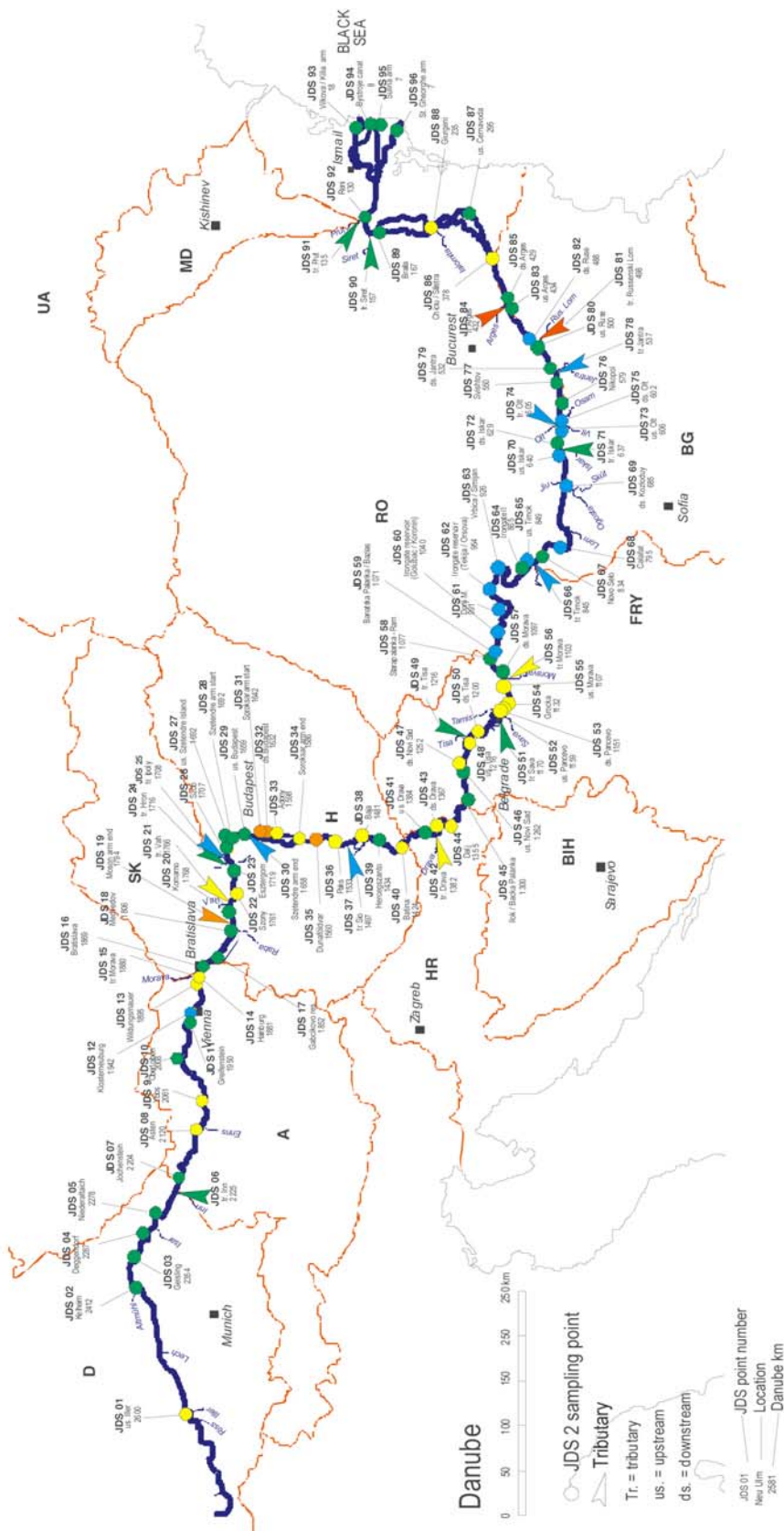
**Figure 30: Microbiological map of faecal pollution (classes of faecal pollution assessed by the concentrations of either E. coli or enterococci)**

The worse classification is represented in the map (one-out – all-out principle): for classification system see Table 8. Key: blue coloured symbols: class 1 - low faecal pollution; green: class II - moderate faecal pollution; yellow: class III - critical faecal pollution; orange: class IV - strong faecal pollution; red: class V - excessive faecal pollution.

### 10.3.4 Comparability and reliability of the chosen parameters

To evaluate the comparability of the results measured on-board the Argus during the JDS2, samples from selected locations were also simultaneously analysed by three reference laboratories. Data for the 19 parallel *E. coli* / Faecal Coliform measurements are shown in Figure 31.
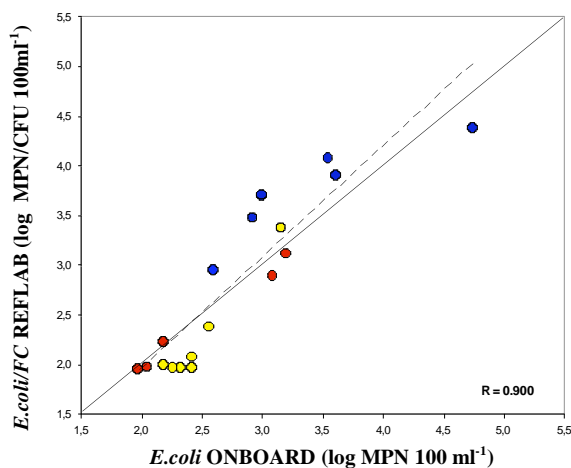


**Figure 31: Comparison of the *E. coli* determination on-board the Argus with the *E. coli* / Faecal Coliform (FC) results from the reference laboratories.**

**Yellow (FC):** Bayerisches Landesamt für Umwelt, Referat 77 - Biotestverfahren, mikrobielle Ökologie (Dir. Dipl. Biol. Willi Kopf, Dr. Margit Schade); **Red (*E. coli*):** Medizinische Universität Wien, Klinisches Institut für Hygiene und Medizinische Mikrobiologie – Wasserhygiene (Univ. Prof. Dr. Regina Sommer). **Blue (*E. coli*):** Water Research Institute Bratislava, Slovak National Water Reference Laboratory (RNDr. Miloslava Prokšova, RNDr. Jarmila Makovinská). The dashed line indicates the regression line and the solid line the 1: 1 relationship.

A highly significant correlation between the data was achieved (R = 0.90, p<0.001, n = 19) and the regression line is close to the 1: 1 relationship, indicating that the data obtained on board with the chosen methodology yielded highly comparable data of *E. coli* detection.

### 10.3.5 Comparison of the data with JDS1 (2001)

Due to financial constraints and a change of legal basis (the previous EU Bathing Water Quality Directive 76/160/EEC, 1976 needs to be replaced by the new EU Bathing Water Quality Directive 2006/7/EC by member states in 2008), in part, different parameters were measured and different methods applied for the three faecal indicators used in the JDS1.

*E. coli* values, measured with the Colilert method in 2007, were compared with the Faecal Coliform (FC) data from 2001 because it is well known that in surface waters, faecal coliforms and *E. coli* determined with membrane filtration methods show a near 1:1 correspondence (e.g. Kirschner et al., 2004). Correlation analysis between *E. coli* (2007) and FC ( ) showed a highly significant relationship (r = 0.82, p <0.001, n = 86) with lower values of *E. coli*.

Due to several limitations, a quantitative comparison between the two years is not admissible. It has to be kept in mind that the methods used during both cruises were different and there is only limited information about the correspondence between FC determination via membrane filtration and *E. coli* determination via the MPN Colilert approach. Differences in natural conditions and changing impacts can influence the monitoring results, especially when only one single measurement per sampling point is available. Moreover, it has to be taken into account that this comparison is based on a comparison between concentrations and not between total load data, which would be necessary for a definitive assessment. However, the high correspondence between the two years corroborated the localisation of hot spots of faecal pollution in the Danube River Basin (including its major tributaries).

### 10.3.6 Microbial Source Tracking

The human marker, BacH, was detectable in 82% (18 of 22) of the samples included in this investigation. The maker equivalent (ME) concentrations ranged from $1.4 \times 10^2$ ME l$^{-1}$ to $5.8 \times 10^7$ ME l$^{-1}$ with a median concentration of $8.9 \times 10^4$ ME l$^{-1}$. A remarkable correlation of the human specific BacH faecal marker to the *E. coli* concentrations in the tributaries was observed (Figure 32). The coefficient of determination in this regression was 0.80, signifying that 80% of the variation in *E. coli* levels could be explained by the variation in the QMST BacH faecal marker. In other words, a dominant part of the observed contamination with *E. coli* in the tributaries can be traced back to human faecal pollution origin. Thus, measures leading to a decrease of faecal pollution emissions from human sources (i.e. establishment or improvement of sewage treatment) will likely lead to an improvement of the water quality in the afflicted tributaries.
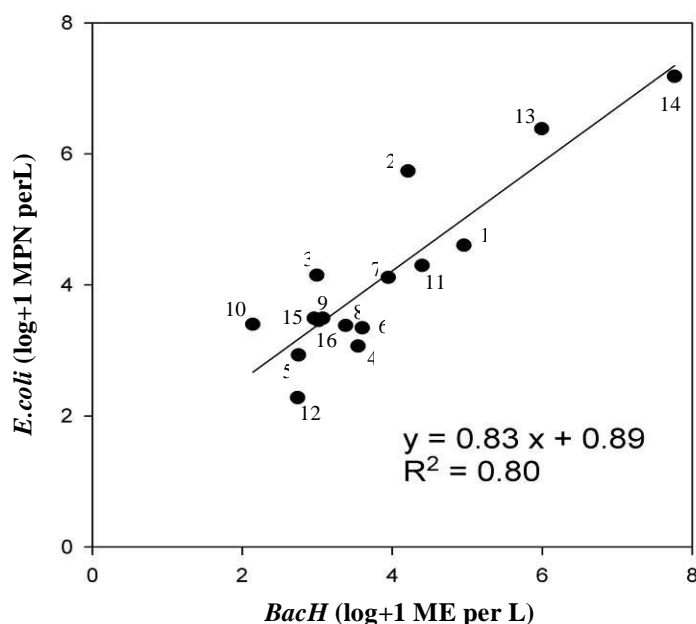


Figure 32: Regression analysis of human specific QMST BacH faecal markers and *E. coli* concentrations in Danube tributaries.

Tributaries: 1, JDS15-Morava; 2, JDS19-Moson Danube; 3, JDS21-Vah; 4, JDS24-Hron; 5, JDS25-Ipoly; 6, T2-Drava upstream; 7, JDS42-Drava; 8, JDS49-Tisza;9, T3-Tisza upstream; 10, JDS51-Sava; 11, JDS56-Velika Morava; 12, JDS66-Timok; 13, JDS81-Russenski Lom; 14, JDS84-Arges; 15, JDS90-Siret; 16, JDS91-Prut.

## 10.4    Conclusions

–    The longitudinal study of the entire course of the Danube River and its tributaries, by applying uniform methods in the on-board laboratory, allowed for a reliable quantitative estimation of the presence of faecal indicators and thus faecal pollution levels.

–    The use of the Colilert system for *E. coli* detection was the most appropriate and robust parameter to predict faecal contamination. Enterococci measured with the microtiter plate technique with four dilutions showed a detection limit which was too high.

–    The comparison of the on-board data with three national reference laboratories revealed a good correspondence with the exception of the methodically caused high detection limits of the enterococci microtiter plate technique.
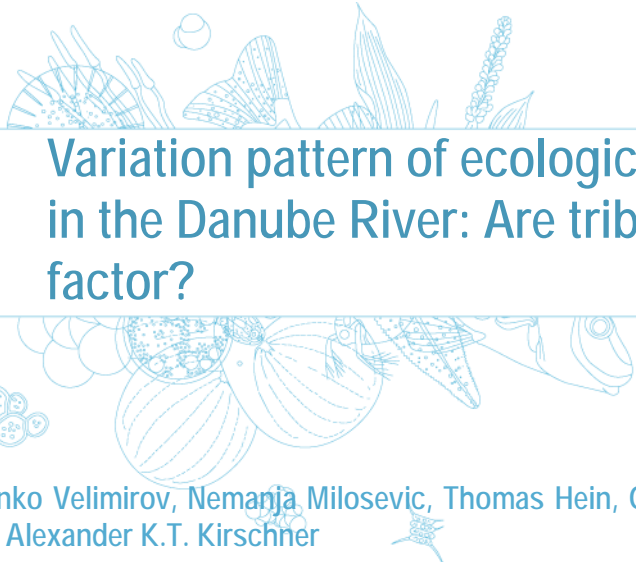
–   Through the application of a "5-level" classification system, which is harmonised with the limit values for good bathing water quality according to the EU Bathing Water Quality Directives (1976 and 2006), the assessment of microbiological water quality regarding faecal pollution became possible. However, it has to be kept in mind that a single determination at one sampling point is not enough to classify a sampling point as being "of sufficient" or "of poor" water quality with respect to the EU Bathing Water Directive.

–   31 JDS sampling sites (22 Danube samples and 9 tributaries/side arms) were classified as critically, strongly or excessively polluted. As sources of highest contamination, the Arges tributary and the Russenski Lom, Rackeve-Soroksar and Moson Danube side arms were identified as hot spots. Lower levels of faecal pollution were found in the uppermost stretches in Germany and Austria; between Estergom and Budapest in Hungary; in the Iron Gate reservoir in Serbia; the Western Pontic Danube up to Russenski Lom in Romania and the Danube Delta itself. Highest contamination levels in the Danube River itself were found in the stretch between Budapest and Belgrade.

–   The comparison of data with the preceding JDS1 in 2001 indicated a high correspondence of the faecal pollution of the Danube River in both years. Due to several limitations, a quantitative comparison of the data between the two years is not admissible. However, the high correspondence between the two years corroborated the localisation of hot spots of faecal pollution in the Danube River Basin including its major tributaries.

–   The application of the human faecal specific quantitative microbial source tracking BacH marker for all tributary and side arm samples demonstrated impressively the importance of human faecal contamination. BacH indicated that the dominant fraction of *E. coli* was derived from human sewage or excreta. Thus, implementation of new water treatment plants or respective improvements will likely have a great benefit for a further reduction of faecal pollution in these areas.

–   Methodical recommendations for a third Joint Danube Survey explicitly imply sampling in the middle and at both river banks to significantly improve the detection probability of faecal contamination from smaller sources. Moreover, a more sensitive protocol for Enterococci detection has to be considered. The integration of more reference laboratories from other countries should be enforced. For a better comparison the use of the same methodology for *E. coli* and Enterococci should be prerequisite in the next JDS, optimally paralleled by on-board measurements of these two parameters with standardised membrane filtration methods. Quantitative microbial source tracking (QMST) to quantify human faecal pollution has shown great potential and should be extended to other relevant faecal source specific markers (e.g. animal sources).

## 10.5   Acknowledgements

## 10.6    References

EU BATHING WATER QUALITY DIRECTIVE (1976): EU Bathing Water Quality Directive 76/160/EEC

EU BATHING WATER QUALITY DIRECTIVE (2006): EU Bathing Water Quality Directive 2006/7/EC

EU WATER FRAMEWORK DIRECTIVE (2000): EU-Water Framework Directive 2000/60/EC

FARNLEITNER A.H., REISCHER G.H., WINTER C., HEIN T., MACH R.L., KAVKA G.G. (2006): Progress in aquatic microbiology: introducing molecular biological approaches on a River Danube scale from Germany to the Black Sea. *Bulletin of the International Association for Danube Research 13-14: 8 - 12*

FARNLEITNER A.H., MACH R.L., REISCHER, G.H., KAVKA, G.G. (2007):  Mikrobiologisch – hygienische Risiken trotz Kläranlagen am Stand der Technik? *Wiener Mitteilungen 201: 209-242*.

KAVKA G.G. AND POETSCH E. (2002): Joint Danube Survey 2001 - Microbiological Results. In: *Joint Danube Survey – Technical Report of the International Commission for the Protection of the Danube River. 138 – 150 pp*

KAVKA G.G., KASIMIR G. D. AND FARNLEITNER A.H (2006): Microbiological water quality of the River Danube (km 2581 – km 15): Longitudinal variation of pollution as determined by standard parameters. In: *Proceedings 36th International Conference of IAD. Austrian Committee for Danube Research / IAD, Vienna. ISBN 13: 978-3-9500723-2-7. pp 415-421*.

KIRSCHNER A.K.T., ZECHMEISTER T.C., KAVKA G.G., BEIWL A., HERZIG A., MACH R.L., FARNLEITNER A.H. (2004): Integral strategy to evaluate the faecal indicator performance in bird influenced saline inland waters. *Appl. Environ. Microbiol. 70: 7396-7403*

REISCHER G.H., KASPER D.C., STEINBORN R., FARNLEITNER A.H., MACH R.L. (2007): A quantitative real-time PCR assay for the highly sensitive and specific detection of human faecal influence in spring water from a large alpine catchment area. *Letters in Applied Microbiology 44, 351–356*

REISCHER G.H., KAVKA G.G., KASPER D.C., WINTER C., MACH R.L., FARNLEITNER A.H. (2008): Applicability of DNA-based quantitative microbial source tracking (QMST) on a large scale in the Danube River and its important tributaries. *FUNDAMENTAL AND APPLIED LIMNOLOGY, SUPPLEMENT 162: 117-125*

# 11 Variation pattern of ecological bacterial parameters in the Danube River: Are tributaries a determining factor?

Branko Velimirov, Nemanja Milosevic, Thomas Hein, Gerhard G. Kavka, Andreas H. Farnleitner and Alexander K.T. Kirschner

## 11.1 Introduction

River networks fundamentally differ from most other ecosystems because (i) they are open systems with tight functional linkages to their adjacent ecosystems and (ii) they are nested systems with their physical and ecological structure and function changing over several spatial and temporal scales. Their hierarchical organisation and their tight link to adjacent terrestrial and subterranean ecosystems have stimulated the development of concepts such as the River Continuum Concept (RCC) and more recent concepts on river-floodplain functioning (Tockner et al., 2000; Thorp et al., 2006). Traditional perceptions during the last 25 years focus on the question of whether the longitudinal continuum (Vannote et al., 1980) or the lateral connectivity driven by the flood pulse (Junk, 1989; Bayley, 1991) control the organic matter supply. While recent concepts discuss the importance of physical discontinuities (Poole, 2002; Benda et al., 2004) and local processes in rivers (Thorp and Delong, 2002) as substantial contribution to understanding habitat structure and carbon cycling in lotic ecosystems.

Since the integration of the microbial loop in aquatic ecosystems (Pomeroy, 1974), it is recognised that a major part of the organic carbon from primary production is channelled through the bacterial compartment, usually quantified as rates of bacterial production and related estimations of required bacterial carbon demand. Only a small percentage of this energy is known to leave the microbial loop to fuel the macro-food chain and while this trend was often confirmed for lentic systems, we notice a lack of studies confirming or rejecting this observation for lotic systems in general and especially for large rivers. In this context it should be emphasized that the effect of the bacterial load of small rivers merging into larger ones was only sporadically investigated; although such information is of primordial importance for the understanding of the functioning of large rivers (Winter et al., 2007).

Large rivers, such as the River Danube, are known to experience the impact of an important number of tributaries. But while there is little doubt that these tributaries may influence the physico-chemical development of the main river, the magnitude of this influence is still a matter of debate. The Danube River Basin is characterised by about 40 major rivers merging into the main stream on its way to the Black Sea and reveals a great number of side arms and oxbow lakes, making the Danube River Basin a unique aquatic system in Europe.

Within the frame of the JDS 2007, we had the opportunity to sub-sample a representative number of stations between Regensburg and the Danube Delta and to determine basic microbial parameters (bacterial numbers - BN, biomass - BBM, morphotypes and bacterial secondary production - BSP), which were so far largely missing in biological records concerning the Danube River. We attempted to address a major issue with this investigation: to provide new and additional data to test and/or reinforce the validity of the River Continuum Concept by investigating whether continuous, linear changes are found for the recorded bacterial parameters or whether discontinuities and local processes control the bacterial performance. For this purpose special attention was given to bacterial input data from tributaries.

### 11.1.1 Aims and goals

Bacteriological data were collected during the JDS2 (2007) along the longitudinal stretch of the River Danube from the upper section (km 2600) to the delta (km 0), with the following aims:

– To monitor the above mentioned microbial-ecological parameters to obtain an overview of the microbial-ecological status of the Danube River and its tributaries;

– To analyse the obtained data in order to detect variation patterns in the Danube River, necessary for a better understanding of carbon cycling in the river;

– To compare bacterial parameters from the tributaries with those from the Danube River in order to assess the potential impact of the tributaries on the bacterial community in the main stream;

– To investigate whether detectable patterns of bacterial parameter values confirm the idea of the River Continuum Concept;

– To evaluate the obtained parameter values for their applicability within the established EU Water Framework Directive (WFD; 2000) as an additional tool to assess the ecological status of large rivers.

## 11.2    Methods

### 11.2.1 Collection of water samples

Water samples were collected from the ship in sterile 1 L Schott-flasks from all JDS 2 sampling stations with a sampling rod at a water depth of approx. 20 to 30 cm. Samples were immediately processed on-board. The water sample was divided into two fractions by filtration through 3 µm pore-size aluminium oxide filters.

### 11.2.2 Bacterial numbers

Bacterial numbers were estimated according to the acridine-orange direct count method (Hobbie et al., 1977), modified after Kirschner and Velimirov (1997). 10 ml of the water sample (total and 3µm filtrate) were fixed with 0.5 ml 37% formaldehyde and stored at 4°C in the dark. Bacterial cells were counted within 25 days and grouped in four morphotype classes (rods, cocci, vibrios and filaments) using an epifluorescence microscope.

### 11.2.3 Biometry

Bacteria were sized by an eyepiece micrometer. Cell volume estimations were derived from the assumption that bacteria have spherical or cylindrical shape with two hemispherical caps. At least 100 bacteria per sample were measured. Cellular carbon content expressed in fg C cell$^{-1}$ was calculated from estimated cell volumes (V; µm³) assuming the allometric relation $C = 120V^{0.72}$ after Norland (1993).

### 11.2.4 Leucine incorporation - bacterial secondary production

Bacterial production was determined for both untreated and 3µm filtered samples (4 samples, 2 blanks). $^3$H-Leucine was used as a tracer for incorporation into the bacterial protein pool, following the protocol of Eiler et al. (2003) and using the conversion factor of Simon and Azam (1989). Additionally, saturation experiments were conducted in order to verify that the administered radioisotope concentration was adequate to saturate the uptake by the respective bacterial population.

### 11.2.5 DOC analysis

Dissolved organic carbon (DOC) analysis was made with Phoenix 8000 TOC Analyser (JVC, Wiener Neustadt, Austria). Samples were frozen on-board ship at -20°C after filtration through 0.2µm filters and kept frozen until processing.

### 11.2.6 Statistical evaluations

All derived correlations were made by Pearson's *r*-test, where compared variables were quantitatively measurable. Parameters not fitting normal distribution were log10 transformed before correlation (**: highly significant).

### 11.2.7 Additional parameters

All additional parameters, namely chlorophyll-a, $O_2$, $NO_2$, $NO_3$, $NH_4$, $PO_4$, total phosphorus, temperature, total suspended solids, inorganic and organic suspended solids, were obtained from the JDS2 database.

## 11.3    Results

### 11.3.1 Bacterial parameter variations for JDS2 sampling points

The majority of the bacteria within the samples from both the longitudinal Danube sections and the tributaries were found in the fraction below 3 μm, which implies that the majority of the cells occur as free living cells. Table 9 shows that in tributaries some 67% of the bacteria are free cells, while in the Danube River this percentage is slightly higher, at 71%. A similar trend is noticed for the bacterial biomass. Bacterial production of the attached fraction, however, is only slightly below the values of the planktonic fraction.

Table 9: Mean percentage of free versus attached bacteria in the Danube River and its tributaries plus side arms (SA).

|                 | BN | | BBM | | BSP | |
|-----------------|--------------|------------|--------------|------------|--------------|------------|
|                 | % planktonic | % attached | % planktonic | % attached | % planktonic | % attached |
| All samples     | 70.1         | 29.9       | 62.5         | 37.5       | 55.8         | 44.2       |
| Tributaries + SA | 67.2        | 32.8       | 58.8         | 41.2       | 50.9         | 49.1       |
| Danube River    | 71.0         | 29.0       | 63.5         | 36.5       | 57.2         | 42.8       |

N = 21 for tributaries and side arms; n = 75 for the Danube River.

The comparison of the mean biomass/production ratios over all section types of free (1.19) and attached (0.88) bacteria indicated that attached bacteria have relatively higher secondary production per unit biomass compared to the free living fraction. BN and BBM values for attached bacteria correlated significantly with total suspended solids (TSS) and particulate inorganic matter (PIM). All three bacterial parameters of the attached fraction correlated significantly with particulate organic matter (POM) (BN r=0.326**, BBM r=0.498** and BSP r=0.336**), which was not observed for free living bacteria.

In Figure 33, it can be seen that most of the peaks for bacterial numbers, biomass and secondary production within the longitudinal Danube transect are derived from tributaries or side arms of the River Danube. Bacterial numbers below $2.0 \times 10^9$ cells $L^{-1}$ and biomass below 110 μg C $L^{-1}$ are characteristic for Danube River stretches, while tributaries display abundances and biomass ranging generally between $2.0 \times 10^9$ cells $L^{-1}$ and $7.3 \times 10^{10}$ cells $L^{-1}$ and 150 μg C $L^{-1}$ to 6140 μg C $L^{-1}$, respectively. However, there are exceptions like the River Inn, which imports amounts to $1.27 \times 10^9$ cells $L^{-1}$, corresponding to some 83 μg C $L^{-1}$. Low bacterial imports are also recorded for the Rackeve-Soroksar arm 2, the rivers Hron, Drava, Tisza, and Sava, where abundances ranged from $1.26 \times 10^9$ cells $L^{-1}$ to $1.8 \times 10^9$ cells $L^{-1}$, and biomass from 33 to 102 μg C $L^{-1}$, respectively.

Correspondingly, high values are noticed for the bacterial secondary production of tributaries with high biomasses and vice versa. The maximum bacterial production value of 14.8 μg C $L^{-1}$ $h^{-1}$ was recorded for the Arges River, which also had the highest bacterial abundance and biomass of all tributaries. For all other tributaries with high abundances and biomass, we recorded production values

between 1.5 and 5.44 µg C L$^{-1}$ h$^{-1}$. It is noteworthy that the Hron tributary which had both a low bacterial abundance and biomass manifested a relative production peak of 2.4 µg C L$^{-1}$ h$^{-1}$ while all other tributaries with low abundance and biomass values gave production values between 0.37 and 0.8 µg C L$^{-1}$ h$^{-1}$. Among the tributaries with production values below 1.0 µg C L$^{-1}$ h$^{-1}$ are also the rivers Timok, Olt, Jantra and Siret despite abundance and biomass values above $2.8 \times 10^6$ cells L$^{-1}$ and 110 µg C L$^{-1}$.

BN, BBM and BSP were highly correlated, despite the heterogeneity along the river (Pearson correlation BN/BBM: r = 0.963**, BN/BSP: r = 0.595**, BSP/BBM: r = 0.631**). Although the overall trend indicates high bacterial abundances, biomass and production values for the majority of the tributaries (but low values for the samples from the Danube River), it was surprising to notice that the water body of the main river remained largely unaffected by the tributaries input. This becomes even more apparent when calculating the specific bacterial biomass input for each tributary by considering the respective discharge rates of the tributaries and when comparing the calculated load of the tributary with the biomass load of the following downstream Danube sample station. This was true for tributaries with both large and small volume discharges (data not shown).

## 11.3.2 Bacterial parameter variations in the main stream

A different pattern of parameter variations becomes obvious when studying the values from Danube sampling points only. Observing the values of Danube samples downstream of the Inn, we can see that bacterial numbers (Figure 34) are constantly increasing. In contrast, the mean cell volume becomes smaller as the river approaches its mouth (Figure 35). As a consequence bacterial biomass reveals only a slight increase over the longitudinal transect. No such continuous trend was observed for bacterial production rates, and it was attempted to group them according to similar magnitude of rates, shown below in the chapter "Magnitude regions of BSP". Allocation of BSP values to the different section types gave no recognisable patterns or trends.

## 11.3.3 Assessment of bacteriological parameters for the sections of the Danube and tributaries

There are ten defined geomorphologic section types along the Danube River. Over those ten sections, abundances vary from 1.4 to $3.9 \times 10^9$ cells L$^{-1}$, biomass from 52 to 124 µg C L$^{-1}$ and BSP from 0.2 to 1.4µg C L$^{-1}$ h$^{-1}$. Yet it should be noted that minimum biomass does not correspond to minimum secondary productions or abundances, indicating a distinct heterogeneity of bacterial parameter between sections.

Section Type 1 (Upper course of the Danube, rkm 2786: – rkm 2581):
Average bacterial number ($2.7 \times 10^9$ cells L$^{-1}$) and biomass values (92.6 µg C L$^{-1}$) are high and secondary production is the highest recorded of all sections (1.41 µg C L$^{-1}$ h$^{-1}$). Since only one sample has been made over a stretch of 200 km within this section, it cannot be decided whether the monitored values are really representative for the bacterial compartment.

Section Type 2 (Western Alpine Foothills: rkm 2581- rkm 2225):
All parameters are extremely high with mean values of bacterial number ($3.05 \times 10^9$ cells L$^{-1}$), biomass (125 µg C L$^{-1}$) and secondary production (0.71 µg C L$^{-1}$ h$^{-1}$). The end of the section is marked by the input of the River Inn, with low biomass and BSP values, but with a large water volume, which doubles the water volume of the main stream.

Section Type 3 (Eastern Alpine Foothills: rkm 2225 - rkm 2001):
In this section a sudden decrease of all mean bacterial parameter values down to half of the previous section values is recorded (BN = $1.44 \times 10^9$ cells L$^{-1}$, BBM = 66.7 µg C L$^{-1}$ and BSP = 0.36 µg C L$^{-1}$ h$^{-1}$).

Section Type 4 (Lower Alpine Foothills: rkm 2001 - rkm 1789.5):
Bacterial parameters keep declining, showing their minimum at Bratislava i.e. Gabcikovo dam (BN= $1.29 \times 10^9$ cells L$^{-1}$, BBM= 52.28 µg C L$^{-1}$ and BSP= 0.33 µg C L$^{-1}$). However, the inputs of the Morava River and Moson arm are characterised by high bacterial numbers, biomass and production,

which are higher by a factor of 6, 8 and 9 respectively. Nonetheless it is remarkable that bacterial parameter values from Danube samples in the vicinity of Bratislava remain low.

### Section Type 5 (Hungarian Danube Bend: rkm 1789.5 - rkm 1497):

The beginning of section 5 is characterised by the presence of the tributaries Vah, Hron and Ipoly. These tributaries are characterised by low discharge volumes (less than 10% of the Danube) but relatively high bacterial parameter values as compared to the main stream. Differences by a factor of 2, 6 and 2.5 for BBM, BN and BSP respectively, are observed. The bacterial parameter values of the first part of this Danube section are lower than mean values from section 4. A contrasting situation is recorded for the samples in the close vicinity of Budapest and thereafter, where all bacterial and most nutrient values show a significant increase as compared to samples taken upstream of Budapest (BN= $1.63 \times 10^9$ cells L$^{-1}$, BBM = 60.3 µg C L$^{-1}$ and BSP= 0.58 µg C L$^{-1}$ h$^{-1}$). The influence of the River Sio is negligible.

### Section Type 6 (Pannonian Plain: rkm 1497 - rkm 1075):

Bacterial parameters in this section are slightly higher than in the previous section (BN= $1.82 \times 10^9$ cellsl$^{-1}$, BBM = 62.68 µg C L$^{-1}$ and BSP = 0.62 µg C L$^{-1}$ h$^{-1}$) with chlorophyll-a showing its maximum after Novi Sad. Due to three big tributaries (Drava, Tisa and Sava) the water volume of the Danube is doubled.

### Section Type 7 (Iron Gate Danube: rkm 1075 - rkm 943):

Bacterial number and biomass keep increasing (BN = $1.96 \times 10^9$ cells L$^{-1}$, BBM = 70.72 µg C L$^{-1}$), whereas bacterial secondary production reveals a sudden decrease comparable to Section Type 3 (BSP = 0.22 µg C L$^{-1}$ h$^{-1}$), again a stretch of dammed water (Iron Gate reservoir).

### Section Type 8 (Western Pontic Danube: rkm 943 - rkm 375.5):

After a stretch of slow water current in the previous section, bacterial numbers remain almost at the same level as in section 7 ($1.99 \times 10^9$ cells L$^{-1}$) but biomass decreases (66.54 µg C L$^{-1}$) together with the average cell volume (8.5% smaller than in Section Type 7). Bacterial secondary production reaches its minimum in the Danube River (BSP = 0.21 µg C L$^{-1}$ h$^{-1}$). This comparably long section (570 km) is characterised by six important tributaries: Timok, Iskar, Olt, Jantra, Russenski Lom and Arges. The first four rivers show low bacterial parameter values, nutrient concentrations and flow rates. Russenski Lom and the Arges River had low discharge volumes, yet bacterial parameter values and nutrient concentrations were conspicuously high, due to city wastewaters from Russe and Bucharest, respectively.

### Section Type 9 (Eastern Wallachian Danube: rkm 375.5 - rkm 100):

Bacterial secondary production values from this section (BSP = 0.31 µg C L$^{-1}$ h$^{-1}$) can be compared with those from section 4, but abundance and biomass values (BN = $2.40 \times 10^9$ cells L$^{-1}$, BBM = 76.89 µg C L$^{-1}$) show a continuous increase along the river, indicating uncoupling between BSP and BBM. Rivers Siret and Prut are the last tributaries before the delta. Their bacterial parameters values are moderate but still higher than the Danube values.

### Section Type 10 (Danube Delta: rkm 100 - rkm 7):

The Danube Delta is characterised by extreme concentrations of particulate organic matter as well as high bacterial abundance and biomass (BN = $3.98 \times 10^9$ cells L$^{-1}$, BBM = 122.66 µg C L$^{-1}$), especially in the attached bacterial fraction. Bacterial secondary production remains at the same level or even slightly lower than in the previous section (BSP =0.25 µg C L$^{-1}$ h$^{-1}$).
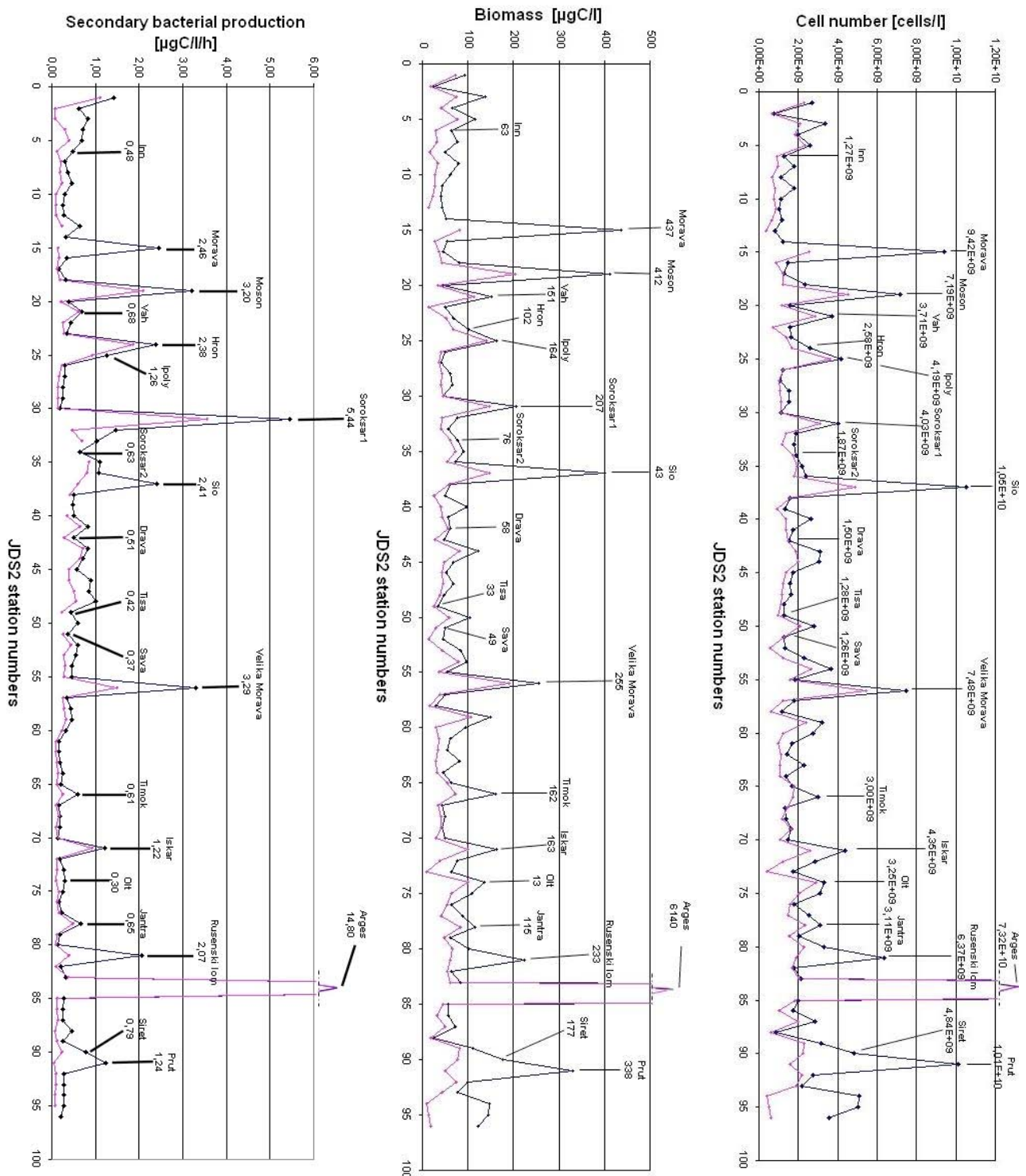
Figure 33: Bacterial parameters from water samples of 75 stations along the Danube River and
21 tributaries and side arms: a) Bacterial numbers. b) Bacterial biomass and
c) Bacterial secondary production

Key: untreated samples (shown by black lines); 3µm filtered samples (purple lines).
Peaks of tributaries and side arms are labelled with specific names and obtained values.

$$y = -4,35E+05x + 2,51E+09$$
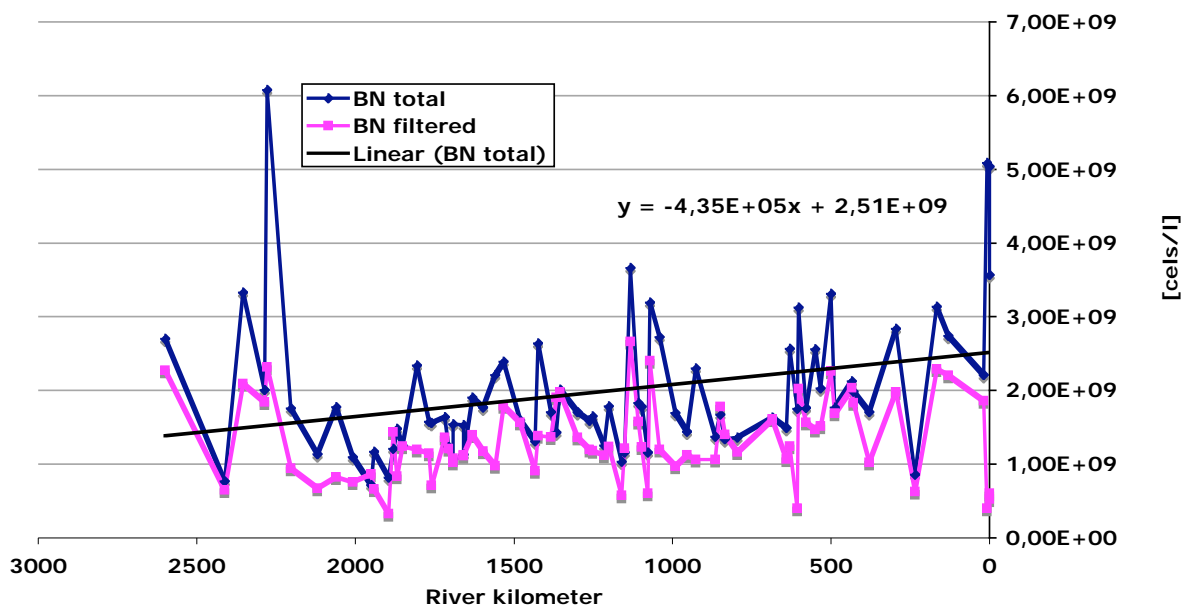
Figure 34: Bacterial numbers in water samples of the Danube River without tributaries



$$y = 0,00003x + 0,15662$$
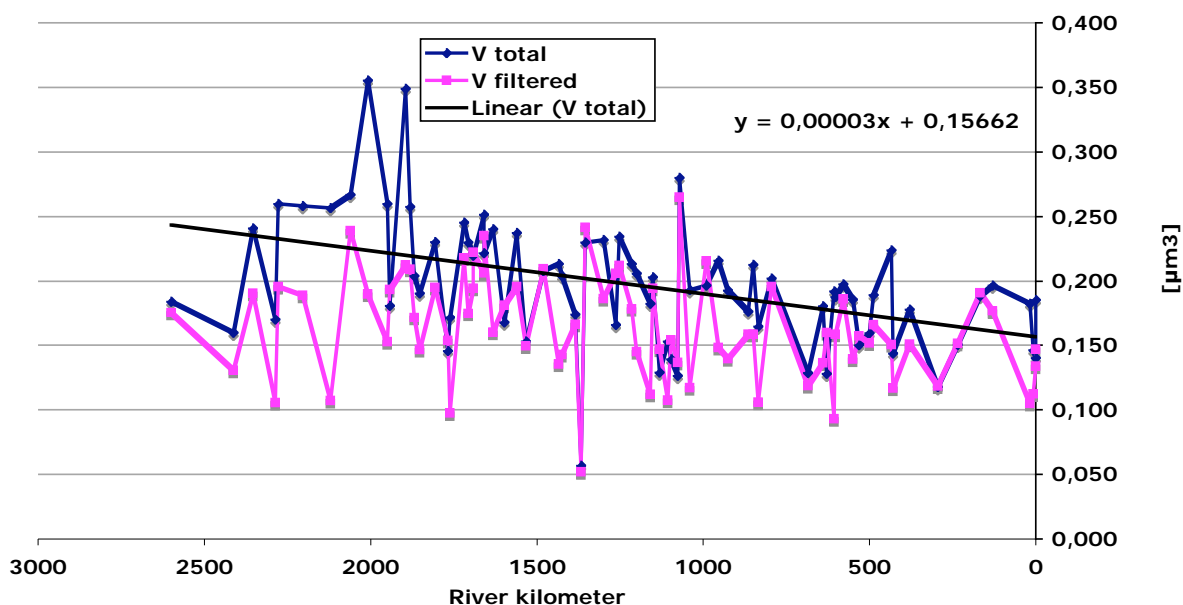
Figure 35: Mean bacterial volume in water samples of the Danube River without tributaries

## 11.3.4 Magnitude regions of BSP

On the basis of regions with comparable bacterial production, we distinguished four magnitude regions of mean bacterial secondary production (Table 10). JDS sampling point 1 was considered to be unrepresentative for the Danube River (see above) and was therefore not included in the estimations of means to characterise the regions. The first magnitude region corresponds to the Upper Danube with a mean BSP of 0.71 μg C L$^{-1}$ h$^{-1}$. This is followed by the region between the River Inn and the sampling point upstream of Budapest (the Alpine foothills), with an average BSP of 0.33 μg C L$^{-1}$ h$^{-1}$. The third region corresponds to the Pannonian Lowland, between the city of Budapest and the Iron Gate (again a

mean BSP of 0.71 µg C L$^{-1}$h$^{-1}$). The fourth magnitude region extends from the Iron Gate downstream to the Danube Delta with a mean BSP of 0.23 µg C L$^{-1}$h$^{-1}$.

### Table 10: Magnitude regions of BSP along the Danube without tributaries.

| Magnitude regions of BSP | JDS points | Average µg C L$^{-1}$ h$^{-1}$ | Standard deviation | Minimum | Maximum | t-test |
|---|---|---|---|---|---|---|
| Upper Danube | 2 - 5 | 0.71 | 0.09 | 0.61 | 0.83 | - |
| Alpine foothills | 7 - 31 | 0.33 | 0.10 | 0.15 | 0.63 | ** |
| Pannonian Lowland | 32 - 60 | 0.71 | 0.28 | 0.35 | 1.46 | ** |
| Lower Danube | 61 - 96 | 0.23 | 0.07 | 0.14 | 0.47 | ** |

** indicates highly significant differences in mean BSP values between consecutive magnitude regions.

## 11.4    Conclusions

– The longitudinal study of the entire course of the Danube River and its tributaries revealed conspicuous differences between bacterial parameter values from the Danube River water samples and the merging tributaries.

– Bacterial numbers, biomass and secondary production values from tributaries were always higher than in the Danube River. Only in the case of tributaries with important discharge volumes, such as the rivers Inn and Drava, did bacterial parameter values remain below those of the Danube River.

– Load calculations for tributaries allowed estimation of expected parameter values for sampling stations downstream of the tributaries. However, in the majority of the cases, the monitored bacterial parameter values were well below the expected values.

– Hence the Danube River remains, as suggested by the obtained data, rather unaffected by the tributaries input. This is a similar conclusion to the earlier findings of Winter et al (2007). As measured parameter values were usually lower than the calculated expected values after the input of tributary waters, it may be assumed that beside the impact of bacterivory by microzooplankton, both the filter feeding macrozoobenthos and cell retention by river bank sediments are responsible for the observed decrease in bacterial biomass. Thus, the Danube River may function as a purification system for bacterial loads imported from tributaries. This is true for the sampling stations in the middle of the river, but the trend could not be extended for the river bancs since they were not included in the sampling strategy. Nonetheless, one should also consider the possibility that the water of the tributaries flows over long stretch distances in the vicinity of river banks and mixing with mid-river water is only achieved at distances of some 30 km or more after the tributary mouths. Testing of this hypothesis is definitely required.

– The evolution of the bacterial parameters along the longitudinal transect reveals clear trends for an increase in bacterial numbers and biomass and a marked decrease in bacterial volume. These observations are in agreement with the River Continuum Concept.

– Bacterial production does not follow a recognisable trend along the longitudinal transect and is therefore not in agreement with the River Continuum Concept. This parameter seems to be more prone to the influence of tributaries and the specific environmental conditions of regions encompassing several section types.

– Free living bacteria are more abundant than attached bacteria in the Danube River and its tributaries but attached bacteria are more productive than free living bacteria, as indicated by mean BBM/BSP ratios.

– Although data on total phosphorus and planktonic primary production, required for the determination of the trophic situation of the various section types are now available, it is not yet

possible to decide whether the bacterial parameters can be used for the assessment of the ecological status in the various section types. Comparative analysis with macrozoobenthos data is definitely required in order to achieve final conclusions. However, the evolution of most parameters along the longitudinal transect is encouraging the planned construction of a new tool for the determination of ecological status classes via bacterial parameters. First attempts in constructing such a tool indicate the potential utility of microbial parameters (possibly within the established EU Water Framework Directive (2000)) to assess the ecological status of large rivers.

## 11.5 Acknowledgements

## 11.6 References

BAYLEY, P. B. (1991): The flood pulse advantage and the restoration of river floodplain systems. Regulated Rivers: Research and Management 6: 75-86.

BENDA, L., POFF, L.R., MILLER, D., DUNNE, T., REEVES, G., POLLOCK, M. & PESS G. (2004): Network dynamics hypothesis: spatial and temporal organisation of physical heterogeneity in rivers. - BioScience 54: 413-427.

EILER A., FARNLEITNER, A.H., ZECHMEISTER, T.C., HERZIG, A., HURBAN C., WESNER, W., KRACHLER, R., VELIMIROV, B. & KIRSCHNER, A.K.T. (2003): Factors controlling extremely productive heterotrophic bacterial communities in shallow soda pools – Microbial Ecology 46, 43-54.

HOBBIE, J.E., R.J. DAYLEY & S. JASPER (1977): Use of nucleopore filters for counting bacteria by epifluorescence microscopy. Appl. Environ. Microbiol. 33: 1225-1228.

JUNK, W. J. & WANTZEN, K. M. (2004): The Flood Pulse Concept: new aspects, approaches, and applications – an update - Proceedings of the second international symposium on the management of large rivers for fisheries. R. L. Welcomme and T. Petr. Bangkok, FAO. 2: 117-149.

KIRSCHNER, A.K.T., VELIMIROV, B. (1997): A seasonal study of bacterial community succession in a temperate backwater system, indicated by variation in morphotype numbers, biomass and secondary production.-Microb Ecol 34: 27-38.

NORLAND, S. (1993): The relationship between biomass and volume of bacteria, p. 303-307 in P.F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole [eds.], Handbook of methods in aquatic microbial ecology, Lewis Publ.

POMEROY L.R. (1974): The ocean's food web, a changing paradigm - BioScience 24, 499 504.

POOLE, G.C. (2002): Fluvial landscape ecology: addressing uniqueness within the river discontinuum. Freshwater Biology 47: 641-660.

SIMON M. & AZAM, F. (1989): Protein content and protein synthesis rates of planktonic marine bacteria. Marine Ecology Progress Series , 51 , 201-213.

TOCKNER, K., MALARD, F. & WARD, J.V. (2000): An extension of the Flood Pulse Concept. Hydrological Processes 14: 2861-2883.

THORP, J. H. & DELONG, A. D. (2002): Dominance of autochthonous autotrophic carbon in food webs of heterotrophic rivers. Oikos 96: 543-550.

THORP, J.H., THOMS, M.C. & DELONG, M.D. (2006): The riverine ecosystem synthesis: Biocomplexity in river networks across space and time. River Res. Applic. 22: 123-147.

VANNOTE, R.L., MISHALL, G.W., CUMMINS, K.W., SEDELL, J.R. AND CUSHING, C.E. (1980): The River Continuum Concept. Can. J. Fish. Aquat. Sci. 37, 130ff.

WINTER, C., HEIN, T., KAVKA, G., MACH R.L. AND FARNLEITNER, A.H. (2007): Longitudinal changes in the bacterial community composition of the Danube River: A whole river approach. *Appl. Environ. Microbiol.* 73: 421-431.

# 12   Ecotoxicity

Lívia Tóthová

## 12.1   Introduction

Water pollution can cross national borders. This necessitates harmonised regulations and standards on a European level. Large numbers of organic and inorganic chemicals are emitted into the environment from anthropogenic sources. Many of them are deposited in the sediments. The significant role that sediments play in aquatic ecosystems is well known. They serve as both a sink and a source of organic and inorganic materials. Sediment contamination can have many detrimental effects on an ecosystem, some of which are evident and others more invisible or unknown. For example, benthic invertebrate communities can be totally lost or converted from sensitive to pollution-tolerant species. (Hoffman et al, 2003). For most determinands, biodegradation is the dominant transformation pathway to remove their environmental concentration significantly. However some degradation products may have significantly higher toxic effects than basic contaminants. Many factors such as contaminant properties, temperature, pH, microbial population density etc. can influence the rate and extent of toxicity. This is especially important for the flowing water systems (rivers, streams, creeks).

The ecotoxicological investigations of the JDS2 focused on the assessment of the toxic effects of Danube sediments on selected species: *Lemna minor*, *Desmodesmus subspicatus* and *Vibrio fischeri*.

## 12.2   Methods

Pore water was recovered from 62 deep frozen raw sediment samples. After the sediment unfreezing, the visible animals were removed and a centrifugation of 15 minutes by 6000 rpm was used to obtain up to 400 ml of pore water. Pore water samples were adjusted to the test temperature and then immediately used for the toxicity tests.

A battery of three tests was used for this study - two tests of producent organisms (7-day growth inhibition test with *Lemna minor* and a 72-hour algal growth inhibition test with *Desmodesmus subspicatus*) and one test of destruent organism (15 and 30 minute inhibition of the light emission of *Vibrio fischeri*). Between two to three replicates were used in each test. Determination of the limit toxic effect of pore water to *Lemna minor* via growth inhibition was based on EN ISO 20 079. Frond number and frond area were measured. Data were evaluated and the percentage of inhibition was calculated. The algal growth limit inhibition test followed EN ISO 8692 with *Desmodesmus subspicatus*. The percentage of inhibition of growth rate was calculated. Both tests (with *Lemna minor* and *Desmodesmus subspicatus*) fulfilled the validity criteria of the tests (e.g. specific growth rate). The inhibition of luminescence of *Vibrio fischeri* was evaluated within the test procedure following the International Standard ISO 11348-2 using liquid-dried bacteria. The percentage of inhibition of the light emission by cultures of *Vibrio fischeri* was calculated for a contact time of 15 and 30 minutes. EC50 was calculated for selected samples of pore water.

Other tests with *Vibrio fischeri* were performed using dry sediment fraction 63 μm in RECETOX Ecotoxicology Laboratories. A whole sediment (solid phase / suspension) toxicity test with FLASH *Vibrio fisheri* bioluminiscence test was done. The test was performed with modifications according to Lappalainen (1999).

## 12.3    Results

The toxicity evaluation of the pore water was based on the interaction of the sample and organisms. The sensitivity of different organisms was detected with biotest batteries. Percentage of toxic effect was calculated. Generally, only a response higher than 10% indicated a positive toxic effect. In some, a test stimulation effect was observed i.e. the scored parameter showed higher growth than in the control samples. In such cases, a positive toxic effect is indicated when stimulation is more than 75%. Stimulation effect is marked with negative number.

### 12.3.1 Species sensitivity and toxic effects evaluation

The samples were taken from 38 sites (right and left side). More than 420 toxicity tests were carried out. Selected results from the toxicity test variables between *Vibrio fischeri* and *Lemna minor* are displayed in Figure 36.
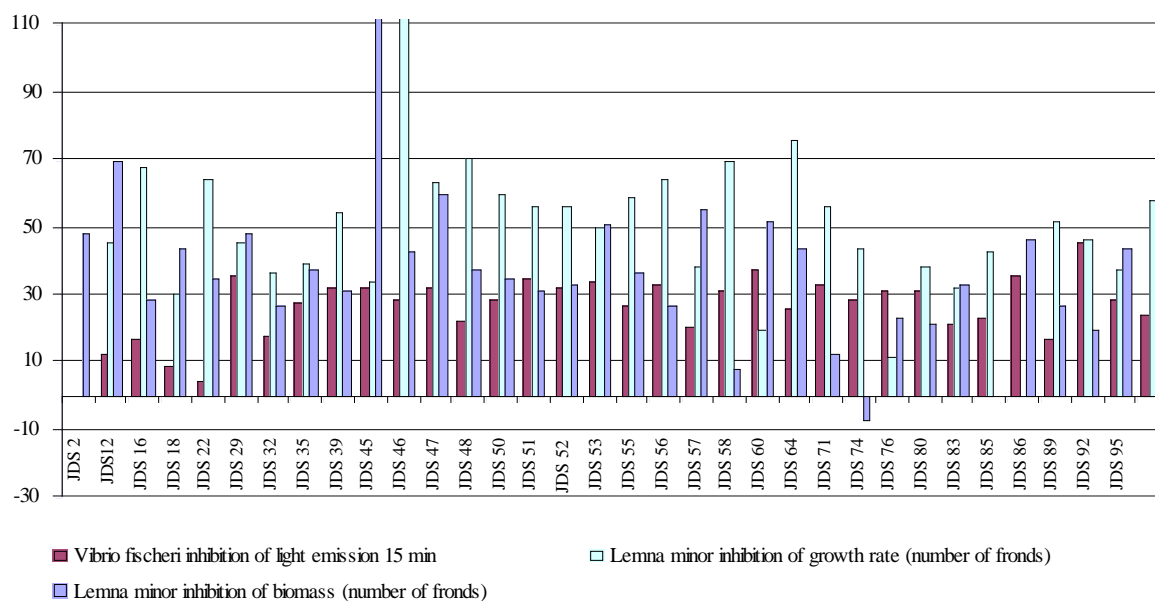


**Figure 36: Toxic effect of pore water to *Lemna minor* and *Vibrio fischeri* in longitudinal profile of the Danube (left side)**

The species *Desmodesmus subspicatus* had the lowest sensitivity. Only one sample in the Middle and Lower Danube showed a toxic effect - 16.9 % of growth rate inhibition. On the other hand, *Lemna minor* was the most sensitive species within this study. Pore water acquisition, presence of benthic organisms and test duration are probably the key factors influencing performance. The toxic effects ranged from 11.6 to 120.1 % of growth inhibition for *Lemna* and from 10,7 to 63 % of luminescence inhibition for *Vibrio fischeri*. The majority of the results were obtained in a limit test. Only four samples influenced inhibition of luminescence at more than by 50%. EC50 could be calculated for only four samples for *Vibrio fischeri* (JDS 16, JDS 18, JDS 45 and JDS 64 - all right banks).

Generally, JDS samples showed lower toxic effect to tested species than samples from the AquaTerra survey in 2004. An example of the toxic response of *Vibrio fischeri* in the 15 minute test of luminescence inhibition is shown on Figure 37. However, no significant correlation was found. AquaTerra pore water samples from left bank sediments indicated stronger toxic effects than samples from the right banks. This tendency was not confirmed by JDS2 samples. A possible reason for the lower toxic effects observed during JDS2 could be a different procedure of pore water collection but also less organic substances identified.
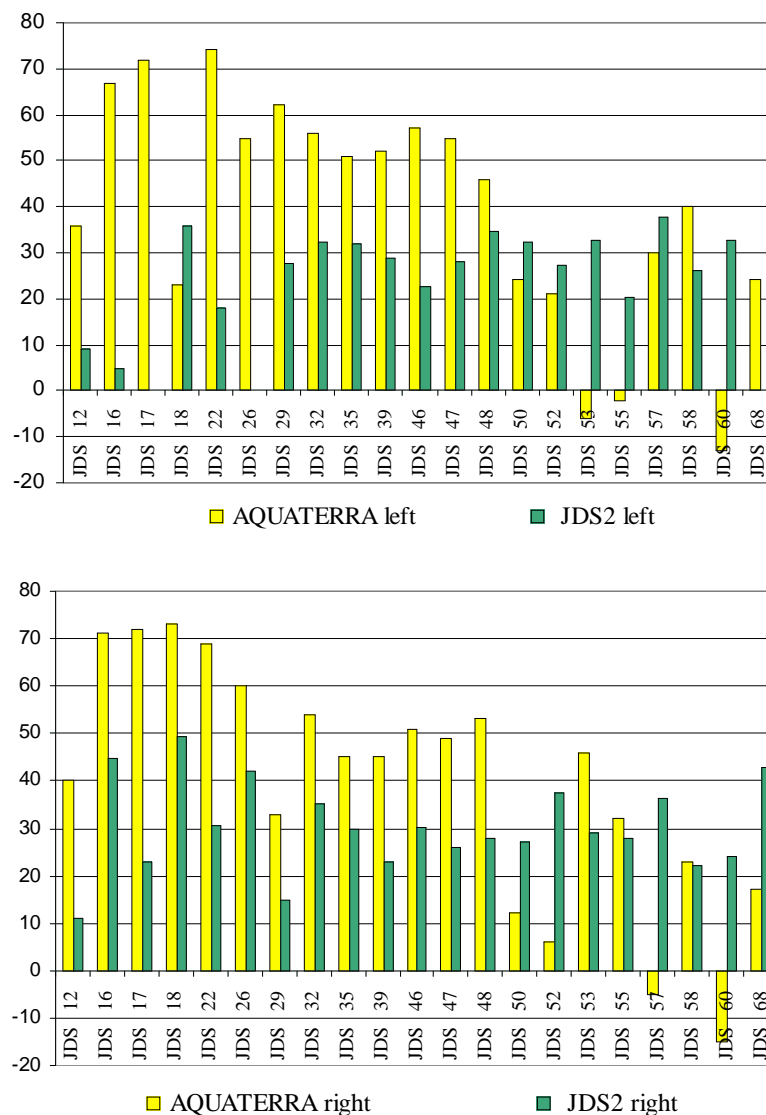
Figure 37: Toxic effect of pore water to *Vibrio fischeri* in longitudinal profiles of the Danube

## 12.3.2 Species sensitivity and pollution

Selected organic substances and heavy metals were measured in dry sediment fractions. However, their concentrations in sediment pore water were not analysed. Therefore sensitivity of species and occurrence of compounds in sediments may be compared only indirectly. Based on statistical analyses, no significant correlation between selected organic compounds and species was found.

## 12.3.3 Different sediment sample procedure and toxicity

Toxic effects to *Vibrio fischeri* using different sediment processing procedures were evaluated. 15 samples were analysed using different test protocols: pore water, whole 63 μm sediment fraction and eluates from dry sediment fraction 63 μm. The effect of the contact time was investigated as well. Results shown in Figure 38 are comparable, however without significant statistical confidence.
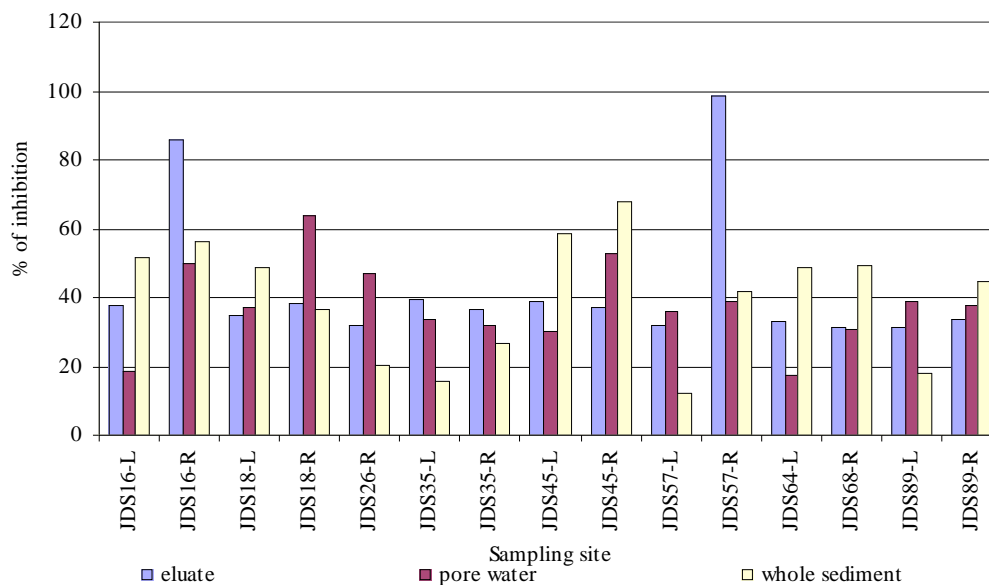
Figure 38: Inhibition of light emission of dry sediment fraction eluate (30 minute exposition) versus pore water (30 minute exposition) and whole sediment (5 minute exposition)

## 12.4    Conclusions

Sediment samples were collected in the period 13.08.2007 - 26.09.2007. Selected toxicity tests were used for pore water and dry sediment fraction 63 μm testing. Based on the results, the following conclusions are formulated:

– More than 420 toxicity tests were done using pore water samples. Sensitivity of tested species (*Lemna minor, Desmodesmus subspicatus and Vibrio fischeri*) differed.

– *Lemna minor* was the most sensitive species.

– Three different toxicity tests with *Vibrio fischeri* are comparable, however without significant statistical confidence.

– EC 50 has been calculated only for four samples using inhibition of luminescence of the *Vibrio fischeri.*

– Danube sediments in longitudinal profile demonstrate a non toxic or slightly toxic effect to the tested organisms based on selected sampling sites.

– JDS2 sediments show a slightly lower toxic effect to *Vibrio fischeri* and higher *Lemna minor* response with comparison to AquaTerra results.

– Analysis of pore water and sediment focussing on heavy metals or organic contaminants is required for a detailed toxicological study.

– Pore water prepared immediately after sediment sampling seems more specific than when prepared from whole frozen sediment because of the presence of benthic organisms. On the other hand pore water prepared from respective sediment fraction prevents presence of undesirable structures.

## 12.5    References

ISO 11348-2 (2007):   Water quality - Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) - Part 2: Method using liquid-dried bacteria.

ISO 11348-3 (1998): Water quality determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent Bacteria) test.

EN ISO 20079 (2005): Water quality - Determination of the toxic effect of water constituents and waste water on duckweed (*Lemna minor*) - Duckweed growth inhibition test.

EN ISO 8692 (2004): Water quality - Freshwater algal growth inhibition test with unicellular green algae.

LAPPALAINEN, J., JUVONEN, R., VAAJASAARI, K., KARP, M. (1999): A new Flash metod for the toxicity of solid and Colorad samples. Chemosphere 5: 1069-1083.

SLOBODNÍK J., HAMCHEVICHI C., LIŠKA I., SHEARMAN A., CSÁNYI B., MAKOVINSKÁ J., PAUNOVIC M., TÓTHOVÁ L., STAHLSCHMIDT-ALLNER P. AND ALLNER B. (2005): Final report on sampling, chemical analysis and ecotoxicological studies - Deliverable BASIN 5.11, October 2005, AquaTerra, EU 6th FP, Integrated Project no. 505428.

# 13 General physico-chemical quality elements (thermal, oxygenation, salinity and acidification conditions)

Carmen Hamchevici and Mary Craciun

## 13.1 Introduction

According to the EU Water Framework Directive (WFD), surface waters should be classified by their ecological and chemical status, which is characterised by different quality elements (physico-chemical, hydromorphological and biological). Implementation of the WFD aims to achieve *good status* in water bodies by 2015 (Council Directive 2000/60/EC of 23 October 2000 establishing a framework for Community action in the field of water policy, 2000; Pollard and Huxham, 1998). One of the groups of quality elements listed in Annex V, Section 1.1.1 (Rivers), of the WFD covers *"chemical and physico-chemical elements supporting the biological elements."* The quality elements under this "general" category include temperature, dissolved oxygen, pH value, conductivity, alkalinity and nutrients (nitrogen (N), phosphorus (P) and silicon (Si)).

This chapter summarises the results of on-board measurements for this group of quality elements (with the exception of nutrients which are discussed in the next chapter).

## 13.2 Methods

### 13.2.1 Sample collection

Using a motorboat, water samples to be analysed on-board were collected directly from the river simultaneously with biological samples.  Since the analysis of these quality elements took place immediately after the sampling, there was no need for preservation and storage of the samples. The longitudinal surveys on the major tributaries were carried out by national experts from the riparian countries (in a given tributary). They also carried out *in-situ* measurements and the results were recorded in the sampling protocols for each sampling location.

### 13.2.2 Analysis of samples

Standard Operational Procedures (SOPs), based on international standardised methods, were used for *in-situ* measurements and on-board analyses, using a portable multiple-probe WTW instrument (temperature, dissolved oxygen, pH value and conductivity) and digital titration system for alkalinity.

### 13.2.3 Analytical quality control

Calibration of the instrument was done according to the manufacturer's instruction manual with the required frequency. Calibrations were recorded in the instrument's logbook.

## 13.3 Results and discussion

Variation ranges for temperature, dissolved oxygen, pH value, conductivity and alkalinity are presented in Table 11.

### Table 11: Variation ranges for general physico - chemical quality elements in the Danube River and its tributaries during the JDS2

| Quality element | Unit | Danube River | | Tributaries | | Longitudinal surveys on the major tributaries | |
|---|---|---|---|---|---|---|---|
| | | Minimum value | Maximum value | Minimum value | Maximum value | Minimum value | Maximum value |
| Temperature | 0C | 17.4 | 24.9 | 11.0 | 26.6 | - | - |
| Dissolved oxygen, (DO) | mg/l | 3.49 | 10.1 | 1.67 | 11.02 | 0.36 | 10.4 |
| DO saturation | % | 39.3 | 114.9 | 17.4 | 127.9 | 3.5 | 112.7 |
| pH value | - | 7.32 | 8.10 | 7.26 | 8.27 | 7.20 | 8.59 |
| Conductivity | µS/cm | 346 | 577 | 280 | 1073 | 136 | 1073 |
| Alkalinity | mmol/l | 2.3 | 4.4 | 1.4 | 7.6 | 0.8 | 7.4 |

Variations in the four major general water quality indicators i.e. temperature, dissolved oxygen, pH value and conductivity, provide information on physical and biochemical processes that control the production and decomposition of organic matter in the water body. These processes also influence the fate and pathways of both organic (primarily bio-resistant) and inorganic priority substances in the aquatic ecosystem. Therefore, the longitudinal profiles of these water quality indicators along the Danube, and the comparison between the results of the JDS1 and JDS2, provide important information for the interpretation of results for several pollution indicators, including the priority substances. Furthermore, the spatial and time variations in these indicators are important for assessing *ecological status*, according to the WFD.

The results of these four indicators are presented together with relevant results obtained during the JDS1 for a better characterisation of the general water quality conditions during the two surveys.

### 13.3.1 Temperature

Variations in water temperature during the JDS1 and JDS2 showed similarities along the Upper Danube reach (see Figure 39).  Higher fluctuations occurred in the Middle Danube reach and particularly downstream of the Iron Gate during the JDS2. This was the result of changes in weather conditions, as well as the increase in water discharge. In general, the temperature varied between 19 to 23 $^{o}$C, typical for the time of year for both surveys (August – September).
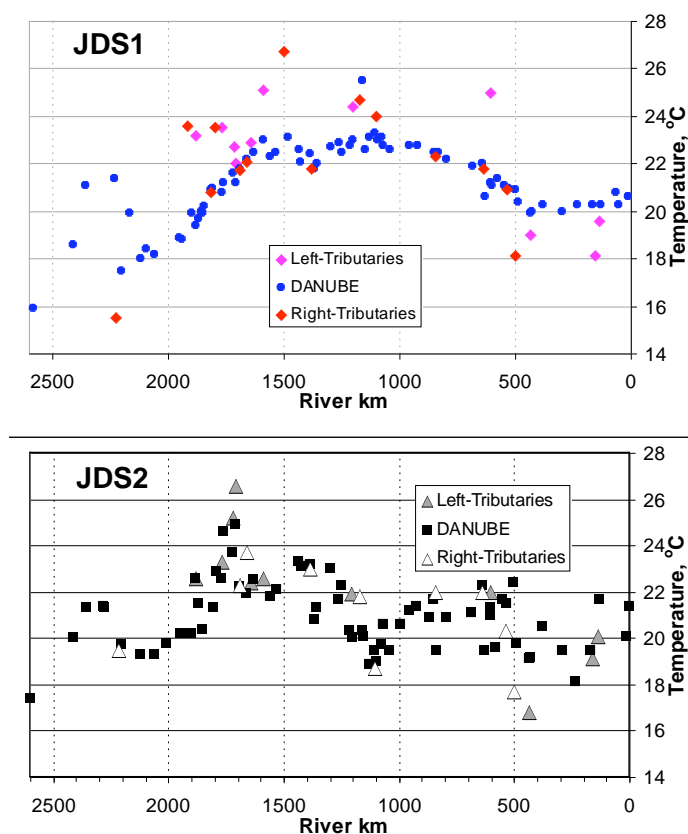
Figure 39:  Variation in water temperature along the Danube during the JDS1 and JDS2

### 13.3.2 Dissolved oxygen (DO)

Figure 40 shows the longitudinal profile of DO, as well as pH values, during the two surveys. During the JDS1, a significant increase in DO concentration at the beginning of the Middle Danube reach resulted from algal blooming; a significant increase in primary productivity and the likely related decomposition of organic matter downstream to the Iron Gate. During the JDS2, DO concentrations represented an almost balanced situation between production and decomposition of organic matter (DO saturation varied between 90 to 110%). Lower DO saturation occurred in some tributaries, particularly along their upper sections.  At one sampling site on the Tisa (JDS-TI5) and one on the Arges (JDS-AR2), extreme low oxygen values were observed (6% and 3.5% DO saturation, respectively). Where the DO saturation exceeded 100%, the concentration of chlorophyl-a also increased, demonstrating higher primary productivity.

### 13.3.3 pH value

During both surveys, the longitudinal profile of pH values was similar to that of the DO (see Figure 40). It is notable that the fluctuations in pH values were more significant than in the case of DO, particularly during the JDS2. This could be the effect of higher fluctuations in water temperature and discharge.
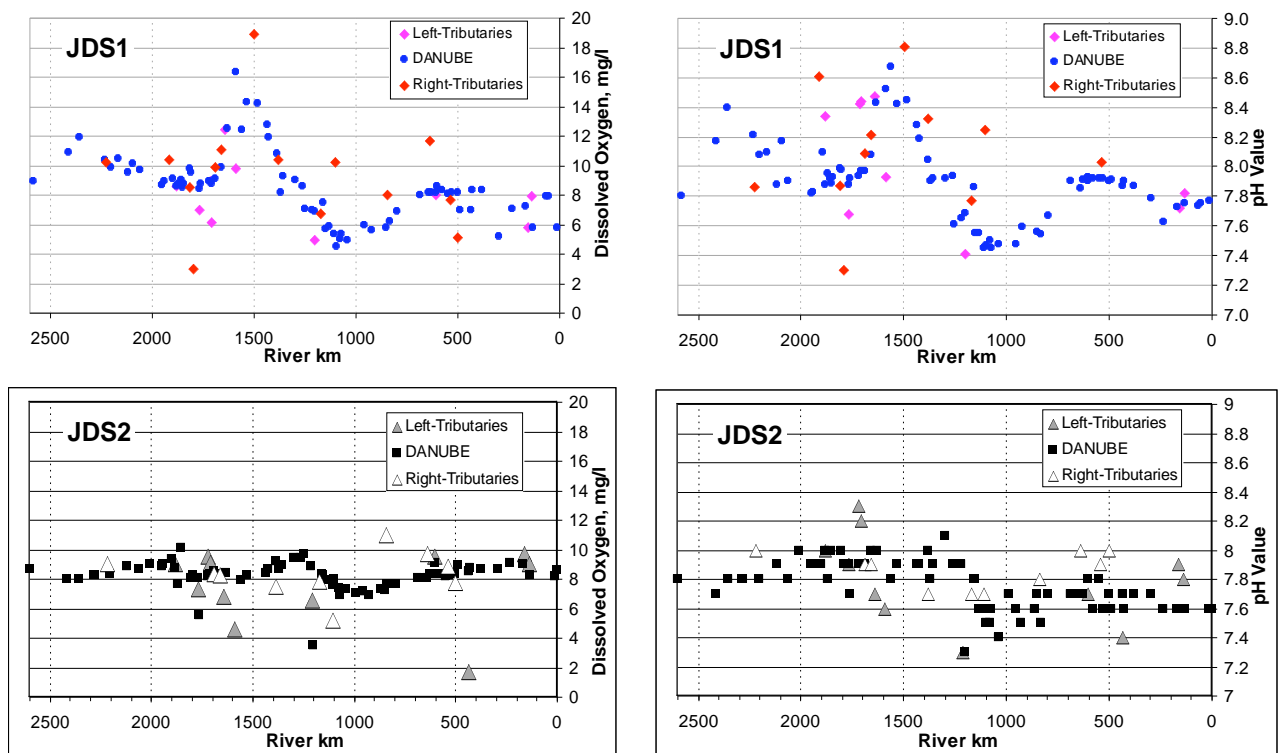
Figure 40: Longitudinal variation in the dissolved oxygen and pH values in the Danube and tributary waters during the JDS1 and JDS2

During both surveys, a significant decrease in pH values (and minimum measurements) were observed in the area of the Iron Gate reservoir, which might be the result of increasing biodegradation activities in the slower-flowing water body.

Along the major tributaries, pH values generally decreased from the upper sections downstream, except in the case of the Morava, where increasing pH values were measured. It is notable that the Morava was under high water conditions at the time of sampling. More detailed interpretation of pH values and DO results for the longitudinal surveys of the tributaries is given in the full paper on the attached CD-ROM.

### 13.3.4 Conductivity

Figure 41 shows conductivity values during the JDS1 and JDS2, indicating very similar trends during both surveys. In the Upper Danube stretch, the low salt content of the Inn significantly influenced the downstream reach of the Danube, due to their similar flow values. In the Lower Danube, even though some tributaries had relatively higher conductivity values at the confluence, no significant influence on the downstream Danube stretch was recorded - except for the Olt, which slightly increased the conductivity at the downstream Danube sampling site (river km 602). The longitudinal surveys on the major tributaries showed an increasing trend from the upper part down the Danube, particularly in the Velika Morava, Iskar, Olt and Russenski Lom rivers. However, a slight decreasing trend was observed in the case of the Tisa, Sava and Prut.
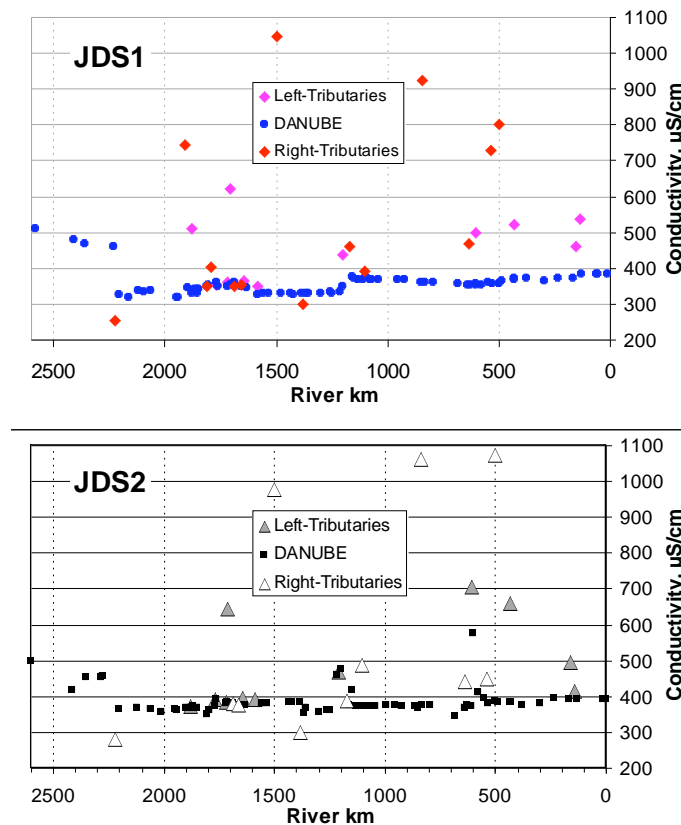
Figure 41: Longitudinal variation in conductivity in the Danube and tributary waters during the JDS1 and JDS2

### 13.3.5 Alkalinity

Alkalinity values in the Danube River and tributaries generally varied within the normal range for natural buffer capacity waters. A slight decreasing profile from upper to middle and lower reaches was observed.

The longitudinal surveys on the major tributaries showed slight decreasing profiles from upstream to downstream in the case of the Tisa and Olt rivers; whereas an increasing trend was observed along the Velika Morava, Jantra, Russenski Lom and Arges.

### 13.3.6 Water quality assessment

Due to the absence of basin-wide standards, the water quality assessment of the JDS2 results was carried out using three different approaches: the Austrian and Czech proposals for WFD-compliant standards and the ICPDR classification system (developed for TNMN purposes only).

#### 13.3.6.1 Water quality assessment according to WFD-compliant criteria

Application of the Austrian and Czech proposed standards to the JDS2 data provided an indication of classification for the Danube and its tributaries using physical-chemical quality elements (see Table 12 and Table 13).

## Table 12: Preliminary ecological class evaluation for the general physico-chemical quality elements based on the *Austrian* proposal

| Parameter to be assessed | Indication of ecological quality class (number of JDS2 sampling sites) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Danube River | | | | Tributaries | | | | |
| | 'High' class | 'Good' class | Not achieving 'good' class | | 'High' class | 'Good' class | Not achieving 'good' class | | |
| | | | Number of sites | JDS2 position | | | Number of sites | JDS2 position | |
| Temperature | 78 | 0 | 0 | - | - | 16 | 2 | 0 | |
| Dissolved oxygen (saturation) | 73 | | 5 | rkm 1761 | Iza/Szony | 14 | 4 | rkm 1215 | Tisa |
| | | | | rkm 1586 | Rackeve-Soroksar Danube Arm - end | | | rkm 1103 | Velika Morava |
| | | | | rkm 1200 | Downstream of Tisa / upstream of Sava (Belegis) | | | rkm 845 | Timok |
| | | | | rkm 1071 | Banatska Palanka/Bazias | | | rkm 432 | Arges |
| | | | | rkm 926 | Vrbica/Simijan | | | | |
| pH | 78 | | 0 | | | 18 | | 0 | |

## Table 13: Preliminary ecological class evaluation for the general physico-chemical quality elements based on the *Czech* proposal

| Parameter to be assessed | Indication of ecological quality class (number of JDS2 sampling sites) | | | |
|---|---|---|---|---|
| | Danube River | | Tributaries | |
| | Meet the EQS | | Meet the EQS | |
| | Yes ('Good' class) | No ('Moderate' class) | Yes ('Good' class) | No ('Moderate' class) |
| Temperature1 | 78 | 0 | 16 | 2 |
| Dissolved oxygen, | 10 | 68 | 9 | 9 |
| pH | 74 | 4 | 15 | 3 |

Analysing Table 12 and Table 13, it can be seen that, while temperature and pH results are relatively similar for both the Austrian and Czech classification schemes, dissolved oxygen results differ significantly. Only five sampling sites on the Danube and four at the mouths of the tributaries do not comply with the Austrian standard for 'good' class.

Using the Czech standard, sixty-eight sites on the Danube and half of the tributaries at their confluence do not comply with the 'good' class criterion. This situation underlines a strong need for the development of a harmonised type-specific classification scheme for assessment of general physico-chemical elements in the Danube River Basin.

1 Given the JDS2 timing (August-September), the water temperature evaluation was done taking into account the EQS as C90 (250C) and not as AA (140C).

### 13.3.6.2 Water quality assessment according to the TNMN five quality classes (not WFD-compliant)

In the TNMN (TransNational Monitoring Network) Water Quality Scheme, five classes are used for the assessment of general physico-chemical elements. Concerning the quality indicators reported in this chapter, TNMN class limits are set for DO concentration only. Therefore, for this indicator, the distribution (in percentage) of sampling sites into the five quality classes for the Danube and selected tributaries is shown in Figure 42. The analysis of DO revealed the following:

- Dissolved oxygen concentrations at most of the sampling sites on the Danube River correspond to Class I (72 sites out of 78);

- Regarding the remaining six sampling sites on the Danube, three still comply with the target value (Class II): Rackeve-Soroksar Danube Arm – start (river km 1642); Banatska Palanka/Bazias (river km 1071) and Vrbica/Simijan (river km 926). Three sites are non-compliant: one belonging to Class III (Iza/Szony - river km 1761); one to Class IV (Rackeve-Soroksar Danube Arm – end - river km 1586) and one to Class V (Downstream of Tisa/upstream of Sava - river km 1200);

- A relatively similar situation is observed in the tributaries: 16 out of 18 are in Classes I or II. Only two tributaries had oxygen concentrations relevant to lower classes: the Velika Morava (Class III) and the Arges (Class V).
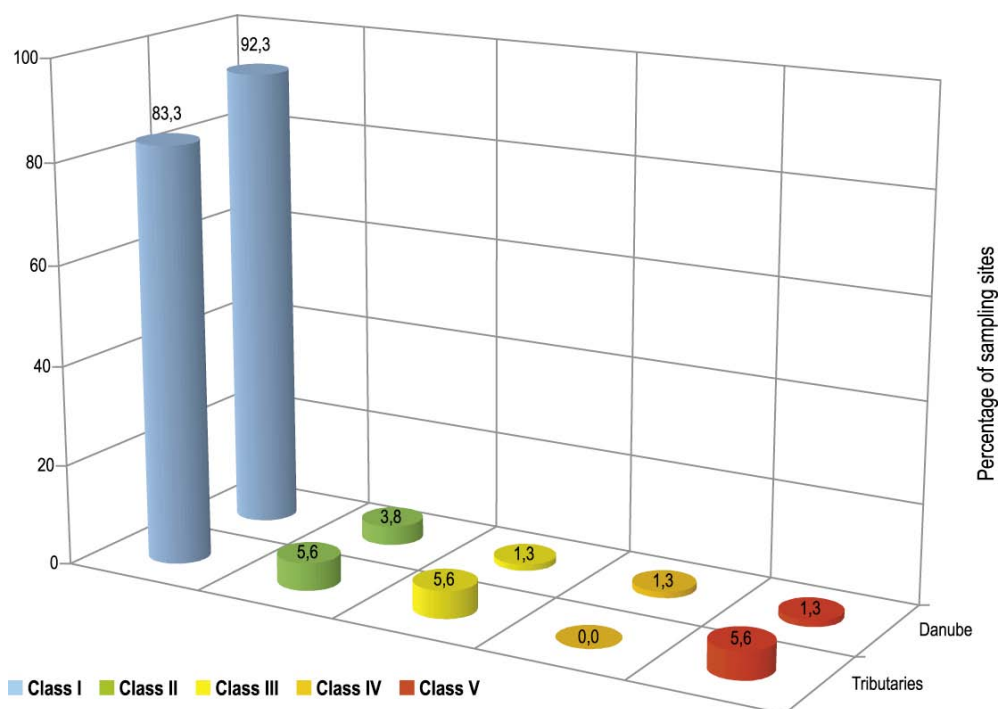


Figure 42: Percentage of sampling sites falling into the five water quality classes for dissolved oxygen concentrations according to the TNMN classification scheme

### 13.4    Conclusions

- Water temperature distribution during the JDS2 ranged within the pattern typical for the timing of the survey (August – September) both in the Danube and in the mouths of selected tributaries. Maximum values were recorded in the middle reach of the Danube River.

- The dissolved oxygen concentration pattern demonstrated near to 100% saturation along the Danube River, with slightly higher values in the upper and middle Danube reaches. Although these higher values corresponded to the primary productivity characterised by chlorophyll-a concentration, there was no such algal blooming during the JDS2 as occurred in the JDS1.

Tributaries at their confluence to the Danube displayed slightly higher dissolved oxygen levels than in the recipient Danube. Significant depletion of dissolved oxygen was recorded at the mouth of the Arges, due to the discharge of untreated municipal wastewater.

–   The longitudinal profile of pH values along the Danube River was similar to the results for dissolved oxygen. The good correlation occurred during both the JDS1 and JDS2, demonstrating the balanced effect of primary production and decomposition of organic matter, or in other words, healthy conditions in this particular aquatic ecosystem.

–   Conductivity values followed a quasi-constant longitudinal profile in the Danube River, with higher values in most of the tributaries at their mouth to the Danube (except for the low salt content of the Inn that significantly influenced the salinity of the downstream Danube reach.

–   Alkalinity values in the Danube River and tributaries represented the normal range of naturally buffered capacity waters.

–   The longitudinal surveys on the major tributaries generally showed increasing trends in conductivity and alkalinity from upper sites in the catchment down to the confluence with the Danube. Similarly to the Danube, the good correlation between DO and pH values was also characteristic for the tributaries.

–   The Austrian and Czech proposals for WFD-compliant standards, as well as the classification applied within the TNMN of the ICPDR, were used for water quality assessment based on the JDS2 results. Although most of the results fell into the 'good' quality classes, the application of the Austrian and TNMN assessment schemes resulted in a rather similar classification, while the results from the Czech system differed from both substantially. This situation underlines a strong need for the development of a harmonised type-specific classification scheme in the Danube River Basin.

## 13.5    References

Council Directive 2000/60/EC of 23 October 2000: Establishing a framework for Community action in the field of water policy, 2000.

ICPDR (2002): Joint Danube Survey, Technical report of the International Commission for the Protection of the Danube River, Vienna, p.23.

POLLARD, P., HUXHAM, M. (1998): The European Water Framework Directive: A new era in the management of aquatic ecosystem health? Aquatic Conservation: Marine and Freshwater Ecosystems. 8, 773-792.

Danube Basin Analysis, WFD Roof Report, 2004.

SMITH, V.H (2003): Eutrophication of freshwater and coastal marine ecosystems: a global problem. Environ Sci Pollut R; 10: 126–39, 2003.

Water quality targets and objectives for surface water in the Danube Basin, Progress Report Phase 2, Delft Hydraulics, 1997.

WETZEL RG (2001): Limnology. 3rd edition. New York: Academic Press.

# 14 General physico-chemical quality elements: Nutrients (N, P and Si)

Carmen Hamchevici and Mary Craciun

## 14.1 Introduction

This chapter continues the analysis of physico-chemical quality elements, focussing on the various nitrogen (N) and phosphorus (P) compounds, as well as the silicates; important nutrients influencing biological and biochemical processes in aquatic ecosystems. In addition to Annex V, the EU Water Framework Directive (WFD) explicitly refers to nutrients in Annex VIII. 12: "substances which contribute to eutrophication (in particular nitrates and phosphates)."

Nutrient pollution has been recognised as one of the important water management issues in the Danube River Basin, also impacting on the Danube Delta and the Black Sea. According to the MONERIS Model, nutrient emissions by point and diffuse sources into the Danube River Basin were 758 kt of N and 68 kt of P (in the period 1998-2000), which is much higher than background conditions (background: 8% for N and 10% for P; Schreiber et al., 2004).

The nutrients issue was also the subject of analysis in the WFD Article 5 report for the Danube River Basin District (Roof Report, 2004). According to the results from the assessment of risk of failure to reach the environmental objectives, a large number of water bodies across the basin are at risk of failing to meet *good ecological status* objectives due to nutrient pollution - 55% of the Danube River and 49% of the Danube tributaries are "at risk" or "possibly at risk" due to nutrient pollution (Roof Report, 2004).

## 14.2 Methods

### 14.2.1 Sampling and storage

#### 14.2.1.1 Water samples
Water samples for nutrient analysis were collected directly from the river using the motorboat used for collecting biological samples. Water samples were stored in amber glasses and polypropylene (PP) containers. Original water samples and filtered samples were preserved and properly stored for analysis (in selected laboratories) for organic nitrogen, total phosphorous and dissolved silicate. Samples from the longitudinal surveys on major tributaries were collected by the national experts from the riparian countries and transported to the laboratory ship within the required period of time, in order to immediately perform the analytical determination.

#### 14.2.1.2 Suspended particulate matter (SPM) and bottom sediment samples
Analysis of SPM and bottom sediment samples for organic nitrogen and total phosphorous were stored in glass or PP containers and properly kept for later delivery to the selected laboratories.

## 14.2.2 Analysis of samples

Standard Operational Procedures (SOPs), based on international standardised methods, were used for analysis of dissolved nutrients on-board the laboratory ship. Water samples were filtered through 0.45μm pore size membrane filters prior to analysis.

## 14.2.3 Analytical quality control

Calibration curves for analysis of the dissolved forms of the nutrients were plotted in graphs according to the specific SOPs. Technical specifications of the on-board analysis of nutrients (correlation coefficient, standard deviation, LOD (Limit of detection) and LOQ (Limit of quantitation) are presented in Table 14.

Depending on the number of sampling stations per sampling day, the samples from one day were analysed in one or more analysis batches in which duplicate and calibration check samples were analysed.

### Table 14: Performance specifications for the analysis of dissolved forms of the nutrients

| Nutrient form | Correlation coefficient | Standard deviation | LOD* | LOQ |
|---|---|---|---|---|
| | | mg/l | mg/l | mg/l |
| Ammoniacal-N ($NH_4^+$ -N) | 0.9991 | 0.0060 | 0.020 | 0.060 |
| Nitrite-N ($NO_2^-$ -N) | 0.9997 | 0.0013 | 0.005 | 0.012 |
| Nitrate-N ($NO_3^-$ -N) | 0.9984 | 0.0331 | 0.100 | 0.300 |
| Orthophosphate-P ($PO_4^{3-}$-P) | 0.9992 | 0.0016 | 0.005 | 0.015 |
| Dissolved silicates ($SiO_2$) | 1.0000 | 0.0180 | 0.057 | 0.114 |

\* LOD (Limit of Detection) for:  $NH_4^+$ -N determination by ISO-7150/1-1984;
$NO_2^-$ -N determination by SMEWW 419; and
$PO_4^{3-}$ -P determination by ISO SMEWW 424.

## 14.3    Results and discussion

For data presentation, the division of the Danube into three sections, as reported in the JDS1, was applied (Joint Danube Survey, Technical Report of the ICPDR, 2002):

– Upper Danube: from river km 2600 to river km 1880 (sampling stations JDS1 - JDS15);
– Middle Danube: from river km 1869 to river km 1077 (sampling stations JDS16 - JDS58);
– Lower Danube: from river km 1077 to river km 0 (sampling stations JDS59 - JDS0).

## 14.3.1 Water

Variation ranges and average values for nutrient forms analysed in water samples during the JDS2 are presented in Table 15.

### Table 15: Concentration ranges and statistical data for the *nutrients forms* analysed in water samples during the JDS2 - Danube River and tributaries

| Quality element | Unit | Danube River | | | Tributaries | | Longitudinal  surveys on major tributaries | |
|---|---|---|---|---|---|---|---|---|
| | | Minimum value | Maximum value | Mean value | Minimum value | Maximum value | Minimum value | Maximum value |
| Ammonium ($NH_4^+$-N) | mg/l | < 0.020 | 0.379 | 0.041 | < 0.020 | 7.200 | < 0.020 | 7.380 |
| Nitrite ($NO_2^-$ -N) | mg/l | < 0.005 | 0.072 | 0.020 | < 0.005 | 0.129 | < 0.005 | 0.236 |
| Nitrate ($NO_3^-$ -N) | mg/l | 0.780 | 3.120 | 1.660 | 0.390 | 8.020 | 0.380 | 9.370 |
| Organic nitrogen | mg/l | nd | 0.365 | 0.035 | nd | 0.131 | nd | 0.510 |
| Orthophosphate-P ($PO_4^{3-}$-P) | mg/l | 0.010 | 0.093 | 0.036 | < 0.005 | 1.000 | < 0.005 | 1.000 |

| Quality element | Unit | Danube River | | | Tributaries | | Longitudinal surveys on major tributaries | |
|---|---|---|---|---|---|---|---|---|
| | | Minimum value | Maximum value | Mean value | Minimum value | Maximum value | Minimum value | Maximum value |
| Total -P | mg/l | 0.060 | 0.910 | 0.110 | 0.070 | 0.800 | 0.060 | 0.780 |
| Dissolved silicates (SiO$_2$) | mg/l | 1.430 | 9.260 | 5.200 | 2.880 | 23.640 | 1.880 | 22.250 |

### 14.3.1.1 Nitrogen forms

#### 14.3.1.1.1 Ammonium

Figure 43 shows the longitudinal variation in the ammoniacal-N concentrations measured during the JDS2. At most of the sampling sites in the Danube, the concentrations were near to the LOQ except for a slight increase in the backwater of the Iron Gate reservoir. Higher concentrations were measured in the dammed Danube arm (Rackeve-Soroksar*)* and in some of the tributaries, reaching an extreme high value (7.2 mg/l!) in the Arges, caused by the secondary discharge of untreated municipal wastewater from the Bucharest sewage system.
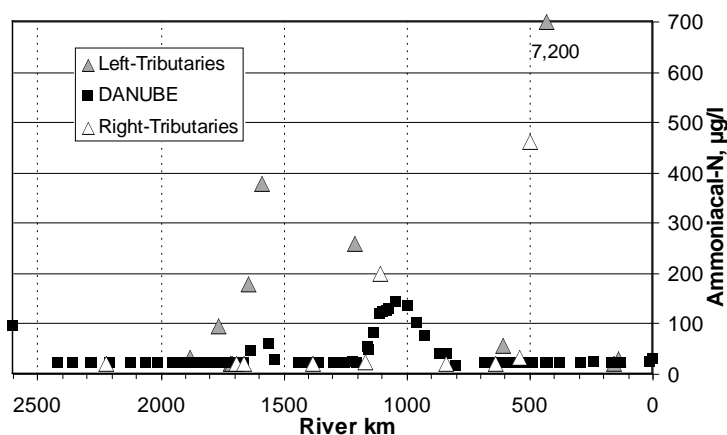


**Figure 43:  Longitudinal variation in the concentration of ammoniacal-N in water samples along the Danube and tributaries during the JDS2**

#### 14.3.1.1.2 Nitrites

The Nitrite-N concentration spatial pattern had a decreasing character in the Upper Danube. The middle reach was characterised by a uniform distribution, followed by a peak in the Iron Gate reservoir, similar to that of ammoniacal-N, but slightly shifted to the headwater of the reservoir and downstream of the dam. No elevated concentrations were measured in the tributaries except in the case of the Sió and Russenski Lom rivers.

#### 14.3.1.1.3 Nitrates

Figure 44 shows the longitudinal variation in the nitrate-N concentrations measured during the JDS2. In the Danube main stream, the highest concentrations were measured upstream of the confluence of the Inn. Downstream of the Inn, the nitrate-N concentration gradually decreased and remained relatively constant downstream of the Iron Gate reservoir. In the majority of the tributaries, nitrate-N concentrations were lower than in the Danube, with the exception of three tributaries. The highest concentration (8.02 mg/l) was measured in the Russenski Lom River.
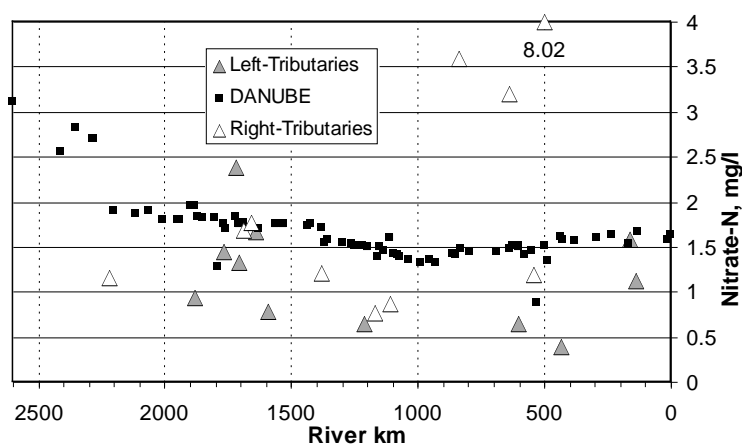
Figure 44:  Longitudinal variation in the concentration of nitrate-N in water samples along the Danube and in its tributaries during JDS2

#### 14.3.1.1.4 Organic nitrogen

The Danube River was characterised by very low organic nitrogen concentrations in water samples. Regarding the tributaries, the organic nitrogen concentrations were below the LOQ in four rivers (the Inn, Hron, Ipoly and Arges), whereas in the others, the concentrations varied in the same range as in the Danube River.

### 14.3.1.2 Phosphorous forms

During the JDS2, water samples were analysed for dissolved orthophosphate-P and total phosphorus (TP). The data interpretation was made on the basis of these results, and for the sake of ease of explanation of the results, the TP was considered as a sum of the orthophosphate-P and organic phosphorus (Org-P), as shown in the Figure 46 in the following discussion of the results.

#### 14.3.1.2.1 Orthophosphates

Figure 45 shows the longitudinal variation in orthophosphate-P concentrations measured during the JDS2. A significant decrease in the orthophosphate-P concentrations occurred downstream of the Inn confluence. In the middle stretch, at first a slight increase was observed, followed by a decreasing profile until the minimum level was found at the confluence of the Tisa tributary. In the backwaters of the Iron Gate reservoir, slightly increasing orthophosphate-P concentrations were measured, and levels basically remained the same up to the Danube Delta. Higher concentrations (between 0.200 and 0.300 mg/l of P) were measured in four tributaries (the Morava, Ipoly, Sió and Iskar), and very high concentrations were found in two tributaries (0.635 and 1.000 mg/l in the Russenski Lom and Arges, respectively).
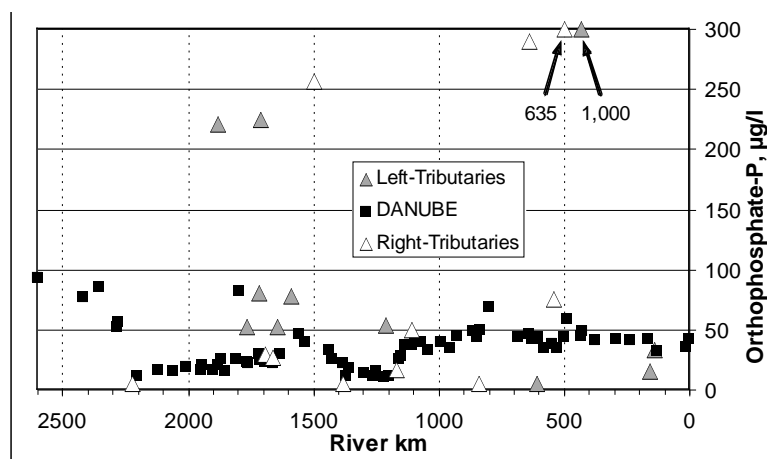
Figure 45:  Longitudinal variation in the concentration of orthophosphate-P in water samples along the Danube and tributaries during the JDS2

### 14.3.1.2.2   Total phosphorus (TP)

In addition to showing orthophosphate-P concentrations, Figure 46 also demonstrates the variations in Org-P in the Danube and its tributaries during the JDS2. As demonstrated in the Figure 46, the TP concentrations were nearly at the same concentration level along the entire length of the Danube River. In the lower reach of the Danube, two high TP concentrations were measured at river km 488 (Downstream of Ruse/Giurgiu) and river km 434 (Upstream of the Arges). Most of the tributaries at their confluence to the Danube had comparable concentration levels with the Danube's main stream. Regarding the Org-P, the highest concentration was measured in the Timok tributary, followed by the Olt.

### 14.3.1.3   Dissolved silicates

The spatial variation of dissolved silicates showed a general decreasing trend from the upper to middle and lower reaches of the Danube. A slightly lower concentration profile was observed in the Iron Gate area, but no significant difference between the upstream part of the reservoir and downstream of the dam could be recognised. As for the confluences of the tributaries, dissolved silicate profiles were scattered. In several watercourses, concentrations were lower than the level in the Danube River; higher values were measured in the Morava, Hron and Ipoly rivers and in the mouth of all tributaries in the lower stretch (except for the Jantra).
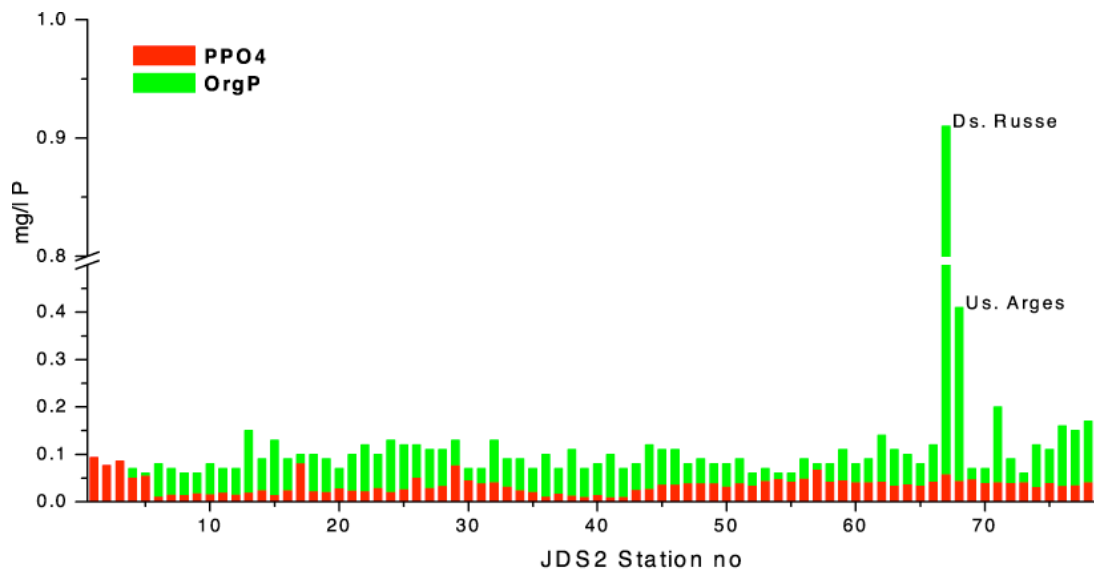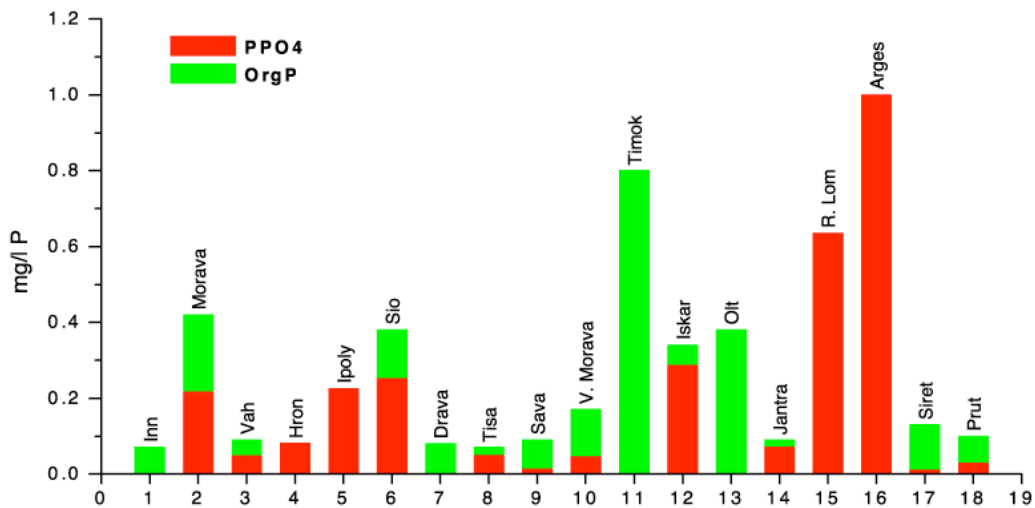
**Figure 46: Variation in the concentration of total phosphorus, indicated as Org-P and orthophosphate-P (PPO4), in water samples collected from the Danube River and tributaries during the JDS2**

### 14.3.1.4  The longitudinal surveys on major tributaries – water samples

Regarding the longitudinal surveys on major tributaries, variation in nitrogen and phosphorous nutrient forms measured in water samples is presented in Figure 47 and Figure 48.

The following conclusions can be made:

–   **N-ammonium**: Undetectable in 11 sampling sites. Relatively constant profiles were observed on the Sava (from JDS-SA1 to JDS-SA2), Jantra and Prut rivers and a decreasing downstream trend was found on the Sava (from JDS-SA3 to JDS51/Sava) and Olt. Increasing downstream concentrations were observed along the Tisa River (from JDS-TI4 to JDS-TI6), Velika Morava (from JDS-VM2 to JDS56/Velika Morava) and Iskar. A very significant downstream increase was recorded in the Arges tributary.

–   **N-nitrites**: Undetectable in seven sampling sites; the most elevated concentrations were measured along the Russenski Lom.

–   **N-nitrates**: A general "V" profile from upper sites down to the confluence with the Danube River was observed; the highest concentrations were recorded along the Russenski Lom.

–   **P-othophosphates**: No significant variations could be distinguished, except for the downstream increase along the Morava, Russenski Lom and Arges.

–   **Total P**: A strong downstream increase was observed in the Iskar and Olt. A marked "V" concentration profile was found in Russenski Lom while an inverted "V" profile occurred along the Sava, Velika Morava, Jantra and Arges rivers.

–   For **dissolved silicates**, a decreasing downstream trend was observed in most of the tributaries.
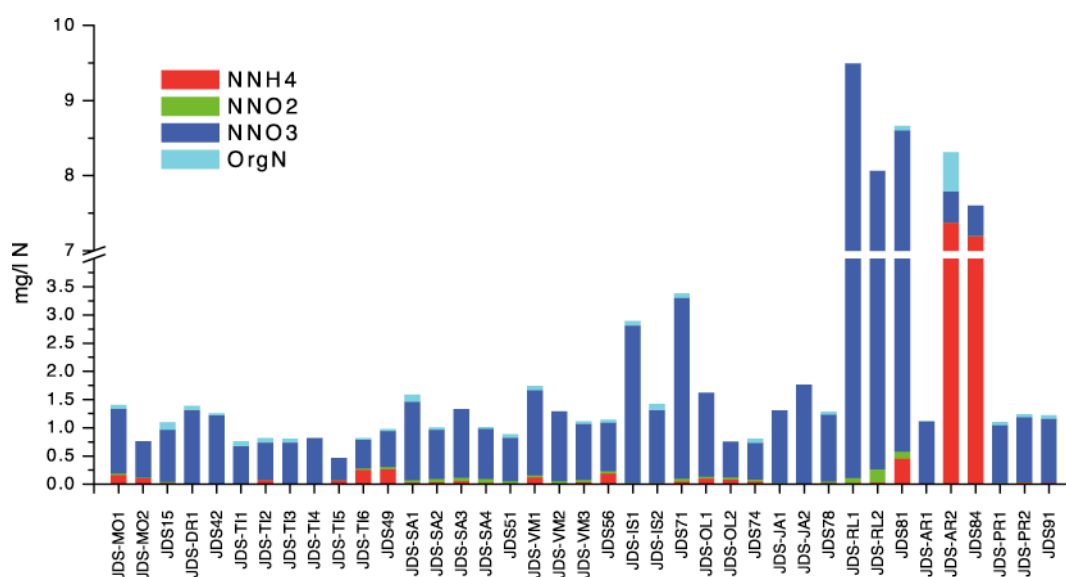


Figure 47: Variation in *nitrogen forms* in water samples from the longitudinal surveys on the major tributaries
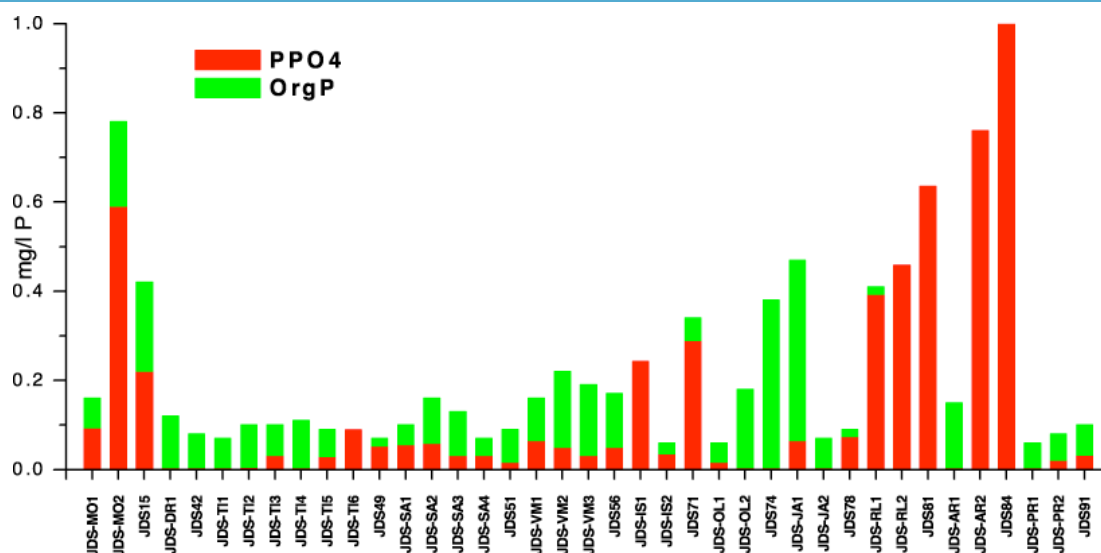
Figure 48: Variation in *phosphorous forms* in water samples from the longitudinal surveys on the major tributaries

### 14.3.2 Suspended particulate matter

#### 14.3.2.1 Organic nitrogen
The longitudinal variation of organic nitrogen in suspended matter showed a relatively "symmetrical pattern" with the maximum profile located in the middle stretch of the Danube. Samples from the mouths of tributaries showed lower content than the Danube itself.

#### 14.3.2.2 Total phosphorus
The variation of TP in suspended particulate matter showed the maximum profile in the middle reach of the Danube and the minimum value in the Gabcikovo reservoir. The TP profile in suspended solids from the mouths of most of the tributaries showed a comparable pattern with that in the Danube. An elevated content of TP in Velika Morava, was probably caused by a high precipitation regime during the sampling period.

### 14.3.3 Bottom sediment

#### 14.3.3.1  Organic nitrogen
The spatial distribution of organic nitrogen in sediment samples showed an increasing profile from the upper to the middle and further to the beginning of the lower Danube stretch. A decreasing profile followed starting downstream of the Iron Gates area, a consequence of the denitrification process in the reservoir. In the Lower Danube, a highly elevated concentration of organic nitrogen was found at river km 429 (Downstream of the Arges - left), a consequence of the highly polluted Arges.

#### 14.3.3.2  Total phosphorus
The longitudinal profile of TP in sediment samples showed an apparent decrease in the Upper Danube stretch. A maximum profile was found in the middle reach of the Danube, followed by a decreasing trend in the Iron Gates area. This result contradicts previous findings that the Iron Gates sediment acts as a phosphorous sink.

#### 14.3.3.3 Longitudinal surveys on the major tributaries – sediment samples
The organic nitrogen variations in sediment samples from the longitudinal surveys in the major tributaries show that, for the Tisa, Sava and Prut, downstream sites have higher concentration than upper ones.

As regards total phosphorous, no significant variation among the sampling sites could be observed, except for JDS-VM3, where the TP concentration in the sediment sample was four times higher than the concentration from the confluence of the Velika Morava tributary with the Danube River.

### 14.3.4 Comparison with the JDS1 (August – September 2001)

#### 14.3.4.1 Water

- **N-ammonium**: Concentrations in the Danube River had a relatively similar profile, except for side arms and the Iron Gates stretch. For tributaries, concentrations were almost at the same level, except for the Arges tributary, in which more than a two-fold higher N-ammonium concentration was measured during the JDS2.

- **N-nitrite**: The pattern from the JDS2 was similar to that recorded during the JDS1. For tributaries, no specific pattern was observed.

- **N-nitrate**: Except for the situation from river km 532 (Downstream of the Jantra), all N-nitrate concentrations measured during the JDS2 in the Danube were higher than those during the JDS1. This trend is also apparent in the selected tributaries, except for the Morava, Ipoly, Sió and Iskar, in which higher concentrations were found during the JDS1.

- **Organic nitrogen**: Concentrations were systematically much lower during the JDS2, both in the Danube River and the tributaries.

- **P-orthophosphate**: Concentrations measured during the JDS2 were generally lower than the ones from the JDS1, with a few exceptions located in the middle reach of the Danube. As for the tributaries, almost a three-fold higher concentration was measured at Iskar in the JDS1. However, remarkably higher concentrations were found during the JDS2 in the Russenski Lom and Arges rivers.

- **Total phosphorous**: Profiles were relatively similar during the two surveys, with few exceptions. For 11 out of 18 of the selected tributaries (Inn, Hron, Ipoly, Sió, Drava, Tisa, Sava, Iskar, Jantra, Siret and Prut), TP values measured during the JDS2 were lower than ones from the JDS1; for five tributaries (Morava, Vah, Olt, Russenski Lom and Arges) the situation was the opposite; while for the Velika Morava tributary, the two values were the same. The biggest difference was recorded in the case of the Timok tributary, in which a ten-fold higher concentration was measured in the JDS2.

- **Dissolved silicates**: With only few exceptions, concentrations measured during the JDS2 were slightly higher than those during the JDS1 in the Danube River. For tributaries, the pattern was similar as for the Danube except for the Sió, Sava, Olt and Jantra. The largest difference occurred in the Velika Morava, where the dissolved silica concentration measured during the JDS2 was fifteen times higher than the one from six years previously.

### 14.3.5 Water quality assessment

Due to the absence of basin-wide standards, the water quality assessment of the JDS2 results was carried out using three different approaches: Austrian and Czech proposals for WFD-compliant standards and the ICPDR classification system (developed for TNMN (TransNational Monitoring Network) purposes only).

#### 14.3.5.1 Water quality assessment according to WFD-compliant criteria

The application of Austrian and Czech proposed standards provided an indication of classification of the Danube and tributaries by physico-chemical quality elements (nutrients).

It should be stressed that the classification of nutrients is supportive of ecological status assessment using biological quality elements. Also, it should be clearly mentioned that the data assessment was

made **for JDS2 sampling sites only and not for water bodies assigned in the Danube River and its tributaries.**

To obtain an indicative overview of nutrient classification, the following limitations have been taken into account:

– The JDS2 data are only momentary data and when compared with the datasets based on C90 statistics, the information provided has only an approximate value with low confidence;

– For the time being, **no reference conditions for nutrients** are established for the Danube Basin;

– In the Czech proposal, only compliance with Annual Average EQS ("the worst case" approach) has been tested.

Table 16: *Preliminary* ecological quality class evaluation for nutrient conditions based on the *Austrian* proposal (assessed by most relevant biological element)

| Parameter to be assessed | Indication of ecological quality class (number of JDS2 sampling sites) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Danube River | | | Tributaries | | | | |
| | | | | | | Not achieving 'good class' | | |
| | 'High' class | 'Good' class | Not achieving good class' | 'High' class | 'Good' class | No. of sites | JDS2 position [rkm] | |
| N-Nitrates (N-NO$_3$) | 77 | 1 | 0 | 17 | 0 | 1 | 498 | Russenski Lom |
| P-phosphates (P-PO$_4$) | 73 | 5 | 0 | 10 | 2 | 6 | 1880 | Morava |
| | | | | | | | 1708 | Ipoly |
| | | | | | | | 1497 | Sió |
| | | | | | | | 637 | Iskar |
| | | | | | | | 498 | Russenski Lom |
| | | | | | | | 432 | Arges |

Table 17: *Preliminary* ecological class evaluation for nutrient conditions based on the *Austrian* proposal (assessed by the toxicity on the aquatic community)

| Parameter to be assessed | Indication of ecological quality class (number of JDS2 sampling sites) | | | | | |
|---|---|---|---|---|---|---|
| | Danube River | | Tributaries | | | |
| | Meets the EQS? | | Meets the EQS? | | | |
| | Yes ('Good' class) | No ('Moderate' class) | Yes ('Good' class) | No ('Moderate' class) | | |
| | | | | Number of sites | JDS2 position [rkm] | |
| N-Ammonium (N-NH$_4$) | 78 | 0 | 17 | 1 | 432 | Arges |
| N-Nitrites (N-NO$_2$) | 78 | 0 | 18 | 0 | | |

Table 18: *Preliminary* ecological class evaluation for the nutrients conditions based on the *Czech* proposal (EQS as Annual Average (AA))

| Parameter to be assessed | Indication of ecological quality class (Number of JDS2 sampling sites) | | | | | |
|---|---|---|---|---|---|---|
| | Danube River | | | Tributaries | | |
| | Meets the EQS (AA)? | | | Meets the EQS (AA)? | | |
| | Yes 'Good' class | No ('Moderate' Class) | | Yes 'Good' class | No ('Moderate' class) | |
| | | No. of sites | JDS2 position [rkm] | | No. of sites | JDS2 position [rkm] |
| N-Ammonium (N-NH$_4$) | 77 | 1 | 1586 / Rackeve-Soroskar arm | 15 | 3 | 1215 / Tisa |
| | | | | | | 498 / Russenski Lom |
| | | | | | | 432 / Arges |
| N-Nitrate (N-NO$_3$) | 78 | 0 | - | 17 | 1 | 498 / Russenski Lom |
| Total Nitrogen (TN) | 78 | 0 | - | 16 | 2 | 498 / Russenski Lom |
| | | | | | | 432 / Arges |
| Total Phosphorous (TP) | 73 | 5 | 488 / Downstream Ruse/Giurgiu | 11 | 7 | 1880 / Morava |
| | | | 434 / Us. Arges | | | 1497 / Sió |
| | | | 295 / Upstream Cernavoda | | | 1103 / Velika Morava |
| | | | 8 / Bystroe canal | | | 845 / Timok |
| | | | 0 / Sf. Gheorghe | | | 637 / Iskar |
| | | | | | | 605 / Olt |
| | | | | | | 498 / Russenski Lom |
| | | | | | | 432 / Arges |

According to Table 16, Table 17 and Table 18 the ecological quality information provided by the two national proposals of WFD-compliant classification schemes is relatively similar. Based on the Austrian scheme, the sampling sites in the Danube River are either in the 'high' or 'good' ecological class, while only six sampling sites in the mouths of tributaries do not comply with the 'good' class criteria. The more restrictive Czech scheme showed that six sampling sites on the Danube and nine sampling sites located at the mouth of tributaries do not comply with 'good' class criteria.

### 14.3.5.2 Water quality assessment according to the ICPDR five quality classes (not WFD-compliant)

In the ICPDR Water Quality Scheme, five classes are used for assessment with the target value being the limit value of class II. In Figure 49 the allocation (in percentages) of the JDS2 sampling sites into the five quality classes for the Danube River and selected tributaries is shown.
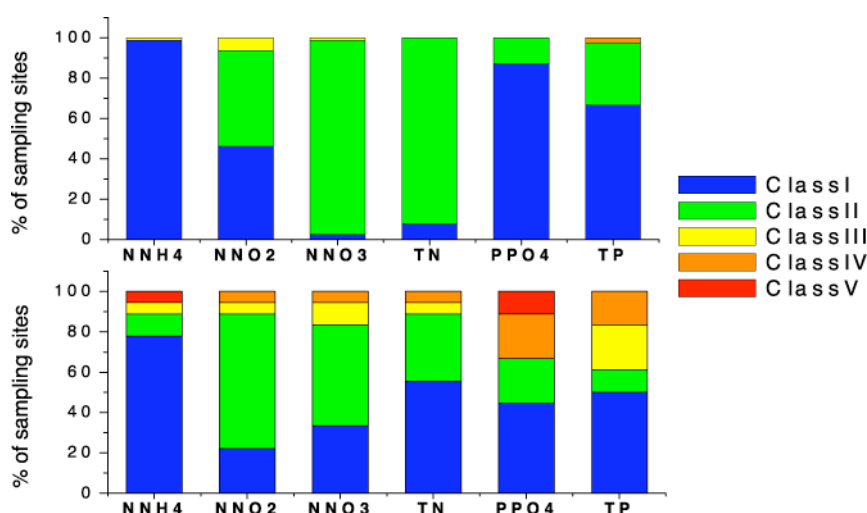
Figure 49: Distribution of sampling sites into five water quality classes
for the Danube River (top) and tributaries (bottom)

Based on the JDS2 data compilation, the following observations can be summarised:

– **Danube River**: The entire course of the Danube River is mainly characterised by nitrogen and phosphorous nutrient levels located in Class I (reference) and Class II (target value), which is in agreement with the general situation as presented in the results of the TNMN;

– **Tributaries**: More than 80% of the tributaries are characterised by Class I and Class II in the case of nitrogen forms; concerning phosphorous forms, more than 60% of the tributaries comply with the target value.

## 14.4    Conclusions

– A relatively constant profile of N-ammonium concentration along the Danube River was observed; the maximum concentration peak was located in the Iron Gates reservoir backwaters. A highly elevated concentration was measured in the mouth of the Arges tributary, caused by untreated municipal wastewater from the Bucharest sewage system.

– The N-nitrite spatial pattern had a decreasing character in the Upper Danube. The middle reach was characterised by a uniform profile, followed by a peak in the Iron Gates reservoir, similar to that found for N-ammonium. With the exception of two sites (the Sió and Russenski Lom rivers), no high concentrations were measured in the mouths of the tributaries.

– A significantly decreasing profile of N-nitrate concentrations from upper to middle and lower Danube reaches was observed. Local variations took place in the side arms of the Danube River, caused by the specific summer pattern in shallow waters (uptake by biological activity and increased organic pollution). The mouths of the tributaries presented a scattered concentrations profile, with a large variation interval.

– The Danube River and the mouths of the selected tributaries were characterised by a very low content of organic nitrogen in water samples.

– A strong decrease in P-orthophosphates concentrations was observed in the Upper Danube, which was followed by a slightly increasing profile in the lower reach, mainly caused by discharges of municipal wastewater with P-containing detergents. Except for two very elevated concentrations (0.635 and 1.000 mg/l in the mouth of the Russenski Lom and Arges, respectively), most of the tributaries had concentration levels similar to the Danube River.

–   A slight increasing profile of TP concentrations from the Upper to Lower Danube was noticed. Most of the tributaries at the confluence had comparable concentration levels with the Danube.

–   The dissolved silicates showed a decreasing trend down the Danube. The spatial distribution of organic nitrogen in sediment samples showed an increasing profile from the upper through the middle Danube stretch. In the Lower Danube, a highly elevated concentration was found downstream of the Arges tributary. Total phosphorous in sediment samples showed a decreasing character in the Upper Danube stretch; the maximum profile was found in the middle reach. A decreasing trend was observed in the Iron Gates area. This fact contradicts the previous findings that the Iron Gates sediment acts as phosphorous sink.

–   Organic nitrogen and TP in suspended matter showed a relatively "symmetrical pattern" with the maximum profile located in the middle stretch of the Danube. Samples from the mouths of tributaries showed lower content than in the Danube itself, except for the Velika Morava River, in which high TP was rather high.

–   The longitudinal surveys on the major tributaries generally showed increasing profiles from upper sites down to the confluence with the Danube River.

–   When compared with the JDS1 results in the Danube water samples, it can be noticed that relatively similar profiles were present in the case of N-ammonium, N-nitrites and TP. N-nitrates and dissolved silica concentrations from the JDS2 were almost systematically higher than those from the JDS1, while in the case of organic nitrogen the situation was opposite. P-orthophosphate concentrations measured during the JDS2 were generally lower than the ones from the JDS1, with a few exceptions located in the middle reach of the Danube.

–   The water quality assessment for the JDS2 results was carried out based on three different approaches: two WFD-compliant proposals for national classification schemes (Austrian and Czech) and the Trans National Monitoring Network Quality Classification System of the ICPDR. Based on the Austrian proposal, all sampling sites located on the Danube River itself are either in the 'high' or 'good' ecological class, while the six tributaries (at their confluence) do not comply with the 'good' class criteria. According to the more restrictive Czech scheme, six sampling sites on the Danube and nine sampling sites located at the mouth of tributaries do not comply with 'good' class criteria. Based on the TNMN classification scheme, the Danube River was mainly characterised by nutrient levels of Class I (reference) and Class II (target value). As regards the mouths of the selected tributaries, more than 80% of nitrogen nutrient forms and more than 60% of phosphorous forms are characterised by Class I and Class II.

## 14.5    References

Council Directive 2000/60/EC of 23 October 2000 establishing a framework for Community action in the field of water policy, 2000;

Council Directive 91/271/EEC concerning urban waste-water treatment (UWWT Directive). 1991;

daNUbs Project, Final report. (2005): Nutrient management in the Danube Basin and its impact on the Black Sea;

DEVLIN, M., PAINTING, S., BEST, M. (2007): Setting the nutrient thresholds to support an ecological assessment based on nutrient enrichment, potential primary production and undesirable disturbance, Marine Pollution Bulletin 55 65–7;

DODDS WK., SMITH VH., LOHMAN K. (2002): Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. Can J Fish Aquat Sci; 59: 865–74;

Environmental Programme for the Danube River Basin: Danube integrated environmental study, Final report, Haskoning, Nijmegen, The Netherland,1994;

FRIEDL, G., TEODORU, C., WEHRLI, B. (2004): Is the Iron Gate I reservoir on the Danube River a sink for dissolved silica? Biogeochemistry, 68: 21-32;

ICPDR: Joint Danube Survey, Technical Report of the International Commission for the Protection of the Danube River, Vienna, p.23, 2002;

Nutrient Balances for the Danube Countries, Project EU/AR/102A/91, Final report, 1997;

POLLARD, P., HUXHAM, M. (1998): The European Water Framework Directive: A new era in the management of aquatic ecosystem health? Aquatic Conservation: Marine and Freshwater Ecosystems. 8, 773-792;

REFCOND Guidance Document, 2003;

SCHMEDTJE, U., BACHMANN, J., BEHRENDT, H., BIRK, S., BIZA, P., D'EUGENIO, J., V. GILS, J., GRATH, J., HAMCHEVICI, C., HANSEN, W., INTERWIES, E., KAMPA, E., LINDINGER, H., LISKA, I., POPESCU, L., POPOVICI, M., POTTGIESSER, T., SIGMUND, G., SOMMERHÄUSER, M., SPECK, S., STUBAUER, I., VOGEL, B., WELLER, P., WINKELMANN-OEI, G. & A. ZINKE (2005): Danube Basin Analysis (WFD Roof Report 2004). The Danube River Basin District - River basin characteristics, impacts of human activities and economic analysis required under Article 5, Annex II and Annex III, and inventory of protected areas required under Article 6, Annex IV of the EU Water Framework Directive (2000/60/EC) - Part A - Basin-wide overview. ICPDR, Vienna: 191 pp.;

SCHREIBER, H., BEHRENDT, H., CONSTANTINESCU, L.T., CVITANIC, I., DRUMEA, D., JABUCAR, D., JURAN, S., PATAKI, B., SNISHKO, S. & ZESSNER, M. (2004): Point and diffuse nutrient emissions and loads in the transboundary Danube River Basin, a modelling approach;

UNDP/GEF, 1. Danube water quality model simulations in support to the transboundary analysis and the Pollution Reduction Programme - Jos van Gils, Delft Hydraulics, Danube Pollution Reduction Programme, UNDP/GEF Assistance, The Netherlands, 204 pp.

# 15 EU WFD organic priority substances in water, suspended particulate matter, sediments and biota and other organic pollutants

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(Co-authors of sections on alkylphenols and alkylphenoletoxylates: Vesna Micic and Thilo Hofmann)

## 15.1 Introduction

One of the key objectives of JDS2 was to obtain a full overview of EU priority substances in the Danube and its major tributaries in the light of current EU legislation. The draft daughter directive (Proposal for a Directive of the European Parliament and of the Council on environmental quality standards in the field of water policy and amending Directive 2000/60/EC) proposes environmental quality standards (EQS) in whole water samples (filtrated samples in the case of heavy metals) as well as EQS for three substances in biota. Environmental quality standards are given for different types of waters as "annual averages" and "maximum allowable concentrations". JDS2 provided not only data for priority substances in water but also data for suspended particulate matter, sediments and biota. All 33 priority substances except C10-C13-chloroalkanes (no method available), as well as eight other pollutants from the proposed EU Directive, were analysed in JDS2 water samples. The draft EU Directive "Laying down technical specifications for chemical analysis and monitoring of water status" (QA/QC Directive) is defining minimum performance criteria for methods of analyses. According to this draft, all methods of analysis shall be based on an uncertainty of measurement of 50% or below (k = 2) estimated at the level of relevant EQS and a limit of quantification (LOQ) equal or below a value of 30% of the relevant EQS.

For most of the priority substances, the limits of quantification (LOQ) of the analytical methods applied were below or at the level of the environmental quality standards (EQS). For some compounds however the LOQ were higher than EQS, so that any indication of compliance with the Water Framework Directive (WFD) was not possible.

**It has to be stressed that EQS for priority substances are defined for an average value of 12 measurements within one year. The JDS2 provided a single sample from August/September, which is certainly not representative for the time period of one year (e.g. pesticide application is carried out only during certain periods of the year). It is not according to WFD rules to assess the chemical status from one single measurement. For this reason the results of JDS2 are reported as an "indication of chemical status at each sampling site".**

The following table gives an overview on determinands, limits of quantification (LOQ), the corresponding EQS and the laboratories involved. For yellow labelled substances, the LOQ was not sufficient for compliance checking. Polycyclic aromatic hydrocarbons (PAH) are discussed in other chapters.

15  EU WFD organic priority substances in water, suspended particulate matter, sediments and biota
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## Table 19: Limits of Quantification (LOQ) of analytical methods for selected WFD priority substances in water samples in comparison to the proposed environmental quality standards (EQS)

| Determinand | Method | Unit | LOQ | EQS | Laboratory |
|---|---|---|---|---|---|
| alachlor | EN ISO 11369 | µg/l | 0.05 | 0.3 | VITUKI |
| atrazine | EN ISO 11369 | µg/l | 0.005 | 0.6 | VITUKI |
| benzene | ISO 10301 (HS, GC-FID) | µg/l | 0.3 | 10 | WRI Bratislava |
| BDE-28 | not analysed | µg/l | Not analysed | 0.0005 | |
| BDE-47 | LLE, GC-NCI-MS | µg/l | 0.002 | 0.0005 | WRI Prague |
| BDE-99 | LLE, GC-NCI-MS | µg/l | 0.002 | 0.0005 | WRI Prague |
| BDE-100 | LLE, GC-NCI-MS | µg/l | 0.002 | 0.0005 | WRI Prague |
| BDE-153 | LLE, GC-NCI-MS | µg/l | 0.002 | 0.0005 | WRI Prague |
| BDE-154 | LLE, GC-NCI-MS | µg/l | 0.002 | 0.0005 | WRI Prague |
| C10-13-chloroalkanes | no method | | no method | | |
| chlorphenvinfos | ISO 6486 (LLE, GC-ECD) | µg/l | 0.005 | 0.1 | WRI Bratislava |
| chlorpyriphos | ISO 6486 (LLE, GC-ECD) | µg/l | 0.005 | 0.03 | WRI Bratislava |
| 1,2-dichloroethane | ISO 10301 (HS, GC-ECD) | µg/l | 0.7 | 10 | WRI Bratislava |
| dichloromethane | ISO 10301 (HS, GC-ECD) | µg/l | 0.5 | 20 | WRI Bratislava |
| DEHP | LC-DAD | µg/l | 0.2 | 1.3 | WRI Bratislava |
| diuron | SPE, LC-MS/MS | µg/l | 0.001 | 0.2 | JRC |
| alpha-endosulfan | ISO 6486 (LLE, GC-ECD) | µg/l | 0.005 | 0.005 | WRI Bratislava |
| hexachlorobenzene | ISO 6486 (LLE, GC-ECD) | µg/l | 0.02 | 0.01 | WRI Bratislava |
| hexachlorobutadiene | ISO 10301 (HS, GC-ECD) | µg/l | 0.1 | 0.1 | WRI Bratislava |
| lindane | ISO 6486 (LLE, GC-ECD) | µg/l | 0.02 | 0.02 | WRI Bratislava |
| isoproturon | SPE, LC-MS/MS | µg/l | 0.001 | 0.3 | JRC |
| 4-iso-nonylphenol | EN ISO 18857-2 | µg/l | 0.02 | 0.3 | WRI Prague |
| p-octylphenol | EN ISO 18857-2 | µg/l | 0.005 | 0.1 | WRI Prague |
| pentachlorobenzene | ISO 6486 (LLE, GC-ECD) | µg/l | 0.018 | 0.007 | WRI Bratislava |
| pentachlorophenol | SBSE, GC-MS | µg/l | 0.1 | 0.4 | WRI Bratislava |
| simazine | EN ISO 11369 | µg/l | 0.01 | 1 | VITUKI |
| tributyltin | ISO CD 17353 | µg/l | 0.0002 | 0.0002 | UBA Vienna |
| 1,2,4-trichlorobenzene | ISO 10301 (HS, GC-ECD) | µg/l | 0.5 | 0.4 | WRI Bratislava |
| trichloromethane | ISO 10301 (HS, GC-ECD) | µg/l | 1.8 | 2.5 | WRI Bratislava |
| trifluralin | ISO 6486 (LLE, GC-ECD) | µg/l | 0.005 | 0.03 | WRI Bratislava |
| DDT total* = sum of 4 | | µg/l | | 0.025 | |
| p,p´-DDT | ISO 6486 (LLE, GC-ECD) | µg/l | 0.007 | | WRI Bratislava |
| o,p´-DDT | ISO 6486 (LLE, GC-ECD) | µg/l | 0.007 | | WRI Bratislava |
| p,p´-DDE | ISO 6486 (LLE, GC-ECD) | µg/l | 0.007 | | WRI Bratislava |
| p,p´-DDD | ISO 6486 (LLE, GC-ECD) | µg/l | 0.007 | | WRI Bratislava |
| p-p´-DDT* | ISO 6486 (LLE, GC-ECD) | µg/l | 0.007 | 0.01 | WRI Bratislava |
| isodrin* | ISO 6486 (LLE, GC-ECD) | µg/l | 0.005 | 0.01 (sum of 4) | WRI Bratislava |
| endrin* | ISO 6486 (LLE, GC-ECD) | µg/l | 0.023 | 0.01 (sum of 4) | WRI Bratislava |
| dieldrin* | ISO 6486 (LLE, GC-ECD) | µg/l | 0.021 | 0.01 (sum of 4) | WRI Bratislava |
| aldrin* | ISO 6486 (LLE, GC-ECD) | µg/l | 0.01 | 0.01 (sum of 4) | WRI Bratislava |
| tetrachloroethylene* | ISO 10301 (HS, GC-ECD) | µg/l | 0.5 | 10 | WRI Bratislava |

| Determinand | Method | Unit | LOQ | EQS | Laboratory |
|---|---|---|---|---|---|
| carbontetrachloride* | ISO 10301 (HS, GC-ECD) | µg/l | 1.2 | 12 | WRI Bratislava |
| trichloroethylene* | ISO 10301 (HS, GC-ECD) | µg/l | 1.7 | 10 | WRI Bratislava |

LOQ > EQS
LOQ = EQS

\* = other pollutants from proposed EU Directive
LLE:                    Liquid-liquid-extraction
GC-NCI-MS:              Gas-chromatography and mass spectrometry using negative chemical ionisation
GC-ECD:                 Gas-chromatography using electron capture detector
HS:                     Head-space-analysis
LC-DAD:                 Liquid chromatography using diode-array-detector

The draft EU Directive ("Proposal for a Directive of the European Parliament and of the Council on environmental quality standards in the field of water policy and amending Directive 2000/60/EC") is also proposing the following three EQS for biota (referring to "prey tissue – wet weight"):

– Hexachlorobenzene          10 µg/kg;
– Hexachlorobutadiene         55 µg/kg;
– Mercury                     20 µg/kg.

Out of these substances hexachlorobenzene and hexachlorobutadiene were analysed in JDS2 fish muscle and liver tissue samples.

In addition to WFD priority substances, the following other substances were analysed as well: alkylphenolethoxylates, di-(iso-nonyl)phthalate, organotin compounds, polychlorinated biphenyls (PCB), HHCB (Galaxolide), triclosan and methyl-triclosan.

To get a full picture on pollution by organic substances including those on the WFD list, chapters 16, 18, 19 and 20 should also be referred to.

## 15.2    Methods

The content of priority substances and other pollutants according to WFD in whole water samples was analysed by international standardised methods whenever available. Only for some determinands were in-house-methods used (see Table 19).

Priority substances in other matrices (suspended particulate matter, sediment and biota) were mostly analysed by validated in-house-methods as there are only a few international standards available.

A detailed description of analytical methods and processes is documented in the full report on organic substances on the attached CD-ROM .

### 15.2.1 Analytical quality control

All the laboratories involved in the JDS2 analysis reported achieving an uncertainty of measurement ≤ 50% (k = 2). The analysis of control samples and/or (certified) reference materials is done on a regular basis. However, it has to be stated that for most of the substances, inter-laboratory tests at the concentration level of the EQS are not available.

## 15.3    Results

### 15.3.1 Alkylphenols

During JDS, 2 p-tert-octylphenol (OP) and 4-iso-nonylphenol (NP) were analysed in whole water samples for the first time. The limits of quantification were well below the WFD environmental quality standards.

OP was found at only three sampling sites in concentrations above the limit of quantification (5 ng/l). The proposed EQS of 0.1 µg/l was not exceeded. NP was found in all water samples at concentrations up to 3280 ng/l (sampling site JDS AR2). The highest NP concentration in the Danube River was found at sampling station JDS47 (downstream of Novi-Sad, 141 ng/l). The NP levels exceeding EQS were found at the three sampling stations where OP was also detected (Arges and Russenski Lom tributaries).

NP and OP concentrations show that Arges and Russenski Lom are the tributaries with the most serious organic pollution caused by direct discharge of untreated or inadequately treated wastewater from industry and municipalities.

### Table 20: Sampling stations with the highest concentration of alkylphenols

| Determinand | JDS2 code | Sampling station | Concentration [ng/l] | WFD EQS [ng/l] |
|---|---|---|---|---|
| octylphenol | JDS AR2 | Arges – downstream of Bucharest | 22 | 100 |
| | JDS84 | /Arges – above confluence | 11 | 100 |
| | JDS81 | /Russenski Lom – above confluence | 5 | 100 |
| nonylphenol | JDS AR2 | Arges – downstream of Bucharest | 3280 | 300 |
| | JDS84 | /Arges – above confluence | 1380 | 330 |
| | JDS81 | /Russenski Lom – above confluence | 418 | 330 |
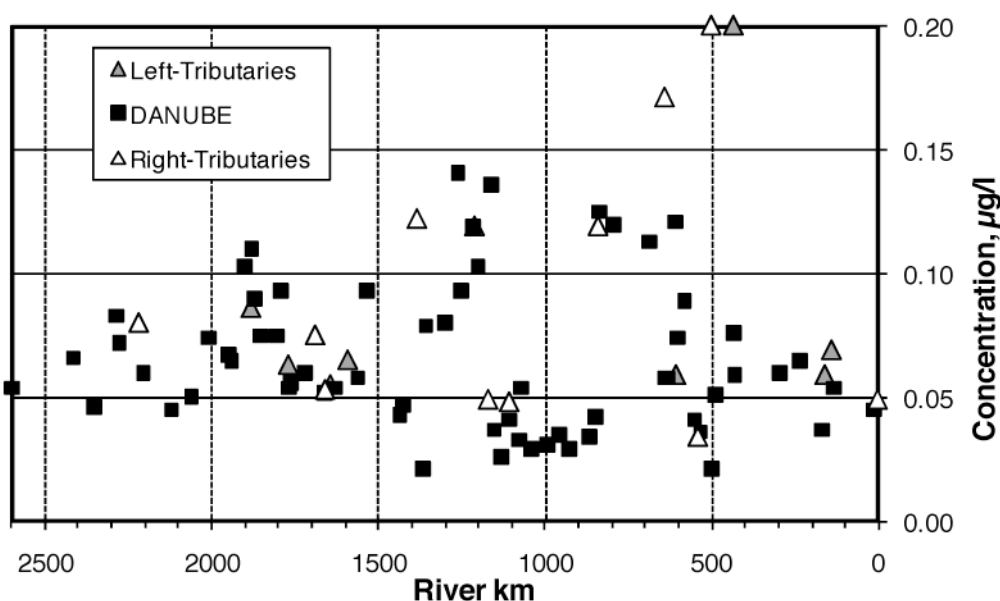


Figure 50: Nonylphenols in water samples

The results for NP in water are in line with the results obtained from suspended particulate matter and sediments.

4-iso-Nonylphenol can be found in all suspended particulate matter samples along the Danube. The highest value (0.280 mg/kg dry matter) was detected downstream of Budapest where the new central sewage plant is still under construction. The impact of Budapest sewage can be seen for more than 200 km. In addition, the Tisza (89 µg/kg dry matter) and Velika Morava (74 µg/kg dry matter) are obviously receiving untreated or insufficiently treated sewage. In the upper and lower parts of the Danube, NP concentrations are always below 0.05 mg/kg dry matter showing only small variations.
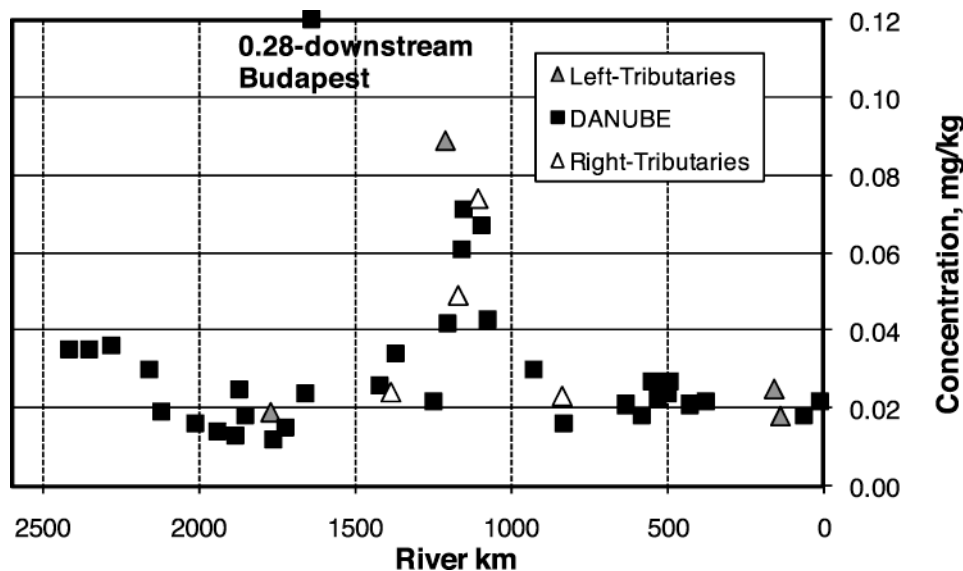


Figure 51: Nonylphenol in suspended particulate matter

Comparing the analytical results from LfU Munich and the University of Vienna at 23 selected sampling sites, about 2/3 of the overlapping samples showed significant differences of more than 35% for NP. These differences might be explained by the analytical methods used. As there is no standard procedure for the analysis of NP and OP in suspended particulate matter, different solvents and extraction techniques were used in the two laboratories.

For OP, only 17 out of 60 samples of suspended particulate matter showed positive results. The highest concentrations were found downstream of Budapest with a maximum value of 0.043 mg/kg dry matter at the Baja station. The concentration of OP is more or less correlated to the concentration of NP indicating the use of mixed tensides. The concentration of OP is usually 2-10 times smaller that the concentration of NP. The ratio NP/OP from 2:1 to 10:1 fits to the production rate of the respective ethoxylates ("NPEOs/OPEOs") of 8:2.

In sediments, OP was only found in 20% of the sediment samples under investigation with a maximum concentration of 0.026 mg/kg dry matter. Positive findings of OP are arbitrarily distributed along the Danube and no hot-spots can be identified. NP could be detected in almost all sediment samples under investigation. Median and maximum concentration of NP were much higher than those for OP, being 0.039 mg/kg dry matter and 1.8 mg/kg dry matter, respectively. NP concentrations for most of the sediment samples were in the range of 0.01 to 0.1 mg/kg dry matter, and only in a few samples higher NP levels were observed. The maximum concentration of NP (1.8 mg/kg dry matter) was found for the sample JDS85 (downstream of the Arges).

### 15.3.2 di-(2-ethylhexyl)phthalate (DEHP)

It is well known that di-(2-ethylhexyl)phthalate (DEHP) is used worldwide in huge quantities and that it can be found in high concentrations in different environmental samples (soil, sewage sludge, water and biota). Due to its ubiquitous presence in plastics, analytical blanks are a serious problem in

laboratories. For this reason a limit of quantification of 0.30 mg/kg dry matter was achieved for suspended particulate matter and 0.2 µg/l for whole water samples.

In all water samples – except four from the upper reach of the Danube – DEHP was detected. The highest concentrations were found at JDS13 (Wildungsmauer, 4.53 µg/l) and JDS35 (Dunavoldfar, 4.42 µg/l). Relatively elevated concentrations of DEHP are present in the middle stretch of the Danube whereas concentrations in the upper and lower parts are mostly <1 µg/l.

In 42 out of 96 water samples (43.8%), the rather high EQS of 1.3 µg/l is exceeded. **Among the priority substances listed in Annexes XI and X of the WFD, DEHP is the most critical substance in water samples and measures have to be taken to reduce its input into the aquatic environment.**
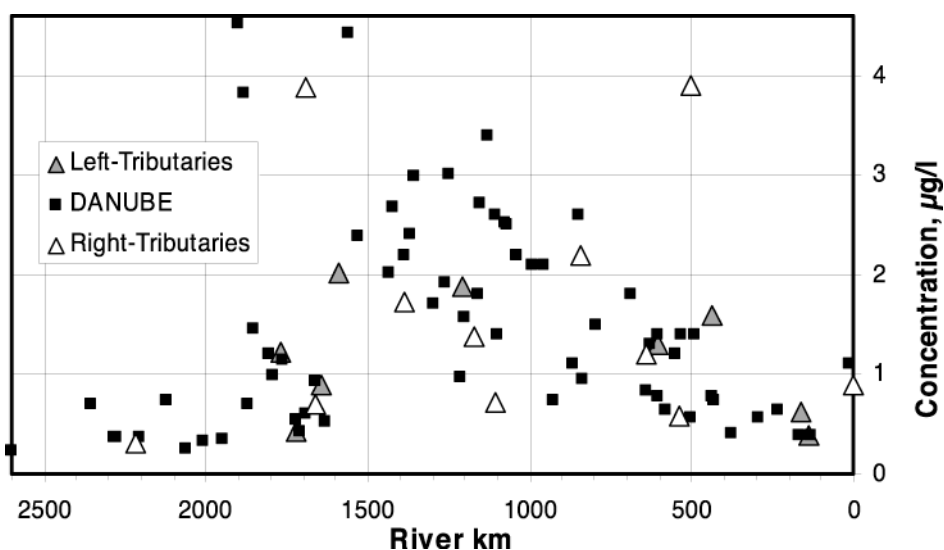


Figure 52: DEHP concentrations in water

In all the JDS2 suspended particulate matter samples, DEHP can be found at concentrations >0.30 mg/kg dry matter. The highest value is almost 10 mg/kg dry matter at the Tisza sampling station (JDS49). The Sava shows a DEHP concentration of 5.03 mg/kg dry matter, which contributes a relevant load to the Danube.

High DEHP concentrations are found in the German stretch of the Danube and again in the middle section. The huge difference in concentrations regarding upstream and downstream Budapest hints at insufficient treated sewage and industrial activities as the main source.
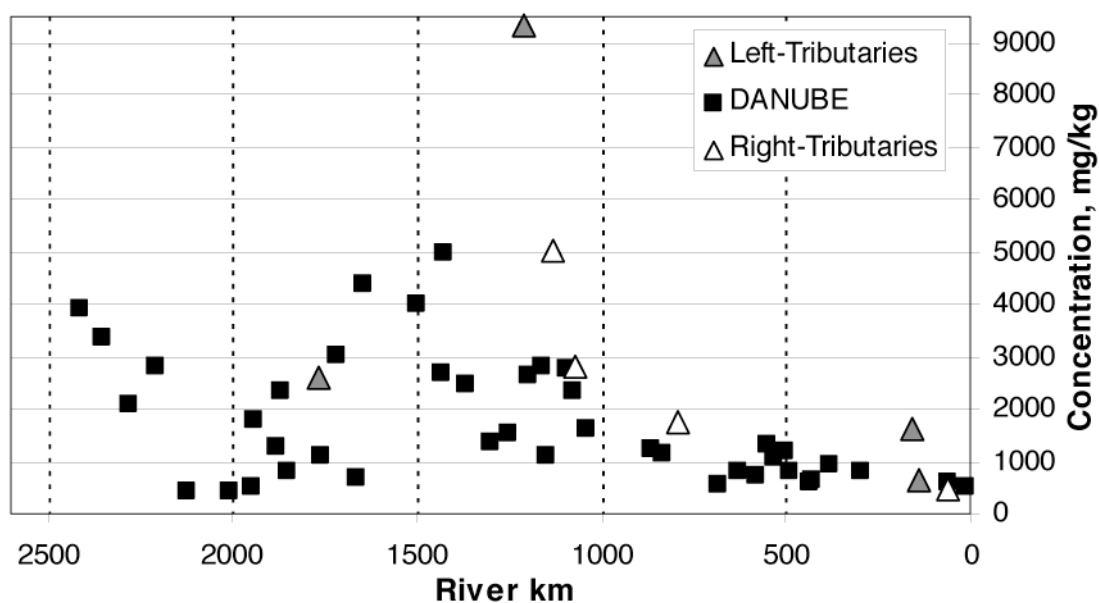
Figure 53: DEHP concentrations in suspended particulate matter

The JDS2 (21 samples >2 mg/kg dry matter) generally showed higher concentrations of DEHP than the JDS1 (3 samples > 2mg/kg dry matter) and the Aquaterra Danube Survey. More sampling stations with relevant concentrations were found in the middle stretch of the Danube during JDS2 than JDS1.

During JDS2, DEHP was found in all sediment samples in concentrations between 0.1 and 1.0 mg/kg dry matter and only a few samples exhibited significantly higher amounts of DEHP. However, no clear trend in DEHP contamination along the course of the River Danube can be identified. Maximum DEHP levels of more than 16 mg/kg dry matter were found for sample JDS85 (downstream of the Arges) i.e. the same sediment that already exhibited elevated amounts of NP.

### 15.3.3 Organotin compounds

23 water samples were selected for the analysis of five organotin compounds. Table 21 shows the statistical evaluation of mean, maximum and minimum values. Mean values were only calculated in the cases where more than 50% of the analysed samples were above the LOQ of 0.2 ng/l.

### Table 21 Results for organotin compounds in water (n=23)

| As cation in ng/l | Dibutyltin | Tributyltin | Tetrabutyltin | Diphenyltin | Triphenyltin |
|:---:|:---:|:---:|:---:|:---:|:---:|
| Mean | 1.1 | | | | |
| Maximum | 9.2 | 14 | < 0.2 | n.d. | < 0.2 |
| Minimum | n.d. | n.d. | n.d. | n.d. | n.d. |

LOD (limit of detection): 0.1 ng/l; LOQ: 0.2 ng/l; n.d. = not detected

Only dibutyltin was found in most of the water samples with a maximum concentration of 9.2 ng/l. In addition, tributyltin was found in 8 out of 23 analysed samples above the LOQ of 0.2 ng/l, which is also the EQS for this substance. The other substances were not detected at levels above the LOQ.

The results of the analysis of suspended particulate matter samples for organotins are shown in Table 22. The maximum concentration of 230 µg/kg dry matter was found for tributyltin at the sampling site JDS53-M. The sample also showed the highest levels for mono- and dibutyltin.

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## Table 22 Results for organotin compounds in suspended particulate matter (n=23)

| As cation in µg/kg dm. | Monobutyl-tin | Dibutyltin | Tributyltin | Tetrabutyltin | Diphenyltin | Triphenyltin |
|---|---|---|---|---|---|---|
| Number < LOQ | 9 | 4 | 3 | 0 | 0 | 4 |
| Maximum | 37 | 75 | 230 | 0 | 0 | 11 |
| Minimum | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |

LOD: 2.5 µg/kg dm.; LOQ: 5 µg/kg dm.

124 sediment samples were analysed for organotin compounds. Monobutyltin and dibutyltin were the most abundant organotin compounds found in the sediment samples. Maximum concentrations for these substances were 38 and 19 µg/kg dry matter. Tributyltin was found in 9 samples with a maximum concentration of 12 µg/kg dry matter.

### 15.3.4 Volatile organic compounds (VOCs)
Only a few VOCs were found in a small number of JDS2 samples at low concentrations close to the limits of quantification.

1,2-Dichloroethane, benzene, dichloromethane, hexachlorobutadiene, trichloromethane, tetrachloromethane and trichloroethylene were not detected in any of the JDS2 samples.

Tetrachloroethylene was found in 2 samples ( JDS2-84M, JDS2-AR2) at a concentration of 0.8 µg/l.

These results confirm the findings of the JDS1 when VOCs were found in only a few samples at low concentrations. As the environmental quality standards for VOCs are relatively high, none were exceeded in JDS2 water samples.

### 15.3.5 Organochlorine compounds
The following organochlorine compounds were analysed: α- endosulfan,  aldrin, dieldrin, isodrin, endrin, trichlorobenzenes, pentachlorobenzene, hexachlorobenzene, chlorfenvinphos, chlorpyrifos, DDT (all isomers and metabolites), lindane, trifluralin and pentachlorophenol.

Organochlorine compounds were not found in water in concentrations above the limit of quantification (LOQ) with the following two exceptions:

i) 1,2,4-Trichlorobenzene was found in only a single sample (JDS2-10M) showing a concentration close to the limit of quantification (0.6 µg/l) which exceeds the proposed EQS of 0.4 µg/l. The other isomers of trichlorobenzene were not detected in any of the JDS2 samples.

ii) Trifluralin was found in a single sample (JDS2-M01) at a concentration of 0.01 µg/l.

For some compounds, the LOQ is higher than the proposed EQS (see Table 19), which disables a proper assessment. In order to have a reliable risk assessment according to the WFD for these substances with a LOQ > EQS the data from other matrices might be taken into regard.

#### 15.3.5.1  Organochlorine compounds in sediment and suspended particulate matter
DDT and its isomer and metabolites analysed in 123 sediment and 23 suspended particulate matter samples showed positive results at only a few sampling sites (mostly in the lower part of the Danube).

Aldrin, dieldrin and isodrin were detected in only very few samples. The highest concentrations of isodrin were found at the German sampling sites JDS1, JDS2 and JDS5. The maximum concentration for isodrin was 94 µg/kg dry matter at JDS1 in sediment from the right bank. Endrin could not be found in any sample.

Trichlorobenzenes, pentachlorobenzene and hexachlorobenzene showed only a few positive results at the lower µg/kg dry matter level. These findings are located in the middle stretch of the Danube.

The insecticides, chlorpyrifos and chlorfenvinphos, also appear at some sites along the middle stretch of the Danube. The highest concentration for chlorfenvinphos was detected at JDS69 at a concentration of 42 µg/kg dry matter.

The only sampling station where trifluralin was found in suspended particulate matter was JDS35 (15 µg/kg dry matter).

Pentachlorophenol (PCP) was not detected in the sediment samples (LOQ being 0.005 mg/kg dry mass). This result is in accordance with the data from the JDS1, where PCP was also not found in the sediments from the Danube. Based on the results of both surveys, the relevance of PCP for the contamination of sediments in the River Danube and its tributaries seems to be rather low.

The results for organochlorine compounds in sediments and suspended particulate matter do not indicate that these substances are relevant pollutants in the Danube catchment area.

### 15.3.6 Polar pesticides

Atrazine, simazin and alachlor were analysed in dissolved water samples. Alachlor was absent in all water samples whereas simazine was found in only three samples at low concentrations (JDS7 0.015 µg/l; JDS25 0.026 µg/l and JDS27 0.055 µg/l). Atrazine could be detected in most of the samples at concentrations around 0.01 µg/l.
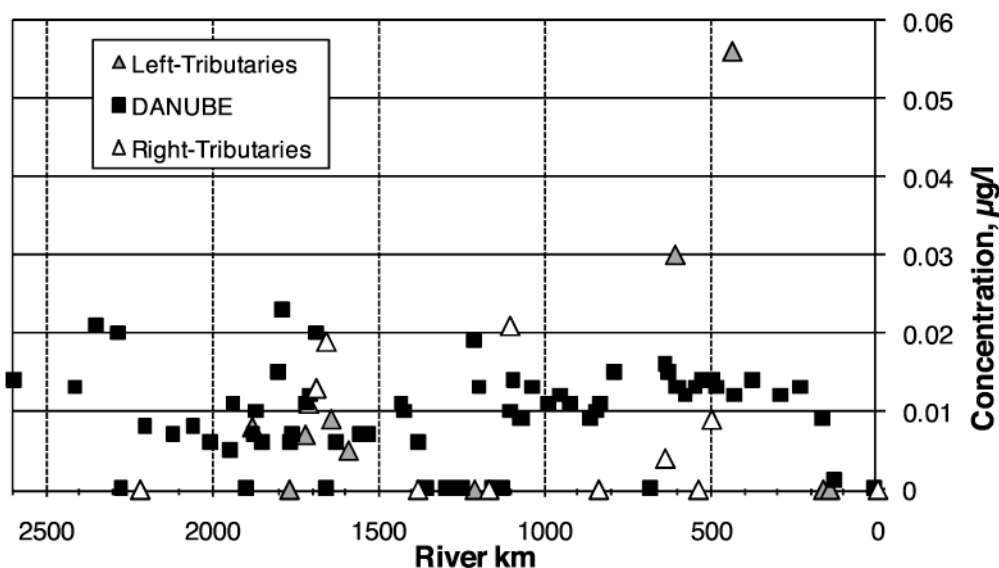


Figure 54: Atrazine concentrations in water samples

The Arges tributary shows the highest concentration (0.056 µg/l) but all findings are well below the proposed EQS of 0.6 µg/l.

The European Commission's Joint Research Centre (EC JRC) in Ispra also analysed desethylatrazine, which is the main metabolite of atrazine (see Chapter 18). The concentrations of desethylatrazine are comparable to the findings for atrazine. This indicates that the atrazine found in river water doesn´t stem from fresh pesticide applications. Comparable concentrations of atrazine and desethylatrazine are found in many groundwaters due to the long-term use of atrazine in agriculture and the degradation process in soils. There is no EQS available for desethylatrazine.

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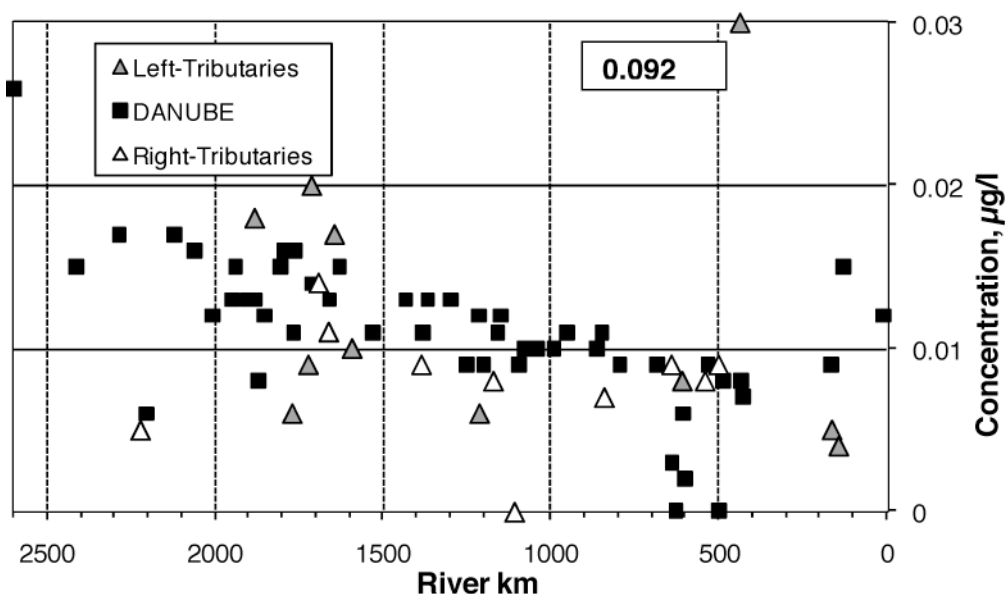


Figure 55: Desethylatrazine concentrations in water samples

Two other herbicides listed in Annex X of the WFD, isoproturon and diuron, were analysed by the EC JRC (see Chapter 18). Using modern LC-MS/MS-techniques, a LOQ of 0.001 µg/l was achieved. In most of the water samples, both isoproturon and diuron were detected in trace amounts below 0.01 µg/l. Isoproturon was found in concentrations of 0.011-0.016 µg/l in three samples and diuron just in a single sample above 0.01 µg/l (JDS11 0,012 µg/l). The EQS for isoproturon and diuron are 0.3 µg/l and 0.2 µg/l respectively. All concentrations are clearly below the EQS.

It has to be noted that the main period of pesticide application is April-July and therefore the JDS results are not representative for this class of compounds.

## 15.3.7 Polybrominated diphenylethers (PBDEs)

For the group of priority substances covered by "brominated diphenylethers" an EQS of 0.0005 µg/l is proposed for the congener numbers  28, 47, 99, 100, 153 and 154 only.

For the JDS2, eight different PBDE-congeners were analysed in water samples. BDE 28 was not analysed.  The limits of quantification for JDS2 samples were 0.002 µg/l (BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, BDE 183) and 0.005 µg/l (BDE 203, BDE 205). The extremely low EQS for PBDEs could not be reached by WRI Prague.

PBDEs were not found in amounts above the limit of quantification in any water sample. In several samples PBDEs were measured in concentrations between the LOQ and the limit of detection (LOD). Pentabromodiphenylether (BDE 99) was detected in four tributaries water sample and two Danube samples.

### Table 23 PBDEs in water samples

| JDS2 Code | Sampling Station | BDE47 ng/l | BDE100 ng/l | BDE99 ng/l | BDE154 ng/l | BDE153 ng/l | BDE183 ng/l | BDE203 ng/l | BDE205 ng/l |
|---|---|---|---|---|---|---|---|---|---|
| JDS54 | Grocka | 0 | 0 | 0 | 0 | 0 | 0 | detected* | 0 |
| JDS82 - M | downstream Ruse/Giurgiu | 0 | 0 | detected* | 0 | 0 | 0 | 0 | 0 |
| JDS84 - M | /Arges | 0 | 0 | detected* | 0 | 0 | 0 | 0 | 0 |
| JDS - AR2 | downstream Bucharest | 0 | 0 | detected* | 0 | 0 | 0 | 0 | 0 |

| JDS2 Code | Sampling Station | BDE47 ng/l | BDE100 ng/l | BDE99 ng/l | BDE154 ng/l | BDE153 ng/l | BDE183 ng/l | BDE203 ng/l | BDE205 ng/l |
|-----------|------------------|------------|-------------|------------|-------------|-------------|-------------|-------------|-------------|
| JDS - RL1 | Basarbovo | 0 | 0 | detected* | 0 | 0 | 0 | 0 | 0 |
| JDS - 91M | /Prut | detected* | 0 | detected* | 0 | 0 | 0 | 0 | 0 |

\* = concentrations between LOD and LOQ.

During the JDS1 neither the bottom sediment nor the suspended particulate matter samples contained PBDEs in concentrations above the limits of detection. Water samples were not analysed in JDS1.

In JDS2 sediment samples from the Danube, only BDE-209 (decabromodiphenyl ether) was found as being relevant. BDE-209, BDE-99 and BDE-100 were detected in only one sediment sample (JDS29, River Danube upstream of Budapest) at a concentration close to the LOQ, whereas all other PBDEs under investigation were not detected at all. During JDS1, only the pentabrominated diphenyl ethers (which are listed in Annex 10 of the WFD) were analysed. These compounds were not detected during either survey, indicating that they have no relevance for Danube sediments.
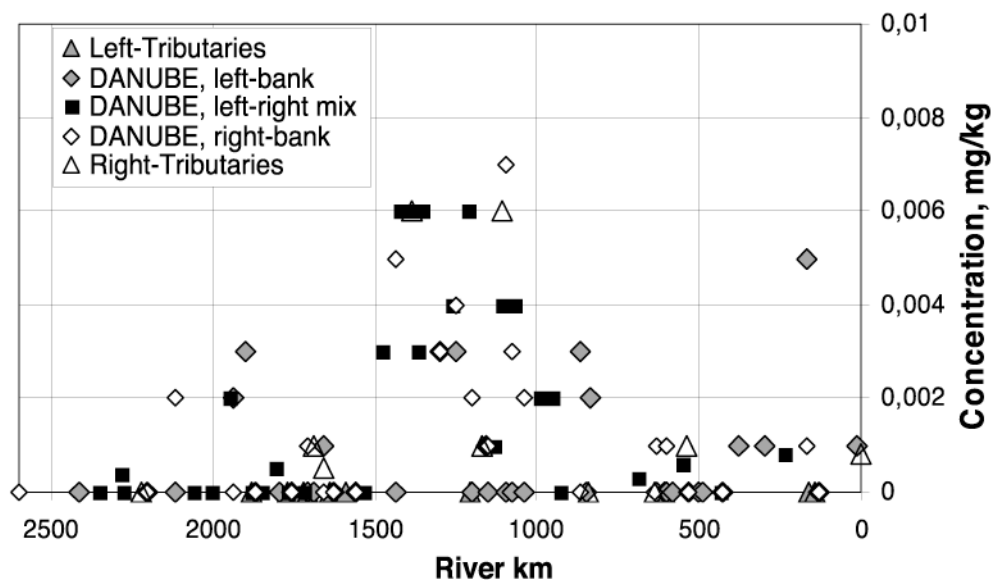


Figure 56: Occurrence of BDE-209 in sediments of the River Danube and its major tributaries

The results for BDE-209 in sediments from the River Danube are summarised in Figure 56. It can be seen that for most samples, concentrations are between <0.00025 and 0.005 mg/kg dry mass with generally higher concentrations in the middle stretch of the Danube. The highest level of BDE-209 was found for a sediment sample from the Velika Morava tributary (sampling location: Varvarin). As these results seem to be the first data on sediment contamination in the Danube with BDE-209, no comparison with former data is feasible.

The EC JRC (Ispra/Italy) was able to analyse WFD-PBDEs in filtered water reaching a LOQ of 0.0000001 µg/l for each single compound (see Chapter 19). Together with results from suspended particulate matter analysis, the concentration for the sum of BDE-congeners 28, 47, 99, 100, 153 and 154 was calculated for 23 sampling sites. The average BDE-concentration was 0.000056 µg/l with a maximum level of 0.000120 µg/l which is still fairly below the EQS of 0.0005 µg/l.

### 15.3.8 Hexachlorobenzene (HCB) and hexachlorobutadiene (HCBD) in fish

The draft "Directive on environmental quality standards in the field of water policy and amending 2000/60/EC" defines EQS for HCB (10 µg/kg wet weight) and HCBD (55 µg/kg wet weight). The wet

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weight refers to "prey tissue", which is not exactly defined. Using JDS2 fish samples, muscle and liver was analysed separately.

When applying the EQS for HCB, not a single sample exceeds a concentration of 10 µg/kg fresh weight and only a few results get close to the EQS.

In most of the samples HCBD was not found. The positive results from the Upper Danube – all far below the EQS - can be explained by a leaching waste dumping site (chemical industry) within the Inn River Basin. This site has been under investigation and decontamination for more than 15 years. Leachate from this dumping ground is treated separately. HCBD concentrations are decreasing from year to year which can also be demonstrated by the low HCBD concentrations in fish muscle tissues.

## 15.3.9 Other organic pollutants

### 15.3.9.1  Alkylphenol ethoxylates

Nonylphenolethoxylates (NP1EOs), nonylphenoldiethoxylates (NP2EOs), octylphenolethoxylate (OP1EO) and octylphenoldiethoxylate (OP2EO) were analysed for the first time in Danube sediments and suspended particulate matter at 23 selected sampling sites.

NP1EOs were found in sediments within a concentration range of 0.021 – 2.096 mg/kg and NP2EO results ranged from 0.024 – 0.280 mg/kg. The highest values were found in the sample from the left bank downstream of the Arges. Concentrations higher than 0.040 mg/kg were mostly found in the middle section of the Danube (rkm 1500-1000) at locations of high NP concentrations.

In the suspended particulate matter samples, NP1EOs were found in a range from 0.020 to 0.125 mg/kg dry matter. NP2EOs were mostly found at a concentration below 0.035 mg/kg dry matter and at only a few sampling sites at concentrations from 0.040 – 0.099 mg/kg dry matter. The highest NP1EOs and NP2EOs concentrations were found at JDS35 (Dunafoldvar) where the highest NP concentration was also found.
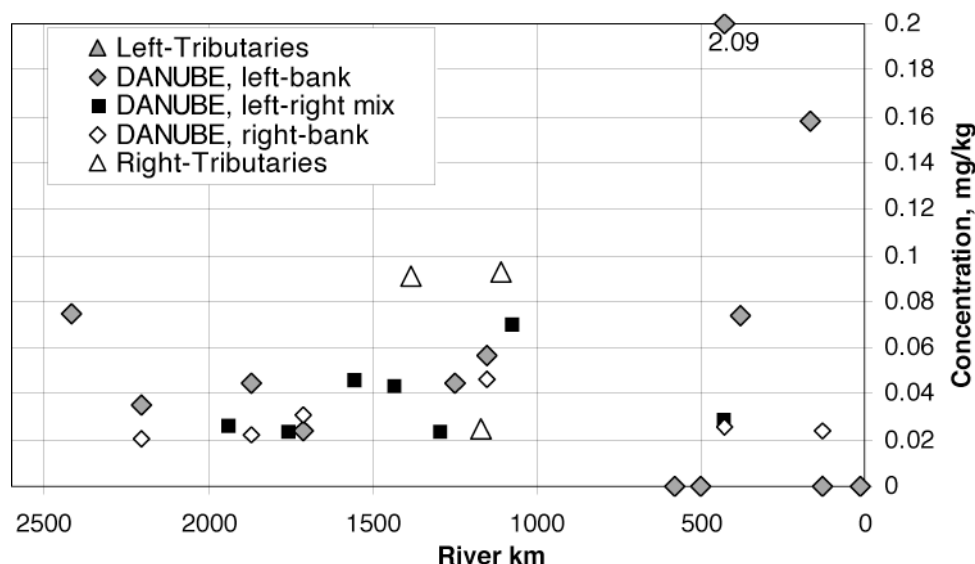


Figure 57: Longitudinal variations of NP1EOs concentrations in bottom sediments
at the selected JDS2 stations

OP1EO and OP2EO were found only once, at the left bank downstream of the River Arges (JDS 85). OP1EO was detected at a concentration of 0.005 mg/kg dry matter and OP2EO at a concentration of 0.007 mg/kg at the same sampling site. In none of the suspended particulate matter samples were OP1EO and OP2EO found.

### 15.3.9.2 Di-(iso-nonyl)phthalate in suspended particulate matter

Di-(iso-nonyl)phthalate was analysed for the first time in Danube suspended particulate matter. The concentrations of di-(iso-nonyl)phthalate and di-(2-ethylhexyl)phthalate (DEHP) in suspended particulate matter show a good correlation. Di-(iso-nonyl)phthalate concentrations are always lower than those of DEHP by a factor of about 3. However there is no EQS available for di-(iso-nonyl)phthalate in suspended particulate matter.
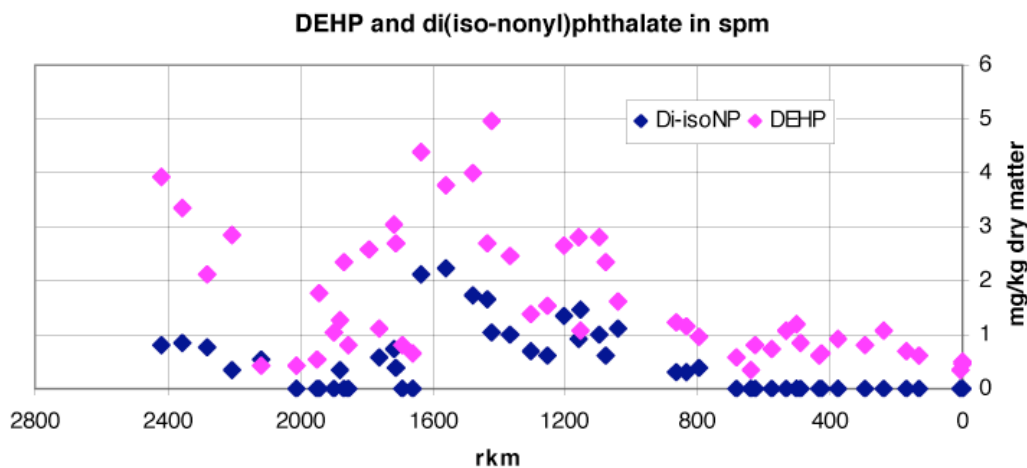


Figure 58 :Comparison of DEHP and di-(iso-nonyl)phthalate in suspended particulate matter

### 15.3.9.3 Chlorinated compounds in fish

Fish muscle and fish liver were analysed for PCB 28, PCB 52, PCB 101, PCB 138, PCB 153, PCB 180, 1,2,4-trichlorobenzene and pentachlorobenzene. At about half of the sampling sites pooled samples were used for analysis.

PCB was found in all fish samples. In most samples the concentrations found in liver were higher than those in muscle tissue. The results for PCB 180 are shown in Figure 59.
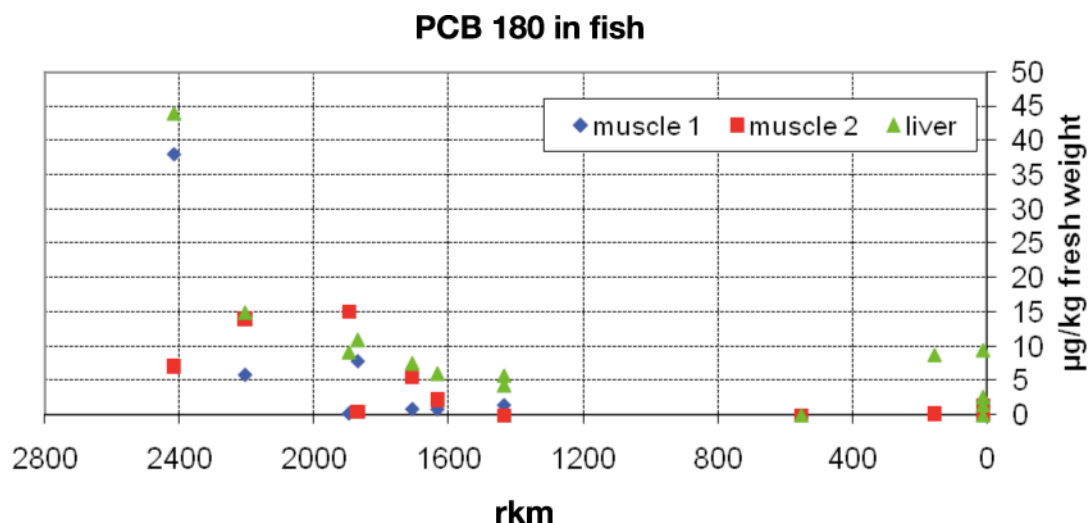


Figure 59: PCB 180 in fish muscle and liver

During JDS1, PCB were not analysed in fish tissue but in mussels. Although fish muscle tissue cannot be compared directly to mussel tissue the order of magnitude of PCB concentration is quite similar.

PCBs were not found in the JDS2 water samples.

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1,2,4-Trichlorobenzene and pentachlorobenzene were found in only a few fish samples at low concentrations. As these concentrations are lower than concentrations for hexachlorobenzene (HCB), HCB can be regarded as an indicator for the group of chlorinated benzenes. The results in fish correspond to low concentrations in the water phase.

### 15.3.9.4 HHCB (Galaxolide), triclosan and methyl-triclosan in suspended particulate matter

HHCB and triclosan are good indicators for municipal waste water discharges to the Danube as they are predominantly used in household and personal care products.

HHCB can be found in suspended particulate matter samples along the Danube down to the Iron Gate reservoir. The highest concentration is found downstream of Budapest where a new wastewater treatment plant is under construction but not yet in operation. In the Lower Danube, HHCB concentrations are below the LOQ of 5 µg/kg dry matter.
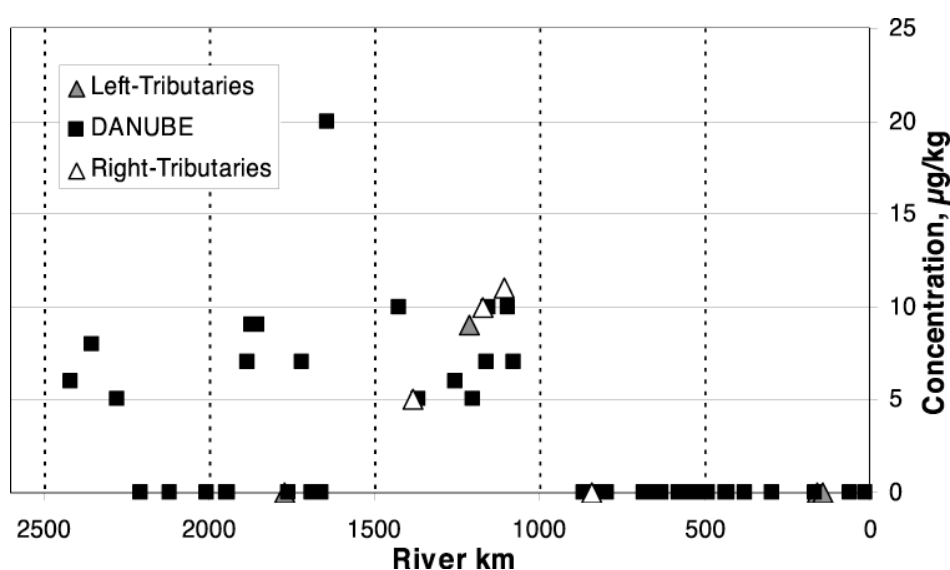


Figure 60: HHCB (Galaxolide) in suspended particulate matter

Triclosan was detected in all samples in the concentration range from 2-46 µg/kg dry matter, whereas the metabolite methyl-triclosan can be found in only a few samples in low concentrations. The highest concentrations for triclosan – similar to HHCB - were found downstream of Budapest.

## 15.4    Conclusions

### 15.4.1 Compliance checking for priority substances according to the WFD

According to Annex 4 of the WFD, surveillance monitoring of priority substances must be done on a monthly basis for the period of one year.

The draft directive ("Proposal for a Directive of the European Parliament and of the Council on environmental quality standards in the field of water policy and amending Directive 2000/60/EC") defines the application of the EQS in the following way:

– "For any given surface water body, applying the EQS-AA ("EQS-annual average") means that, for each representative monitoring point within the water body, the arithmetic mean of the concentrations measured at different times during the year does not exceed the standard";

– "For any given surface water body, applying the EQS-MAC ("EQS-maximum allowable concentration") means that the measured concentration at any representative monitoring point within the water body does not exceed the standard".

For about half of the priority substances the EQS-MAC is marked as "not applicable." In these cases the EQS-AA values are considered protective against short-term pollution peaks in continuous discharges since they are significantly lower than the values derived on the basis of acute toxicity.

The draft directive "Laying down technical specifications for chemical analysis and monitoring of water status" defines the procedure for the calculation of mean values. For results below the limit of quantification, half of the value of the limit of quantification shall be used.

## 15.4.2 Assessment of the indication of the chemical status from JDS2 results

For JDS2 a single water sample was taken at each sampling site. For this reason the assessment of the chemical status cannot be carried out in line with WFD requirements.

The following procedure was decided:

− An **indication** of the chemical status is given for each sampling site (not water body);
− For compliance checking, the proposed EQS-AA for inland waters is used.

Table 24 shows the sampling sites where EQS are exceeded as well as the corresponding parameters.

## Table 24 Sampling sites and parameters exceeding EQS for priority substances

| Sampling station | Parameter/s > EQS | Sampling station | Parameter/s > EQS |
|---|---|---|---|
| JDS10 | 1,2,4-trichlorobenzene | JDS57 | DEHP |
| JDS13 | DEHP | JDS58 | DEHP |
| JDS14 | DEHP | JDS59 | DEHP |
| JDS17 | DEHP | JDS60 | DEHP |
| JDS26 | tributyltin | JDS61 | DEHP |
| JDS34 | DEHP | JDS62 | DEHP |
| JDS35 | DEHP, tributyltin | JDS65 | DEHP |
| JDS36 | DEHP | JDS66 | DEHP |
| JDS38 | DEHP | JDS68 | DEHP |
| JDS39 | DEHP | JDS69 | DEHP |
| JDS40 | DEHP | JDS72 | DEHP |
| JDS41 | DEHP | JDS73 | DEHP |
| JDS42 | DEHP | JDS74 | DEHP |
| JDS43 | DEHP | JDS76 | tributyltin |
| JDS44 | DEHP | JDS79 | DEHP |
| JDS45 | DEHP, tributyltin | JDS80 | tributyltin |
| JDS46 | DEHP | JDS81 | nonylphenol, DEHP |
| JDS47 | DEHP | JDS82 | DEHP |
| JDS49 | DEHP | JDS83 | tributyltin |
| JDS50 | DEHP | JDS84 | nonylphenol, DEHP |
| JDS51 | DEHP | JDS92 | tributyltin |
| JDS52 | DEHP | JDS94 | DEHP |
| JDS53 | DEHP | JDS95 | tributyltin |
| JDS54 | DEHP | | |
| JDS55 | DEHP | | |

## 15.4.3 Assessment of other organic pollutants

For some of the other organic pollutants, only national EQS or EQS proposals can be used for assessment. From the compounds analysed, HHCB and di-(iso-nonyl)phthalate should be discussed as candidates for a revision of the priority substances list.

# 16  PAH and petroleum hydrocarbon contamination in water, suspended particulate matter, sediments and biota

Peter Literathy, Michal Pavonic and Vera Ocenaskova

## 16.1  Introduction

The draft directive ("Proposal for a Directive of the European Parliament and of the Council on environmental quality standards in the field of water policy and amending Directive 2000/60/EC") concerns priority substances. It gives proposals for environmental quality standards (EQS) for eight PAH (polycyclic aromatic hydrocarbon) compounds in water samples, in addition to other organic substances, and four heavy metals, as well as EQS for three compounds in biota. Environmental quality standards are given for different types of waters as "annual averages" and "maximum allowable concentrations."

All 33 priority substances except C10-C13-chloroalkanes (where no method is available) were analysed in the JDS2 water samples. Most of the organic WFD priority substances are discussed in Chapter 1. However, PAHs (several of which are among the 33 priority substances) are discussed together with other indicators of petroleum hydrocarbon contamination (e.g. fluorescence fingerprints and Total Extractable Matter (TEM)).

In addition to the analysis of water samples, in-kind contributions from laboratories provided data for different substances (including both listed priority substances and others) and also for suspended particulate matter, sediments and biota.

For most of the priority substances the limits of quantification (LOQ) were below or at the level of the environmental quality standards (EQS). For some compounds the LOQ did not meet the EQS, so that an assessment of water quality is not possible according to WFD rules.

Furthermore, **since EQS values are defined for the annual average (i.e. mathematical average of 12 measurements within one year), and the JDS2 provided data for only single samples during August/September,, the results of JDS2 are reported as an "indication of chemical status at each sampling site".**

Table 25 gives an overview of determinands, limits of quantification (LOQ), corresponding EQS and the laboratories involved. For substances labelled in yellow, the LOQ was not sufficient for compliance checking except where at least one of the substances was quantified.

### Table 25: Limits of Quantification (LOQ) of analytical methods for PAHs in water samples in comparison to the proposed environmental quality standards (EQS)

| Determinand | Method | Unit | LOQ | AA-EQS | Laboratory |
|---|---|---|---|---|---|
| Anthracene | ISO 17993 (LLE, LC) | µg/l | 0.005 | 0.1 | WRI Bratislava |
| Fluoranthene | ISO 17993 (LLE, LC) | µg/l | 0.004 | 0.1 | WRI Bratislava |
| Naphthalene | ISO 17993 (LLE, LC) | µg/l | 0.250 | 2.4 | WRI Bratislava |
| Benzo(a)pyrene | ISO 17993 (LLE, LC) | µg/l | 0.002 | 0.05 | WRI Bratislava |
| Benzo(b)fluoranthene | ISO 17993 (LLE, LC) | µg/l | 0.005 | 0.03 (sum of 2) | WRI Bratislava |
| Benzo(k)fluoranthene | ISO 17993 (LLE, LC) | µg/l | 0.005 | | WRI Bratislava |
| Benzo(ghi)perylene | ISO 17993 (LLE, LC) | µg/l | 0.002 | 0.002 (sum of 2) | WRI Bratislava |
| Indeno(1,2,3-cd)pyrene | ISO 17993 (LLE, LC) | µg/l | 0.002 | | WRI Bratislava |

LOQ > EQS

## 16.2    Methods

PAHs and TEM were analysed in the original water samples by international standardised methods wherever possible. For fluorescence fingerprints, recording the total fluorescence spectra in 3D (in-house-method) was used.

PAHs, fluorescence fingerprints and TEM in other matrices (suspended particulate matter, sediment and biota) were mostly analysed by validated in-house-methods because there are only a few international standards available.

At the laboratory of the EC JRC/IES, 16 EPA priority PAHs plus benzo(e)pyrene and benzo(j)fluoranthene were analysed in water, suspended particulate matter (SPM) and sediments. Further details of the analysis are given in Chapter 19. A detailed description of the analytical methods and processes are documented in the full report on the CD-ROM attached to this report.

## 16.3    Results

### 16.3.1 PAHs in water

Most of the PAHs in the water samples were below the LOQ and far below the AA-EQS values. Danube laboratories quantified only some of the PAH substances. The results revealed that the AA-EQS values were exceeded as follows:

- benzo(g,h,i)perylene exceeded the AA-EQS (for sum with Indeno(1,2,3-cd)pyrene) in the Velika Morava River (JDS56); and

- the sum of benzo(b)fluoranthene plus benzo(k)fluoranthene, and benzo(g,h,i)perylene plus indeno(1,2,3-cd)pyrene exceeded the AA-EQS in the upper section of the Tisa tributary (JDS-TI4).

In addition to the Danube laboratories, the EC JRC/IES laboratory also analysed water samples for PAHs and the results revealed that the sum of benzo(g,h,i)perylene and indeno(1,2,3-cd)pyrene exceeded the AA-EQS value in five Danube water samples out of 23 analysed samples.

### 16.3.2 PAHs in suspended particular matter and bottom sediments

In the samples collected along the Danube from the 96 JDS2 stations, the concentrations of total PAHs varied between 260 to 1230 μg/kg in the SPM and between 130 to 1850 μg/kg in the bottom sediments. The highest value of total PAHs in bottom sediment samples was observed in the Morava tributary (JDS-MO1, at Lanzhot). The concentration of total PAHs was 5150 μg/kg. This is significantly lower than the maximum (~ 16000 μg/kg) measured during JDS1.

The proposed replacement of Annex X of Directive 2000/60/EC recommends the priority substance fluoranthene as an indicator of other, more dangerous PAHs. In addition, the proposed Priority Substance data sheets (under preparation) provide EQS for benzo(a)pyrene and benzo(k)fluoranthene for SPM. The corresponding concentrations of these substances for protecting the pelagic community in freshwater are 2940 and 326 μg/kg, respectively. Taking into account these proposals, Figure 61 and Figure 62 show the longitudinal variation in the concentration of fluoranthene,  and benzo(a)pyrene and benzo(k)fluoranthene, respectively, in the SPM.
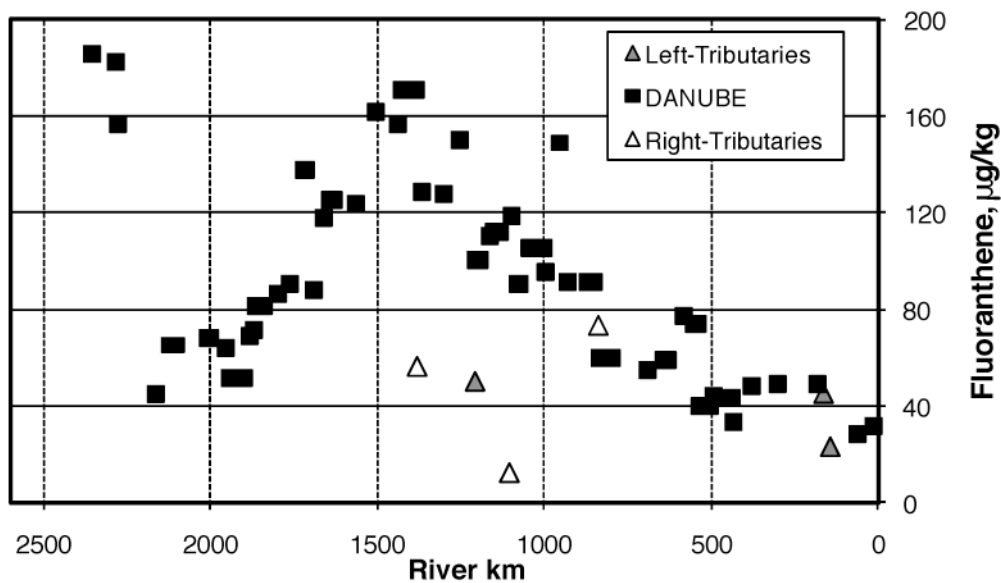
Figure 61: Longitudinal variation in fluoranthene determined in SPM during JDS2

The longitudinal profile of fluoranthene (Figure 61) indicates the highest concentration in the Upper Danube reach; however, after a sudden decrease a "hump" developed along the middle section of the Danube. This distribution pattern is typical for the sum of PAHs as well as the variation in concentration of the other two PAH compounds, benzo(a)pyrene and benzo(k)fluranthene as it is shown in Figure 62.

Comparing the concentrations of these two PAH compounds with the proposed EQS for SPM, the results indicated that even the maximum concentrations were far below the recommended limit values, i.e. the maximum concentration of benzo(a)pyrene was more than 20-times less than the EQS, and the concentration of benzo(k)fluoranthene was about one-fifth of the proposed EQS for SPM.
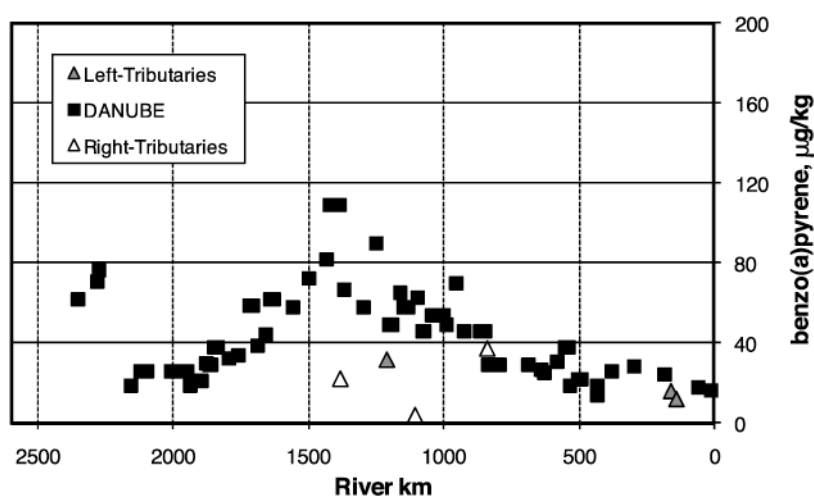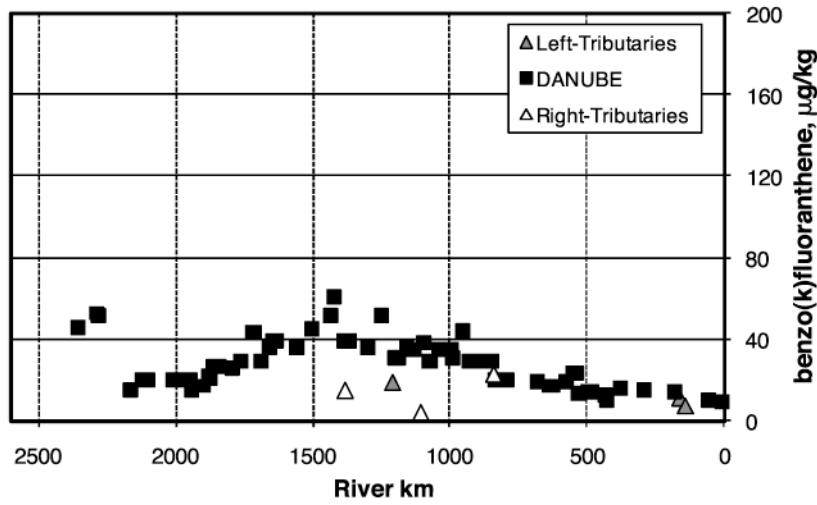
Figure 62: Longitudinal variation in benzo(a)pyrene and benzo(k)fluoranthene determined in SPM during JDS2

The concentration of all three PAH compounds was higher in the SPM of the Danube than in the tributaries. The variation in the concentration of the characteristic compound, fluoranthene, in the bottom sediment is shown in Figure 63. Although the overall concentration levels were very similar in water and SPM, bottom sediment of the tributaries contained significantly higher concentrations compared to the Danube, reaching the highest measured value of 853 μg/kg in the Iskar River. This indicates the fact that PAHs are accumulating and reaching high concentrations in the bottom sediments of the tributaries, which were not re-suspended and transported through the SPM during the low water conditions of the survey. However, the sediment-bound PAHs can be re-suspended and transported to the Danube during floods.
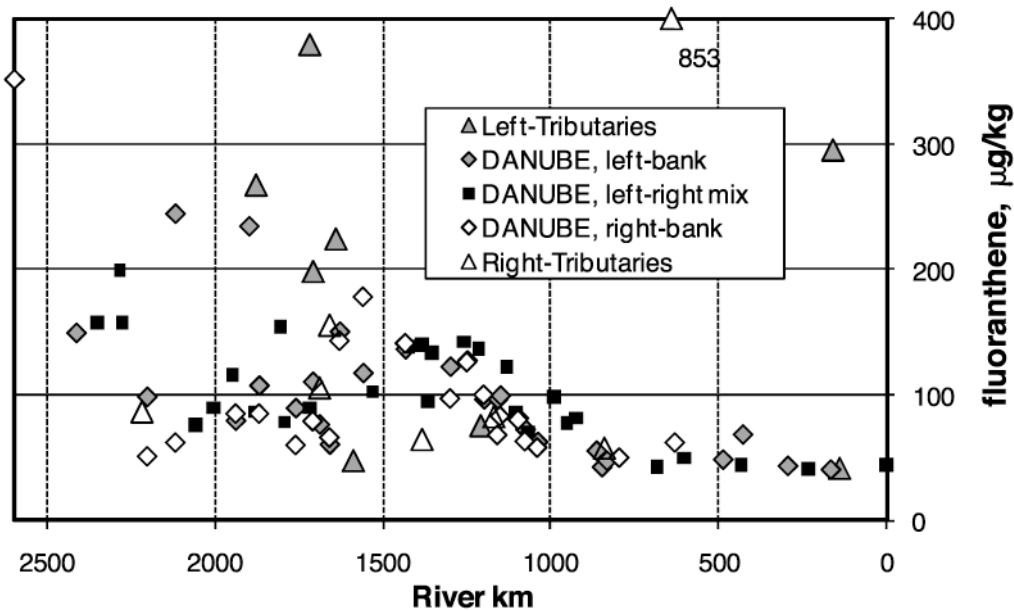


Figure 63: Longitudinal variation in fluoranthene determined in bottom sediment during JDS2

In general, the concentration levels of the characteristic PAH compounds in both the SPM and the bottom sediment do not show significant problems. Comparison with the data obtained within the JDS1 indicates that there is a certain improvement in the central part of the Danube itself, while the tributaries showed higher fluctuations during the JDS2.

### 16.3.3 PAHs analyzed by EC JRC/IES

In addition to the Danube laboratories, EC-JRC also analysed PAHs in different matrices at selected JDS2 sites. The results are given in Chapter 19.

### 16.3.4 Total Extractable Matter (TEM)

In addition to the PAHs, the characterisation of water pollution by oil, petroleum products, etc., can be estimated with the measurement of TEM. The measurement results for TEM in SPM and bottom sediments are demonstrated in Figure 64 and Figure 65 respectively.
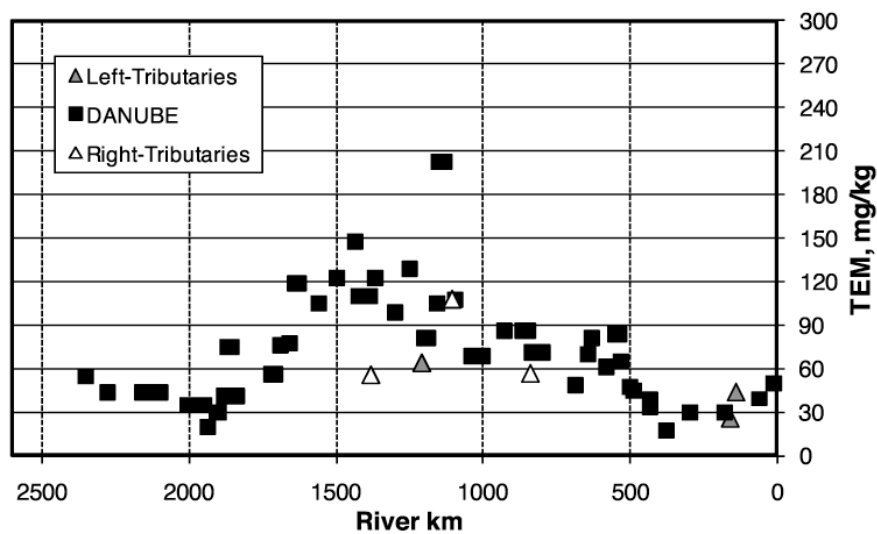


Figure 64: Variation in the TEM concentration in the SPM in the Danube and its major tributaries
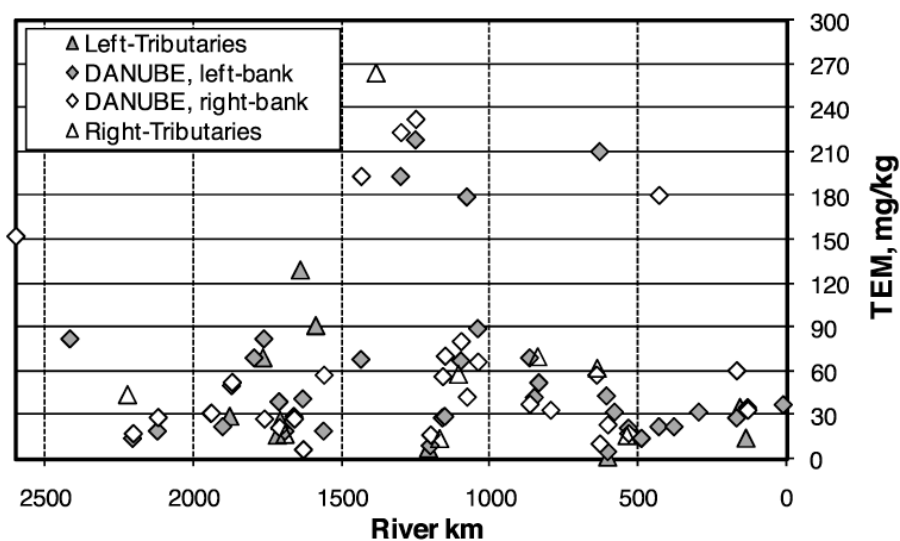


Figure 65: Variation in the TEM concentration in the bottom sediments in the Danube and its major tributaries

The concentration distribution patterns of TEM in both matrices show similarity to the fluoranthene concentrations, supporting the relationship between oil contamination and PAHs.

### 16.3.5 Fluorescence fingerprints

Fluorescence fingerprints were successfully used for characterising petroleum-related contamination and screening for unknown trace organics during JDS1. The total (3D) fluorescence spectra of the cyclohexane extract of water, SPM and bottom sediments were compared to arbitrary standards e.g. gasoline, diesel and crude oil. The character of the pollutants was evaluated by comparing the concatenated fluorescence spectra of the samples to the arbitrary standards. As an example, Figure 66 shows the correlation coefficients obtained for water and SPM samples.
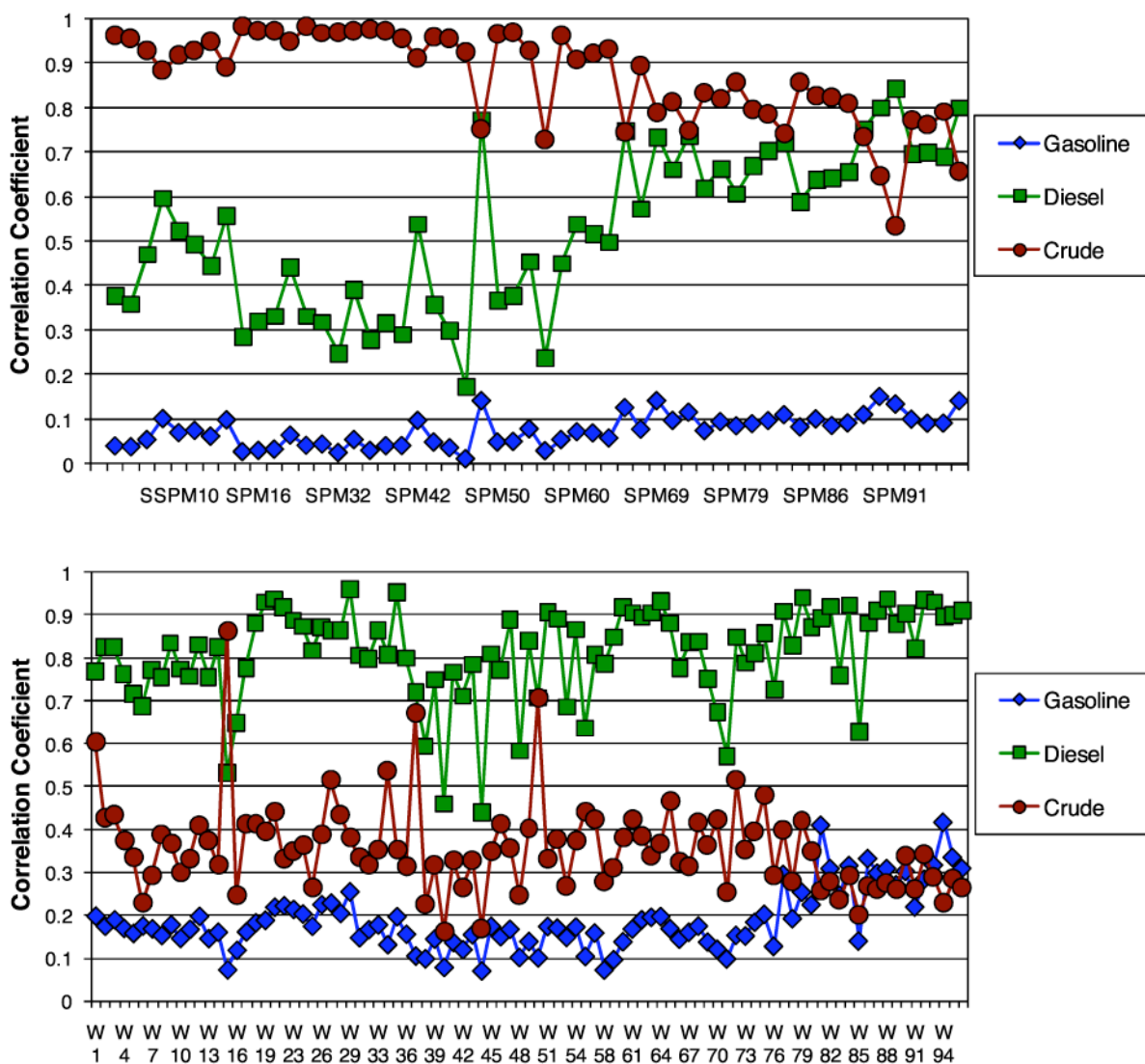


**Figure 66: Characterisation of hydrocarbon contamination by correlating fluorescence spectra of water (W) and SPM sample extracts with the arbitrary standards**

The correlation coefficients obtained in the JDS2 water and SPM samples reveals the characteristics of the oil pollution. According to the results shown in Figure 66 diesel oil type pollution was characteristic in the water column and crude oil type in the SPM, as well as in the bottom sediments. The fluorescence characteristics of the SPM and bottom sediment samples are in line with the PAH

results, showing the presence of the  PAHs with more rings (characteristic of crude oil) in the SPM and bottom sediments.

## 16.4    Conclusions

### 16.4.1 Compliance checking for priority substances according to WFD

The requirements of the WFD surveillance monitoring of priority substances aredescribed in more detail in the Chapter 1.

### 16.4.2 Assessment of indication of chemical status from the JDS2 results

Most of the PAH in water samples were far below the AA-EQS values and the values for sediments were about one order of magnitude lower than those typically found in the River Elbe.

Table 26 shows the sampling sites where AA-EQS are exceeded in the case of the corresponding PAHs.

### Table 26: Sampling sites and parameters exceeding AA-EQS values for the priority PAHs

| JDS2 code | PAH compound | Sampling station | Concentration [µg/l] |
|---|---|---|---|
| JDS56 | ∑Benzo(g,h,i) perylene & Indeno(1,2,3-cd)pyrene | /Sava 7.0 rkm | 0.004 <0.002 |
| JDS-TI4 | ∑Benzo(b)fluoranthene & Benzo(k)fluoranthene | /Tisa, upstream Danube | 0.074 |
| | ∑Benzo(g,h,i)perylene & Indeno(1,2,3-cd)pyrene | | 0.052 |
| From analytical results of 23 sites' samples analysed by EC JRC/IES | | | |
| JDS2 | ∑Benzo(g,h,i)perylene & Indeno(1,2,3-cd)pyrene | Kelheim –  2415 rkm | > 0.002 |
| JDS16 | ∑Benzo(g,h,i)perylene & Indeno(1,2,3-cd)pyrene | Bratislava – 1869 rkm | > 0.002 |
| JDS39 | ∑Benzo(g,h,i)perylene & Indeno(1,2,3-cd)pyrene | Hercegszanto – 1434 rkm | > 0.002 |
| JDS92 | ∑Benzo(g,h,i)perylene & Indeno(1,2,3-cd)pyrene | Reni – 130 rkm | > 0.002 |
| JDS95 | ∑Benzo(g,h,i)perylene & Indeno(1,2,3-cd)pyrene | Sulina-arm – 0 rkm | > 0.002 |

# 17 Heavy metals and arsenic in water, suspended particulate matter, sediments and biota

Peter Literathy, Ioana Enache, Michal Pavonic, Vera Ocenaskova and Jürgen Diemer

## 17.1 Introduction

The water solubility of heavy metals and arsenic is limited in natural water, and most of them are readily associated with the solid phase (particulate matter) either in suspension or after settling in the bottom sediment. Therefore during JDS2, they were analysed in water, suspended particulate matter (SPM) and bottom sediments as well as in mussels and fish. The investigated elements were categorised into three groups as follows:

– Group 1: Heavy metals included in the Priority List of the Water Framework Directive (WFD): Cadmium (Cd), lead (Pb), mercury (Hg) and nickel (Ni);

– Group 2: Other heavy metals and arsenic: Arsenic (As), chromium (Cr), copper (Cu) and zinc (Zn) as well as bismuth (Bi), cobalt (Co), molybdenum (Mo) in SPM only, and Co, titanium (Ti) and vanadium (V) in bottom sediments only;

– Group 3: Other metals (important for overall assessment): Aluminium (Al), iron (Fe) and manganese (Mn).

Table 27 gives an overview on the limits of quantification (LOQ); the corresponding EQS for the relevant heavy metals dissolved in water and the laboratory involved.

## Table 27: LOQs of analytical methods for WFD target compounds in water samples in comparison with EQS (including tentative Austrian EQSs for Cu and Zn)

| Determinand | Unit | LOQ | AA-EQS |
|---|---|---|---|
| Cadmium | µg/l | 0.2 | |
| If Total Hardness is: | | | ≤ 0.08 |
| Class 1: <40 mg/l CaCO$_3$ | µg/l | | 0.08 |
| Class 2:  40 to <50 mg/l CaCO$_3$ | µg/l | | 0.09 |
| Class 3:  50 to <100 mg/l CaCO$_3$ | µg/l | | 0.15 |
| Class 4: 100 to <200 mg/l CaCO$_3$ | µg/l | | 0.25 |
| Class 5: ≥200 mg/l CaCO$_3$ | µg/l | | |
| Lead | µg/l | 0,004 | 7.2 |
| Mercury | µg/l | 0.05 | 0.05 |
| Nickel | µg/l | 0,5 | 20 |
| Copper | µg/l | 2.0 | |
| If Total Hardness is: | | | |
| < 50 mg/l CaCO$_3$ | | | 1.1 |
| 50 to 100 mg/l CaCO$_3$ | | | 4.8 |
| >100 mg/l CaCO$_3$ | | | 8.8 |

| Determinand | Unit | LOQ | AA-EQS |
|---|---|---|---|
| **Zinc**<br>If Total Hardness is: | µg/l | 5.0 | |
| <50 mg/l CaCO$_3$ | | | 7.8 |
| 50 to 100 mg/l CaCO$_3$ | | | 35.1 |
| >100 mg/l CaCO$_3$ | | | 52 |
| | | LOQ > EQS | LOQ = EQS |

For yellow labelled substances the LOQ was not sufficient for compliance checking. There are different recommended national, and in some cases regional and/or international standards, for these elements in the sediments and biota, which will be considered during evaluation of the JDS2 results

In addition to the EQS for priority heavy metals dissolved in water, according to paragraph 3 of Article 2 (Environmental quality standards) in the proposed directive on environmental quality standards in the field of water policy and amending Directive 2000/60/EC, the Member States shall ensure that the methyl-mercury concentration of 20 µg/kg in prey tissue (wet weight) for biota (fish, molluscs, crustaceans and others) is not exceeded.

Table 28 shows the involvement of various laboratories in performing element analysis of different matrices, including overlaps in several cases.

## Table 28: Laboratories performing heavy metals and arsenic analysis in different matrices

| Environmental matrix | Element analysed | Laboratory |
|---|---|---|
| Water, dissolved | Cd, Pb, Hg, Ni<br>As, Cr, Cu. Zn | Water Research Institute T.G.M.<br>Prague, Czech Republic |
| Suspended Particulate Matter | Cd,Pb,Hg, Ni<br>As, Bi, Co, Cr Cu, Mo, Zn<br>Mn | Bavarian Environment Agency<br>Augsburg, Germany |
| | Al, Fe, Mn | VITUKI, Budapest, Hungary |
| Bottom Sediment | Cd, Pb, Hg, Ni<br>As, Cr, Cu. Zn<br>Al, Fe, Mn | ICIM, Bucharest, Romania |
| | Cd, Pb, Hg, Ni<br>As, Co, Cr, Cu.Ti, V, Zn<br>Al, Fe, Mn<br>Si, Ca, Mg, Na, K | EC JRC/IES, Ispra, Italy |
| Biota (mussel, fish) | Cd, Pb, Hg, Ni<br>As, Cr, Cu. Zn | Water Research Institute T.G.M.<br>Brno, Czech Republic |

## 17.2    Methods

<u>Surface water samples</u> were filtered through 0.45 µm pore-size membranes on-board, acidified to pH <2 with nitric acid and stored in a plastic bottle. The samples for mercury analysis were stored in a glass container.

<u>Suspended particulate matter and bottom sediment samples</u> (wet sieved for less than 63 micron grain-size) were freeze-dried ready for analysis.

Biota samples were prepared differently: (a) mussels were freeze-dried and the dry material analysed (results expressed in "dry weight") and (b) fish were analysed as frozen samples (results expressed in "wet weight").  NB: Methyl-mercury was not analysed in biota.

The various laboratories used different analytical methods including:

–    AAS with Graphite Furnace for Cd, Pb, Ni, Cu and Cr in water and sediment;

–    AAS with Cold Vapour for Hg (for Hg, AMA method was also used);

–    Hydride AAS for As;

–    AAS with flame for Zn, Al, Fe, Mn;

–    ICP with Optical Emission Spectrometry;

–    ICP with Mass Spectrometry for all metals, except Hg, in SPM;

–    WDXRF (extreme high LOQ) for all elements except Hg.

Detailed descriptions of the analytical procedures as well as the analytical quality control measures are given in the full report on the attached CD-ROM.

## 17.3    Results

### 17.3.1 Heavy metals and As in water

Results of the determination of the dissolved heavy metals in the surface water samples are summarised in Table 29 and Table 30.

### Table 29: Minimum and maximum concentration of dissolved heavy metals and As in water

| Elements | Concentration [µg/l] | | | |
| --- | --- | --- | --- | --- |
| | Danube | | Tributaries | |
| | Minimum | Maximum | Minimum | Maximum |
| Cadmium (Cd) | <0.2 | < 0.2 | <0.2 | < 0.2 |
| Lead (Pb) | <2.0 | < 2.0 | <2.0 | 5.07 |
| Mercury (Hg) | <0.05 | 0.071 | <0.05 | < 0.05 |
| Nickel (Ni) | <2.0 | 12.2 | <2.0 | 33.3 |
| Arsenic (As) | <0.8 | 4.31 | <0.8 | 13.2 |
| Chromium (Cr) | <0.5 | 1.26 | <0.5 | 1.73 |
| Copper (Cu) | <2.0 | 4.59 | <2.0 | 34.5 |
| Zinc (Zn) | <5.0 | 16.1 | <5.0 | 67.9 |

Table  27 demonstrated the problem of evaluation of the Cd results pertaining to the AA-EQS because the LOQ of the method used is higher than the relevant total hardness dependent EQS values. Although the total hardness in most water samples is in the range of 100 to 200 mg/l $CaCO_3$, Table 29 shows that the Cd has not been quantified in any of the water samples; therefore, the exceedance of the EQS for Cd can not be evaluated.

## Table 30: Highest concentrations of heavy metals and As in water

| Element | JDS 2 code | Sampling station | Concen-tration [µg/l] | AA-EQS* [µg/l] | AA-EQS** [µg/l] |
|---------|-----------|------------------|-----------------------|----------------|-----------------|
| Pb | JDS-IS2 | Before the Iskar Reservoir | 5.07 | 7.2 | - |
| Hg | JDS32 | Downstream of Budapest | 0.071 | 0.05 | - |
|  | JDS33 | Adony/Lórév | 0.063 | 0.05 | - |
| Ni | JDS66 | /Timok (rkm 0.2) | 33.3 | 20 | - |
|  | JDS32 | Downstream of Budapest | 12.2 | 20 | - |
|  | JDS15 | /Morava (rkm 0.08) | 7.63 | 20 | - |
| As | JDS24 | /Hron (rkm 0.5) | 13.2 | - | - |
|  | JDS-MO2 | Dyje - Pohansko | 7.53 | - | - |
|  | JDS37 | Sio (rkm 1.0) | 7.44 | - | - |
| Cr | JDS-RL2 | Beli Lom, Pisanetz | 1.73 | - | - |
|  | JDS-IS2 | Before the Iskar Reservoir | 1.67 | - | - |
|  | JDS81 | /Russenski Lom | 1.26 | - | - |
| Cu | JDS66 | /Timok (rkm 0.2) | 34.5 | - | 8.8 |
|  | JDS85 | Downstream of Ruse/Giurgiu | 14.6 | - | 8.8 |
|  | JDS81 | /Russenski Lom | 11.2 | - | 8.8 |
| Zn | JDS-IS2 | Before the Iskar Reservoir | 67.9 | - | 35.1 |
|  | JDS83 | Upstream of Arges | 16.1 | - | 35.1 |
|  | JDS66 | /Timok (rkm 0.2) | 9.3 | - | 52 |

*Proposal for amending Directive 2000/60/EC    **Austrian proposal, hardness dependent

Concerning the other heavy metals on the list of Priority Substances, Pb did not reach the EQS at any sampling sites. The concentration of Hg slightly exceeded the EQS (by 30-40%) at two sites downstream of Budapest, measuring 0.071 and 0.063 µg/l at JDS32 and JDS33, respectively. The concentration of Ni exceeded the EQS by 67% in the Timok River at its confluence with the Danube.

Two more heavy metals, Cu and Zn, require further attention. Austria and Slovakia are preparing tentative EQS for these two metals depending on the total hardness of the water. The JDS2 results for the two metals revealed the following:

− In the case of Cu, the exceedance of the proposed Austrian EQS was observed in three water samples: in the Danube downstream of Ruse/Giurgiu (JDS85) and in two tributaries, the Timok (JDS66) and the Russenski Lom (JDS81). The total hardness in these water samples was 101, 311 and 226 mg/l $CaCO_3$, respectively, and accordingly, the EQS for Cu was 8.8 µg/l for each sample. The results showed that the Cu concentration in the Timok River exceeded the Austrian EQS by around four times.

− In the case of Zn, a relatively high concentration was measured in three samples, but only one exceeded the hardness dependant Austrian EQS (in the upstream section of the Iskar tributary - station JDS-IS2). (NB: The total hardness at JDS66 (Timok), JDS83 (Danube upstream of the Arges) and JDS-IS2 was 311, 99 and 100 mg/l $CaCO_3$, respectively).

Most of the other heavy metals and As were present in concentrations below the LOQ or slightly above this limit. Chromium was found at few stations, mostly in the Lower Danube tributaries e.g. Russenski Lom and Iskar.

It should be taken into account that these results for water samples characterise the dissolved substances. However, the major transportation route of the heavy metals is in the SPM, which could accumulate in the bottom sediment. Therefore, characterisation of heavy metals in the solid phase is equally important.

### 17.3.2 Heavy metals and As in suspended particulate matter (SPM)

#### 17.3.2.1  Quality standards
For the evaluation of heavy metals in SPM and sediments, there are various sources of environmental quality standards available (see Table 31):

–    Quality targets used for the evaluation of JDS1 data;

–    Dutch quality standards – target values according to "Staatscourant, The Netherlands, June 2000;"

–    Target values from the International Commission for the River Rhine.

### Table 31: Quality targets for heavy metals and As in sediments (including both SPM and bottom sediments)

| Element | Sediment quality target during JDS1 [mg/kg] | Dutch target value* [mg/kg] | Rhine target value [mg/kg] |
|---|---|---|---|
| Cadmium | 1.2 | 0.8 | 1 |
| Lead | 100 | 85 | 100 |
| Mercury | 0.8 | 0.3 | 0.5 |
| Nickel | 50 | 35 | 50 |
| Arsenic | 20 | 29 | 40 |
| Chromium | 100 | 100 | 100 |
| Copper | 60 | 36 | 50 |
| Zinc | 200 | 140 | 200 |
| Molybdenum | - | 3 | - |

* The Dutch target values are given for normalised sediment [10% TOC and 25% lutum (clay)]

As there is a rather high coherence between all the above mentioned quality standards, those used for JDS1 evaluation were also used this time.

#### 17.3.2.2  Longitudinal profile of the Group 1 heavy metals (Cd, Pb, Hg and Ni)
Particular attention was given to WFD priority substances Cd, Pb, Hg and Ni during the data interpretation. Figure 67 shows the longitudinal variation of these heavy metals in suspended particulate matter in the Danube and its tributaries.
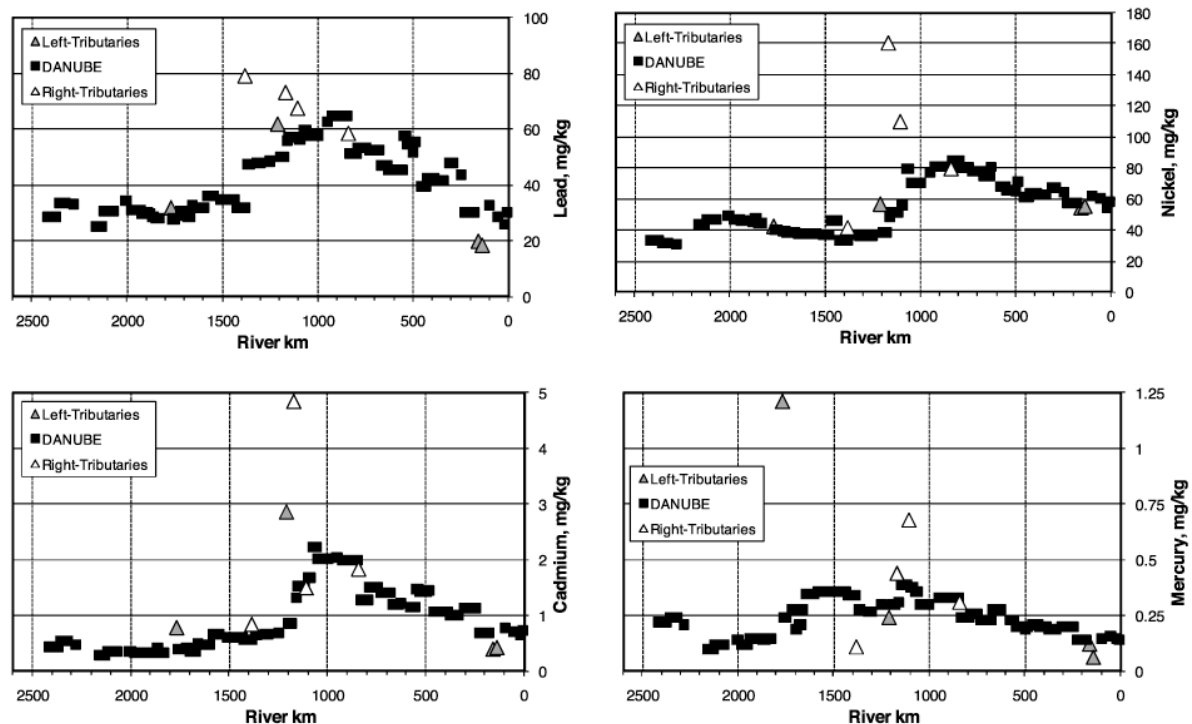
Figure 67: Distribution of Cd, Pb, Hg and Ni in the SPM along the Danube River

Figure 67 clearly demonstrates the significant impact of the two major tributaries, the Tisa and the Sava, in increasing the concentration of Cd in the SPM along the Lower Danube reach. The 1.2 mg/kg sediment standard was significantly exceeded in the two rivers. As a result of the transport of these metals to the Danube, their impact on the Danube SPM was obvious along a 1,000 km Danube reach downstream of the confluence with the Sava River.

The concentration of Pb in the SPM did not reach the 100 mg/kg value of the sediment standard but the polluting effect of the tributaries, the Drava, Tisa, Sava and the Velika Morava, is demonstrated by the elevated Pb concentrations in the Danube SPM.

The Hg concentration in the SPM exceeded the sediment standard (0.8 mg/kg) in the Vah tributary only. An increased concentration was also found in the Velika Morava; however, this was slightly below the sediment standard. The Hg concentration in the Danube SPM slightly exceeded the Dutch target value of 0.3 mg/kg from the confluence with the Vah down to the Iron Gate. The Ni contamination profile in the SPM clearly demonstrates the significant effect of the SPM transported through the Sava (161 mg Ni/kg)  and Velika Morava (110 mg Ni/kg).

### 17.3.2.3  Longitudinal profile of the Group 2 heavy metals  (Cr, Cu, Zn, Bi and Mo) and As
Figure 68 shows the longitudinal variation in the concentration of As, Cr, Cu, Zn, Bi and Mo in the SPM along the Danube and its tributaries.
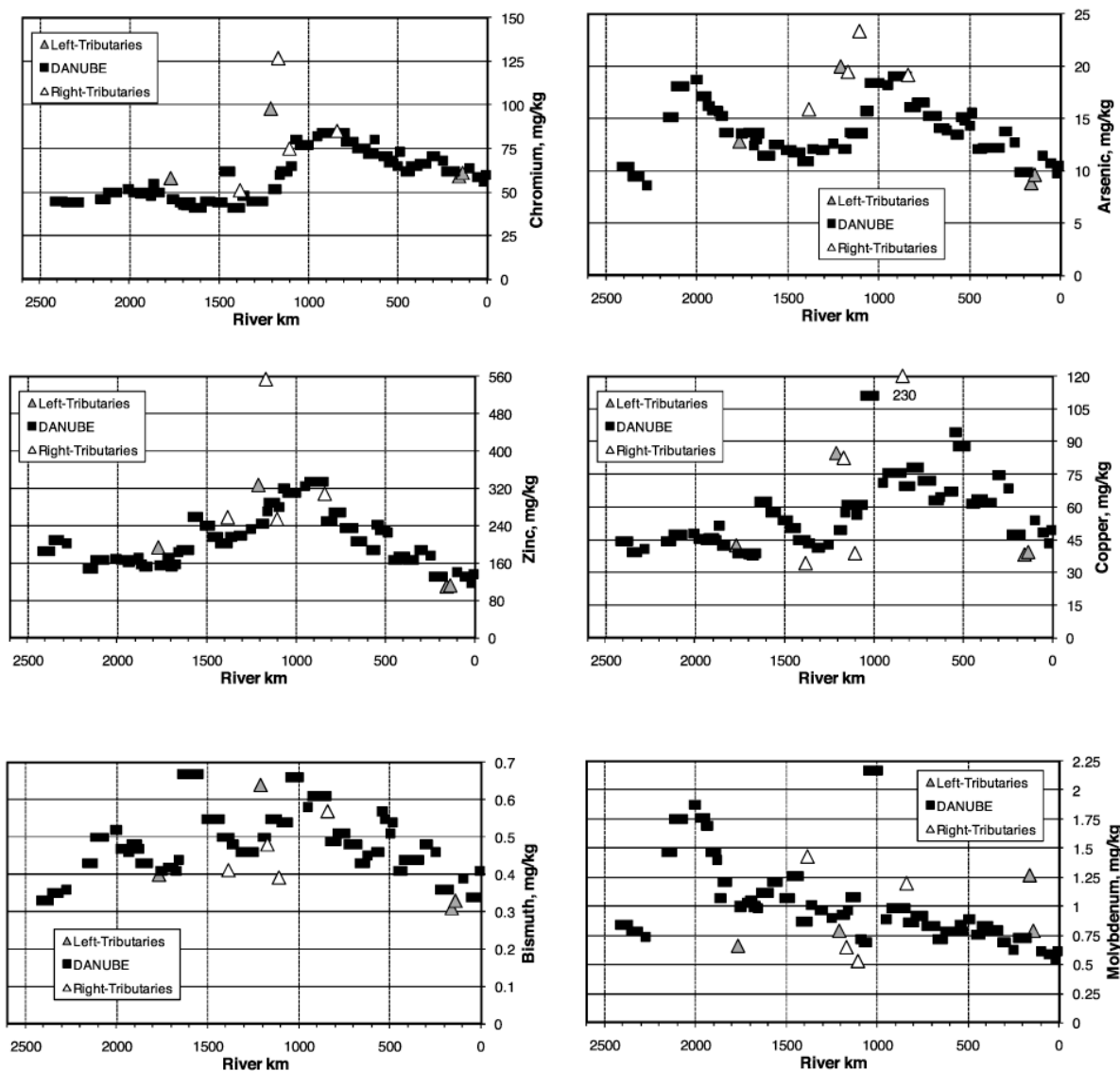
Figure 68: Distribution of As, Cr, Cu, Zn, Bi and Mo in the SPM in the Danube river during JDS2

With the exception of Velika Morava, the concentrations of As in the SPM were below the 20 mg/kg in all samples from the Danube and its tributaries. In the Upper Danube reach, it is likely that the River Inn caused a significant increase in the As concentration downstream of the Inn confluence (NB: No SPM sample was taken from the Inn). As a result of the input from the three tributaries (the Tisa, Sava and Velika Morava) the As concentration in the Danube increased nearly reaching the concentration of the sediment quality standard.

The longitudinal profile of the Cr concentration in the SPM shows similarity to that of Ni. A characteristic "hump" was recorded downstream of the Tisa and Sava confluences, the latter was the only one in which the sediment standard for Cr was exceeded (JDS51, 127 mg Cr/kg). The 60 mg/kg sediment standard was basically exceeded in all SPM samples downstream of the Tisa and Sava confluences. The overall longitudinal concentration profile was also similar to the Cd and Ni, which may indicate a mainly geochemical origin for these metals.

Zn is the fourth heavy metal joining the group of Cd, Ni and Cu regarding the characteristics of the longitudinal profiles. The 200 mg Zn/kg sediment standard in the SPM was exceeded in the same samples as was the case for Cu, showing the major impact of the Tisa and Sava.

The spatial distribution for Bi shows a more scattered picture in comparison to the other metals but the concentrations were within a relatively narrow 0.3 to 0.7 mg/kg range. Mo has a clear peak in the Upper Danube, which similarly to the As, could be related to input through the Inn or to anthropogenic influences. However, the concentrations of Mo were below the Dutch target value of 3 mg/kg at all sampling sites.

### 17.3.2.4  Longitudinal profile of the Group 3 heavy metals (Al, Fe and Mn)
Figure 69 shows the concentrations of Al and Fe in the SPM, demonstrating significantly different trends upstream and downstream of the Iron Gate.
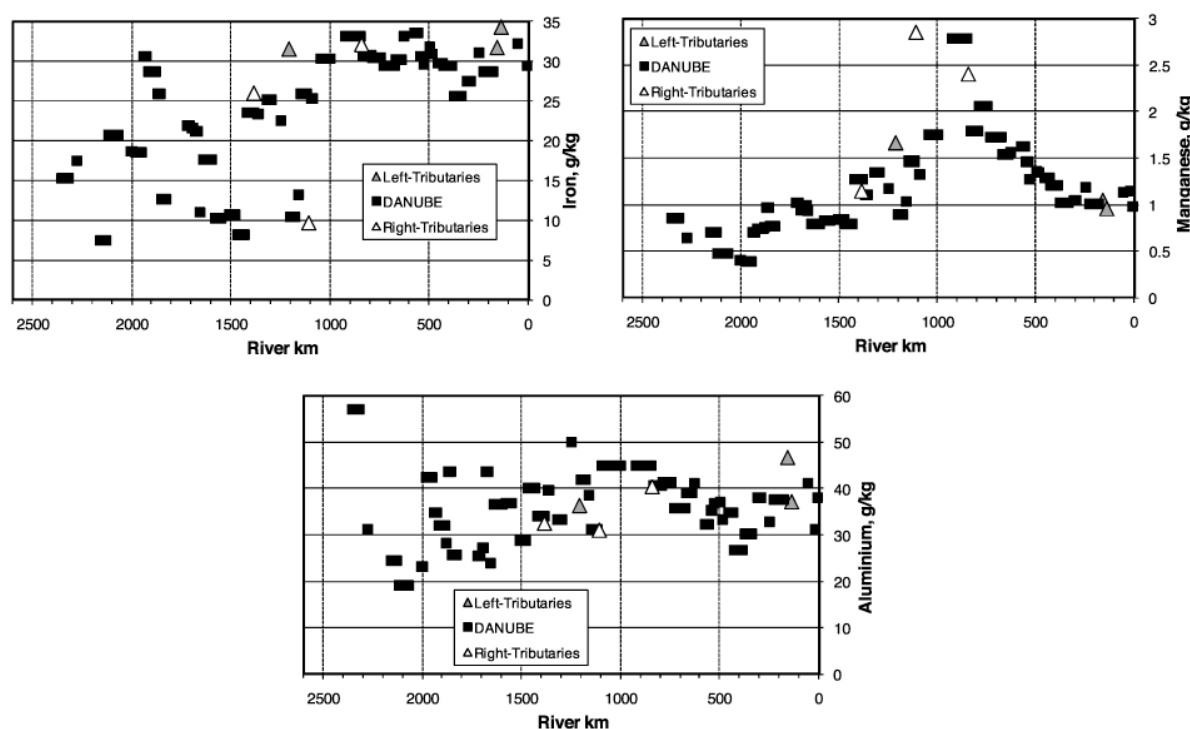


Figure 69: Distribution of Al, Fe and Mn in the SPM along the Danube River during JDS2

Low concentrations of Al and Fe with a significant variation were recorded along the Upper Danube while relatively constant values were observed from the Iron Gate down to the Danube Delta. These metals are mainly of geochemical origin and are controlled by a different mineralogical composition of the SPM, which is more variable upstream of the Iron Gate due to inputs from sub-basins with different geochemical characteristics. The variation in Al is useful for normalisation of the other heavy metal results (see the full report on the CD-ROM).

The concentration of Mn in the SPM showed a similar longitudinal trend as was the case for Cu and Zn. A significant increase was observed downstream of the Velika Morava and the Iron Gate reservoir, which gradually decreased downstream to the Danube Delta.

### 17.3.2.5  Comparison of the concentration of heavy metals and As in SPM between JDS1 and JDS2
Regarding the four heavy metals in the list of WFD priority substances, minimum and maximum concentrations in the SPM are summarised in Table 32. In general, lower maxima of Cd and Pb were observed; other changes were not very significant.

Table 32: Range of element concentrations in the SPM samples of the Danube River and some of its tributaries, during JDS1 and JDS2

| Element | Concentration [mg/kg] | | | |
|---|---|---|---|---|
| | Danube | | Tributaries | |
| | JDS1 | JDS2 | JDS1 | JDS2 |
| Cd | < 1.1 – 7.6 | 0.294 – 2.23 | < 1.1 – 25.6 | 0.394 – 4.85 |
| Pb | 18.2 – 85.0 | 25.3 – 64.6 | 17.3 – 214.9 | 18.5 – 79.1 |
| Hg | < 0.10 – 0.55 | 0.102 – 0.388 | < 0.10 – 0.79 | 0.060 – 1.21 |
| Ni | 23.2 – 89.8 | 30.9 – 85.0 | 32.6 – 170.9 | 41.4 – 161.0 |
| | | | | |
| As | 9.4 – 32.1 | 8.62 – 19.0 | 10.4 – 29 | 8.83 – 23.4 |
| Cr | 32.9 – 107.5 | 40.8 – 94.3 | 55.0 – 148.9 | 38.0 – 127.0 |
| Cu | 28.3 – 193.7 | 37.7 – 111.0 | 26.9 – 95.5 | 34.4 – 230.0 |
| Zn | 99 – 398 | 117 - 335 | 87 - 2224 | 111 - 553 |
| Al | 17900 – 52800 | 19000 - 57000 | 15300 – 54100 | 31200 - 49800 |
| Fe | 14300 – 38300 | 7180 - 35400 | 21300 – 37200 | 9700 - 34300 |
| Mn | 565 – 4028 | 770 - 3150 | 963 – 3340 | 1060 - 4120 |

Regarding the other elements, the spatial distribution of Cu and Zn (similarly to the Priority Substances Cd and Ni) was very similar to the distribution of Al and Fe during JDS1 and JDS2. This comparable trend was interpreted in both surveys as a reflection of geochemical background. Significant difference can only be observed in the case of maximum values such as (a) Cu concentration decrease in the Danube; increase in the tributaries and (b) Zn concentration decrease in the tributaries. All other metal concentrations were very similar during the two surveys.

### 17.3.3 Heavy metals and As in the bottom sediment

Bottom sediment samples were collected from both left and right banks of the Danube; a single mixed sediment sample was taken from the tributaries. Figure 70 shows the concentrations of priority heavy metals, i.e. Cd, Pb, Hg **and** Ni. Results for the other heavy metals and As are given in detail in the full report on the CD-ROM.
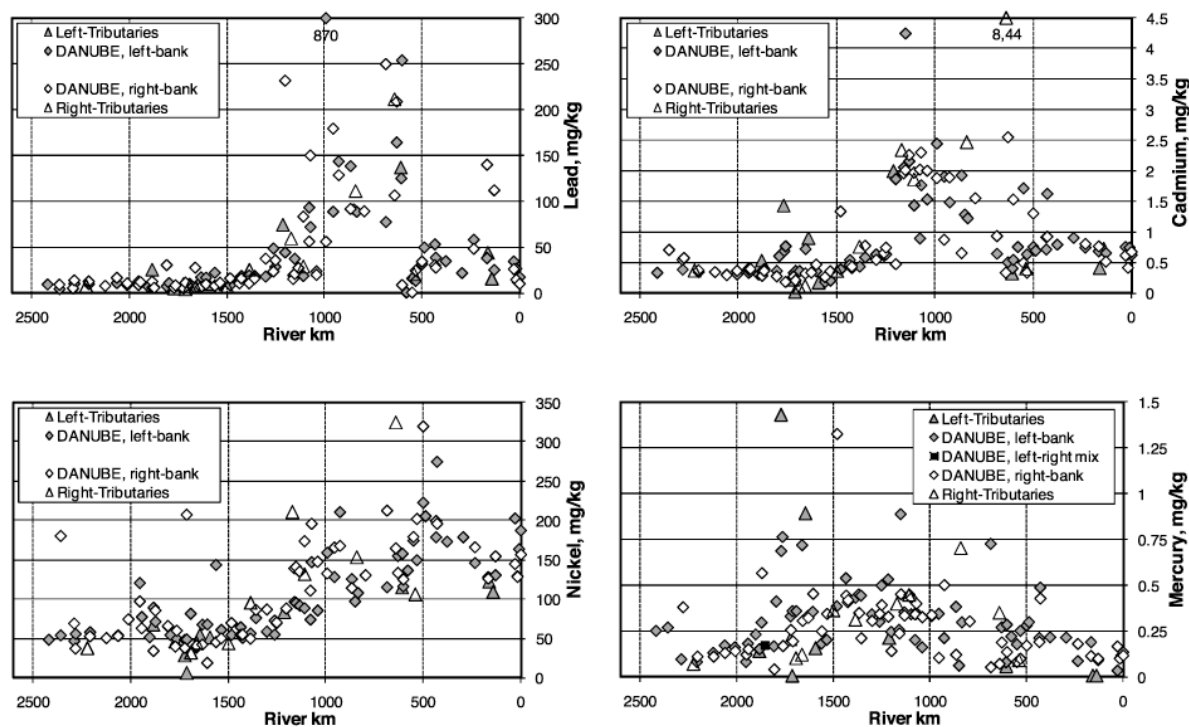
Figure 70: Variation in the concentration of Cd, Pb, Hg and Ni in the bottom sediment during JDS2

The exceedance of sediment standards and longitudinal trends were as follows:

−   Cd: The longitudinal trend for Cd is very similar to that in the SPM; a significant variation was observed downstream of the Tisa and Sava. The distribution pattern is similar to that of the JDS1.

−   Pb: Significantly higher concentrations of Pb were recorded than those in the SPM. The low concentrations along the Upper Danube were followed by an elevation downstream of the Tisa and Sava. The sediment standard was exceeded in a relatively large number of samples.

−   Hg: The impact of the major pollution source from the Vah tributary and other inputs along the middle section of the Danube can be recognised in the pattern of this typically anthropogenic pollutant. It is notable, however, that the sediment standard (0.8 mg/kg) was exceeded at only a few sampling sites.

−   Ni: In the case of Ni, contrary to the results for the SPM, the concentration in the bottom sediment exceeded the 50 mg/kg sediment standard at most sampling sites. As with several other heavy metals, the longitudinal trend was typical, showing significant increase downstream of the confluences of the Tisa and Sava rivers.

## 17.3.4 Heavy metals and As in mussels and fish

### 17.3.4.1  Mussels
Mussels are used frequently for pollution monitoring e.g. the "mussel-watch" programme is one of the major monitoring programmes particularly for detecting heavy metals. The only difficulty can be the abundance of identical species at different sites along the surveyed river.

When comparing the concentration of heavy metals in mussels with those in fish, it should be taken into account that results in mussels are expressed in dry weight, whereby those in fish are given in

mg/kg wet weight. If we consider that mussels have around 80-85% water in their tissue, the concentration in dry weight could be divided by around 6 to refer to wet weight.

During JDS2, mussel samples (dependent on the abundance at a given site) were collected from 20 Danube sites and from two tributaries - the Vah (JDS21) and Prut (JDS91), which is significantly less than was undertaken for JDS1. Altogether 33 mussel samples were collected during JDS2 compared to 136 during JDS1. This difference in the number of samples during the two surveys makes comparison difficult. Therefore, the range of concentrations of the different elements during JDS1 are given and compared with the maximum measured values during JDS2. These are summarised in Table 33. This table also shows the quality targets used during JDS1.

Table 33: Range of element concentrations in mussel samples (minimum - maximum) of the Danube River and its tributaries during JDS1; highest concentrations during JDS2 and quality targets used during JDS1

| Element | Concentration ranges during JDS1 [mg/kg dry weight] | | Highest concentration during JDS2 [mg/kg dry weight] | Quality targets during JDS1 [mg/kg dry weight] |
|---|---|---|---|---|
| | Danube | Tributaries | | |
| As | 0.08 - 1.23 | 0.06 - 0.81 | 2.7  (JDS39) | 20 |
| Cd | 0.1 - 35.9 | 0.2 - 16.4 | 29.6  (JDS50) | 4 |
| Cr | 0.5 - 11.7 | < MQL - 24.12 | 4.9  (JDS93) | 6 |
| Cu | 4.5 - 178.4 | 4.3 - 54.0 | 37.5  (JDS93) | 20 |
| Pb | 0.5 - 49.9 | 0.7 - 31.7 | 9.8  (JDS93) | 10 |
| Hg | 0.055 - 0.412 | 0.037 - 0.742 | 0.3  (JDS21) | 0.4 |
| Ni | 0.44 - 4.69 | 0.49 - 9.43 | 5.16  (JDS17) | 10 |
| Zn | 120 - 2680 | 160 - 1360 | 1880  (JDS50) | 400 |

The results indicate decreasing trends in the case of priority heavy metals Pb, Hg and Ni, as well as in the case of Cr, Cu and Zn. However, increasing concentrations were found in the case of the priority heavy metal Cd and also As. Particular attention has to be paid to the concentration of Hg (even the total form) because of the specified strict limit values in the proposed EC Directive indicated earlier. Unfortunately the specified methyl-mercury was not analysed during the survey, but, according to several scientific references, the aquatic biota, particularly fish and mussels, may contain over 60 to 80% of total mercury in alkylated form. Therefore, even total Hg concentrations in the biota samples require special attention.

Figure 71 shows the results for total Hg concentrations in the two most abundant species, *Unio tumidus* and *Anodonta anatina*, and Figure 72 shows the results for the other heavy metals and As.
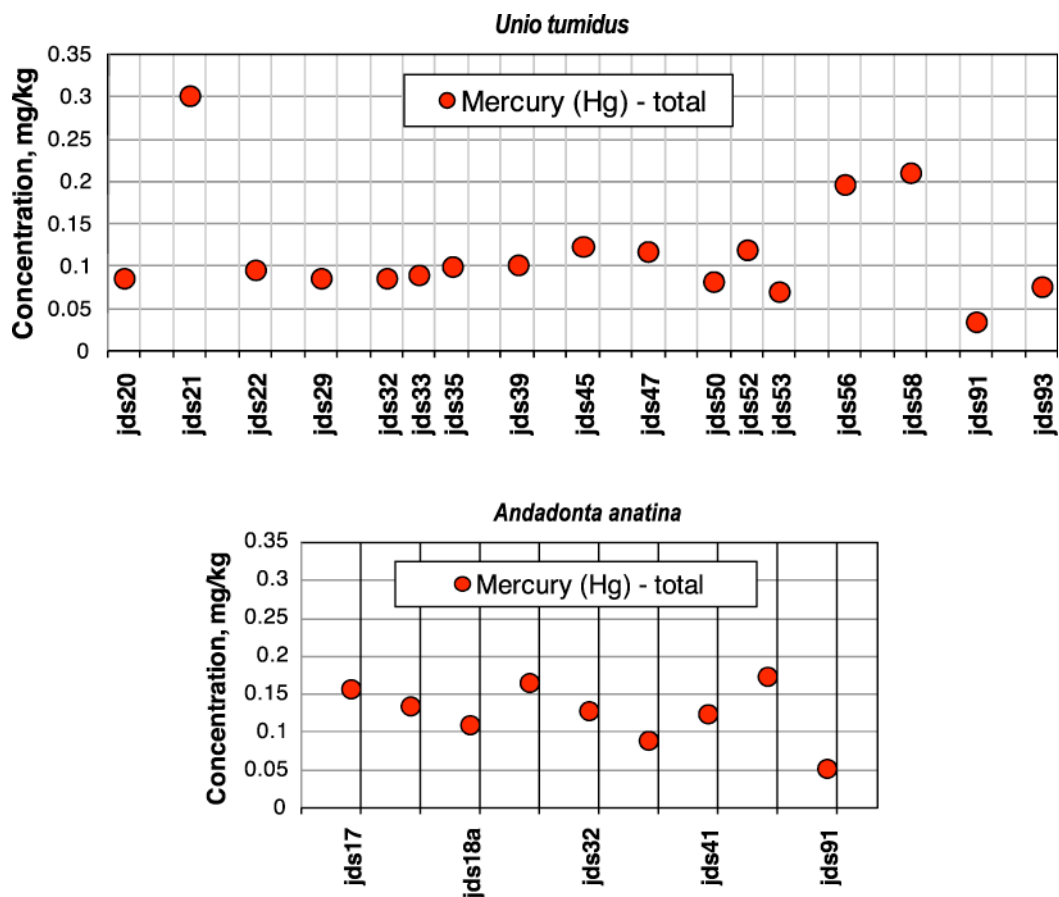
Figure 71: Total Hg concentrations in the two most abundant mussel species
(all results are in mg/kg dry weight)

The maximum total Hg concentration (0.3 mg/kg) was measured in a *Unio tumidus* mussel sample. As the results of the mussel samples are expressed in dry weight, this concentration could be around 0.05 mg/kg in wet weight.

Comparing the total concentration of heavy metals and As with the quality targets used during JDS1 (see Table 33), Cd and Zn significantly exceeded the relevant quality target value, whereby Cu exceeded it only slightly.
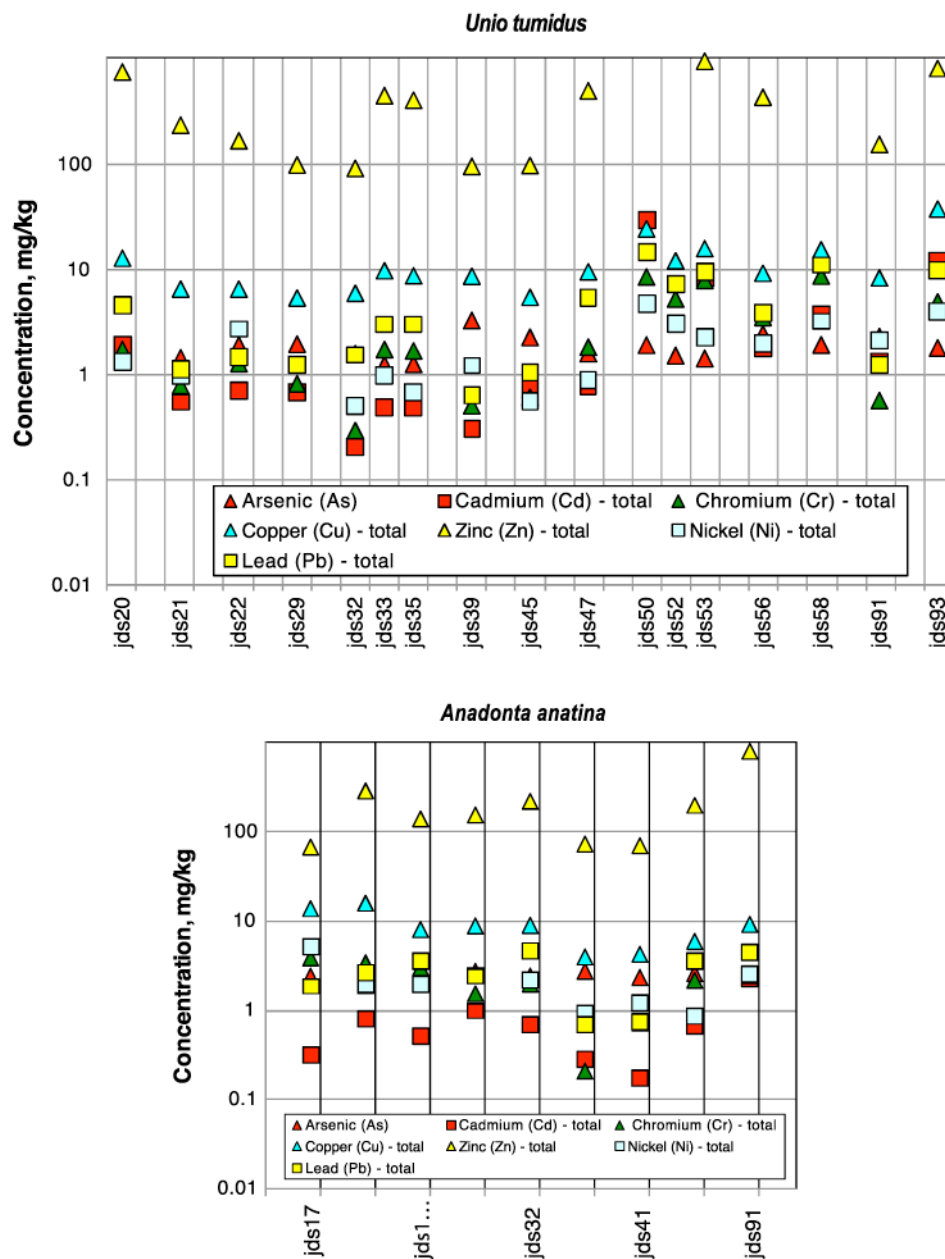
**Figure 72: Total heavy metals and As concentrations in the two most abundant mussel species during the JDS2 (all results are in mg/kg dry weight)**

## 17.3.4.2 Fish

Samples of fish tissue (*Abramis brama*) were taken from 11 characteristic sites along the Danube starting at Kelheim, Germany, downstream to the Danube Delta. Concentrations of total Hg in *Abramis brama* from the Danube River (as well as the other heavy metals and As) are shown in Figure 73 and Figure 74, respectively.
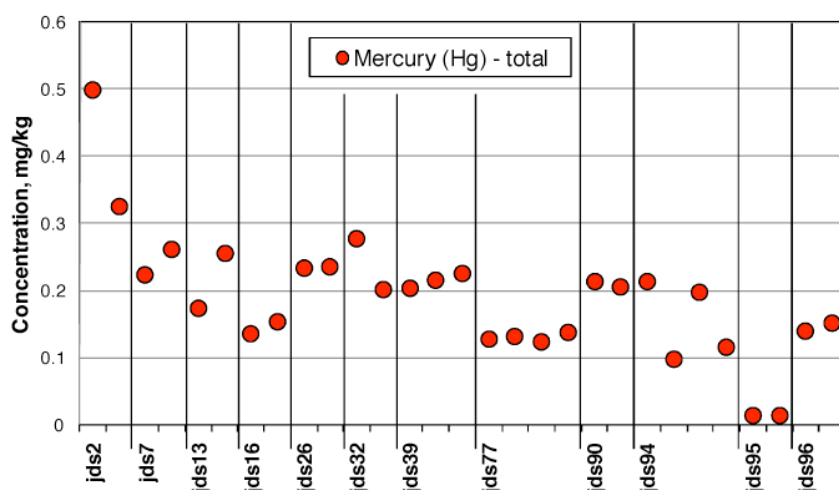
Figure 73: Total Mercury in *Abramis brama* in the Danube
(all results are in mg/kg wet weight)
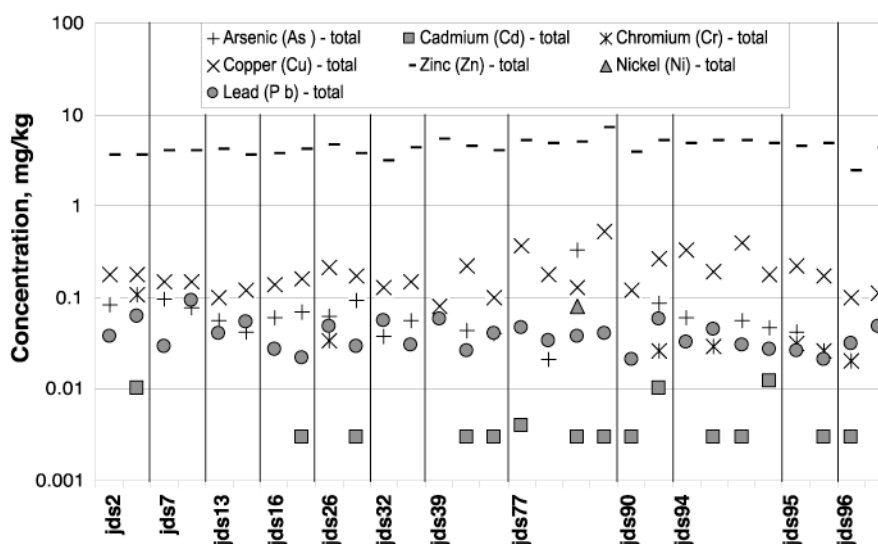


Figure 74: Heavy metals and As in *Abramis brama* in the Danube
(all results are in mg/kg wet weight)

Out of seven heavy metals and the As analysed in the given group, the most serious problem appeared to be in the case of mercury. In the 29 analysed samples, Hg content varied from 0.015 to 0.499 mg/kg of tissue. The highest individual value was found in the most upper section of the measured stretch of the Danube (at Kelheim).

Despite the fact that these results are below the maximum value of 1.0 mg Hg/kg of wet weight set in Commission Regulation No 221/2002 amending Regulation No 446/2001 (setting maximum levels for certain contaminants in foodstuffs), the values would significantly exceed the quality target of JDS1 (0.4 mg/kg in dry weight) because concentrations would be around six times higher if expressed in dry weight.

Copper showed similar longitudinal variation, between 0.08 to 0.52 mg/kg, with an increase downstream of the Iron Gate. The concentrations of two other heavy metals – Cd and Pb – limited by

the corresponding Commission Regulation were significantly lower in all samples than the given limit values (most Cd values were below the LOQ and this is why the values are not recognisable in the figure). The concentration of both heavy metals varied slightly (<0.003–0.012 mg/kg for Cd and 0.021–0.092 mg/kg for Pb) without showing any trends along the Danube. The other heavy metals and As were near to the LOQ in most of the samples.

In summary, because total Hg concentrations found in the fish samples may indicate accumulation in the fish tissue, and likely exceedance of the newly proposed EQS, it is important that the Hg contamination issue is further investigated and considered.

## 17.4    Conclusions
In general, the concentration ranges of heavy metals and arsenic in water, suspended and bottom sediments during the JDS2 were very similar to those observed in the JDS1 samples.

Regarding the water samples, the dissolved Cd could not be quantified because of a relatively high LOQ even exceeding the respective WFD EQS.

### 17.4.1 Compliance checking for priority substances according to the WFD
Compliance checking principles are described in more details in Chapter 1. For Pb and Ni, and their compounds, the MAC-EQS is marked as "not applicable." Therefore, AA-EQS values were selected for all four heavy metals as being protective against short-term pollution peaks in continuous discharges.

The draft directive also includes an EQS for methyl-mercury in aquatic biota (fish, molluscs, etc.) but this substance was not on the list of JDS2 parameters. Results for total mercury determined in mussels and fish, however, indicate relatively high concentrations, particularly in fish tissue, which should be a consideration for future monitoring.

### 17.4.2 Assessment of indication of chemical status from JDS2 results
Similarly to the assessment of other priority substances, an indication of the chemical status is given for each sampling site by using the proposed AA-EQS for inland waters. Table 34 shows the sampling sites where AA-EQS are exceeded.

### Table 34 Sampling sites and priority heavy metals exceeding AA-EQS values for inland waters

| JDS2 code | Heavy metal | Sampling station | Concentration [µg/l] | AA-EQS[1] [µg/l] |
|---|---|---|---|---|
| JDS32 | Hg | Budapest downstream | 0.071 | 0.05 |
| JDS33 | | Adony/Lórév | 0.063 | 0.05 |
| JDS66 | Ni | /Timok (rkm 0.2) | 33.3 | 20 |

[1] Proposal for amending Directive 2000/60/EC

### 17.4.3 Assessment of other heavy metals and As
For some of the other heavy metals, only national EQS or EQS proposals may be used for their assessment. Accordingly, the tentative Austrian EQS for dissolved Cu and Zn (both dependent on total hardness) was considered and the exceedances are shown in Table 35. Most other elements present in dissolved form were around natural background levels.

## Table 35 Sampling sites and parameters exceeding the proposed Austrian EQS values

| JDS2 Code | Heavy Metal | Sampling Station | Concentration [µg/l] | EQS[1] [µg/l] |
|-----------|-------------|------------------|----------------------|---------------|
| JDS66 | Cu | /Timok (rkm 0.2) | 34.5 | 8.8 |
| JDS85 | | Downstream of Ruse/Giurgiu | 14.6 | 8.8 |
| JDS81 | | /Russenski Lom | 11.2 | 8.8 |
| JDS-IS2 | Zn | Before the Iskar Reservoir | 67.9 | 35.1 |

[1] Austrian proposal, hardness dependent.

# 18 Polar water-soluble contaminants in the liquid water phase by SPE-LC-MS[2]

Robert Loos, Giovanni Locoro and Serafino Contini

## 18.1 Introduction

Polar water-soluble organic compounds were analysed in the dissolved (liquid) water phase by solid-phase extraction (SPE) followed by triple-quadrupole LC-MS[2]. The extraction volume for the River Danube and tributary samples was 400 ml. In total, 34 different compounds were analysed. The analysis included six WFD priority compounds (atrazine, simazine, isoproturon, diuron, nonylphenol and octylphenol).

All tributary water samples (23 close to the Danube and 28 further upstream in individual member states) and 53 River Danube water samples were analysed by the EC JRC-IES.

Focus was given on pharmaceutical compounds (such as ibuprofen, diclofenac, sulfamethoxazole and carbamazepine); pesticides and their degradation products (e.g. bentazone, 2,4-D, mecoprop, atrazine, terbutylazine and desethylterbutylazine); perfluorinated acids (PFOS; PFOA) and endocrine disrupting compounds (such as nonylphenol, $NPE_1C$, bisphenol A and estrone).

## 18.2 Methods

The detailed analytical method has been published elsewhere (Loos, 2007, 2008). In summary: 500 ml water was decanted; an internal standard was added; extraction of 400 ml water was then performed by solid-phase extraction using 200 mg Oasis HLB cartridges and elution was carried out with 6 ml methanol. The extract was evaporated to 0.5 ml and analysed by triple-quadrupole LC-MS[2]. Quantification was performed by isotope dilution using labelled internal standards.

## 18.3 Results

### 18.3.1 Compound classes

In general, concentrations of the target organic compounds were relatively low in the Danube River. In the tributaries higher levels were detected. The most contaminated tributary river was the Arges. The highest levels for most chemicals in the Danube were detected in the area around Budapest.

The chemicals detected in the Danube at the highest median concentrations were: benzotriazole (213 ng/l), tolyltriazole (81 ng/l), caffeine (80 ng/l), nonylphenoxy acetic acid $NPE_1C$ (49 ng/l), carbamazepine (37 ng/l), sulfamethoxazole (17 ng/l), perfluorooctanoate PFOA (14 ng/l) and desethylatrazine (11 ng/l).

### 18.3.2 Perfluorinated acids (PFAs)

The highest perfluorooctanoate (PFOA) level was detected in the River Inn (Germany); a concentration of 60 ng/l was measured. This river is the major PFOA source for the Danube. In the Danube downstream of this influent, PFOA levels up to 46 ng/l were found. PFOA was diluted along the river course, reaching levels around 14 ng/l at the end of the river. It has to be mentioned that the

emission of PFOA to the Inn was stopped in 2008 as PFOA has been replaced in the relevant technological process.

The perfluorooctansulfonate (PFOS) concentration was quite constant in the upstream part of the Danube (~ 10 ng/l). At the river delta in Romania, 6 ng/l was reached. In the following tributaries elevated PFOS concentration levels were detected: Morava (20 ng/l), Jantra (57 ng/l) and Arges (101 ng/l).
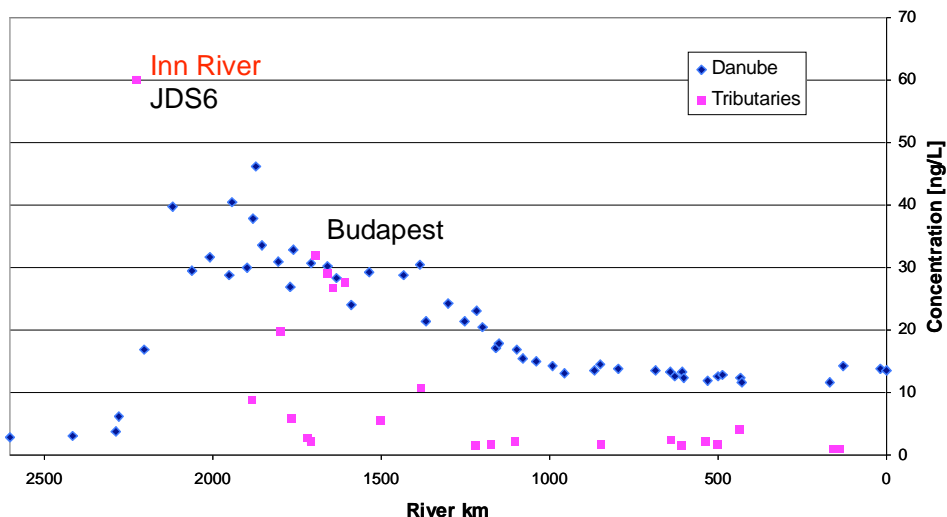


Figure 75: PFOA in the Danube River and tributaries

### 18.3.3 Pharmaceuticals

The highest pharmaceutical levels were found for carbamazepine and sulfamethoxazole, which can be explained by their persistency. For carbamazepine in the Upper Danube, slightly higher levels were detected than in the lower reach (around 50-60 ng/l around Budapest and ~30 ng/l in samples downstream of JDS50, which corresponds to the Tisa and Sava tributaries). For sulfamethoxazole levels ~ 20 ng/l were detected along the whole river. These anthropogenic substances are "discharged" along the whole river.

Concentration levels observed for bezafibrate, ibuprofen (5-10 ng/l), and diclofenac (< 5 ng/l) were lower. Further downstream in Romania, diclofenac and bezafibrate were not detected anymore, which could be explained by the biodegradability of these compounds. Ibuprofen was found after the Velika Morava tributary (JDS56) in concentration levels between 9-27 ng/l, and at the Danube Delta ~ 5 ng/l.
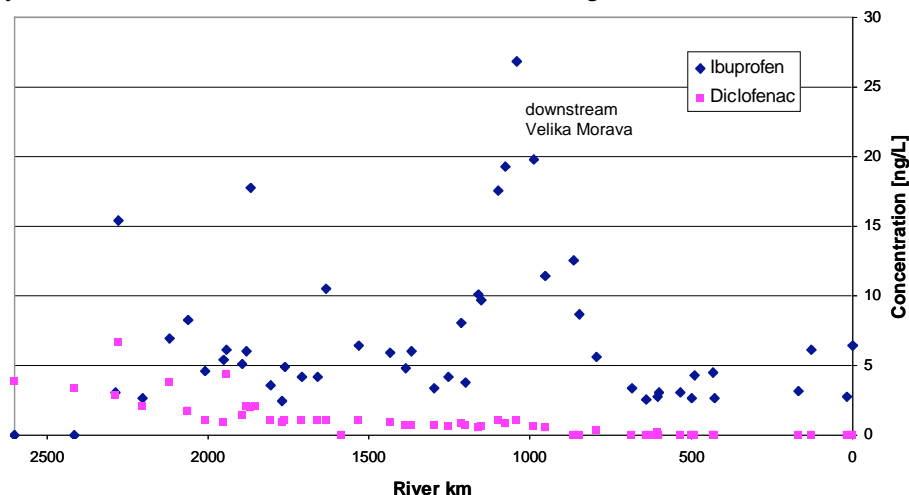


Figure 76: Ibuprofen and diclofenac in the Danube River

### 18.3.4 Pesticides

The highest concentrations of 2,4-D, one of the most widely used herbicides in the world, were found in the area around Budapest (~ 50 ng/l). In the Austrian part of the Danube concentrations were measured at ~ 20 ng/l and in the downstream part ~ 10 ng/l were found. Bentazone levels were ~ 5-10 ng/l in the whole Danube. Simazine, atrazine, isoproturon and diuron levels were low (< 10 ng/l) in the whole Danube. Terbutylazine levels were slightly higher, between 10-20 ng/l around Budapest, and between 5-10 ng/l in the downstream region of the Danube. The concentrations for the metabolites desethylatrazine and desethylterbutylazine were in the range of 5-20 ng/l with maximum levels around Budapest.
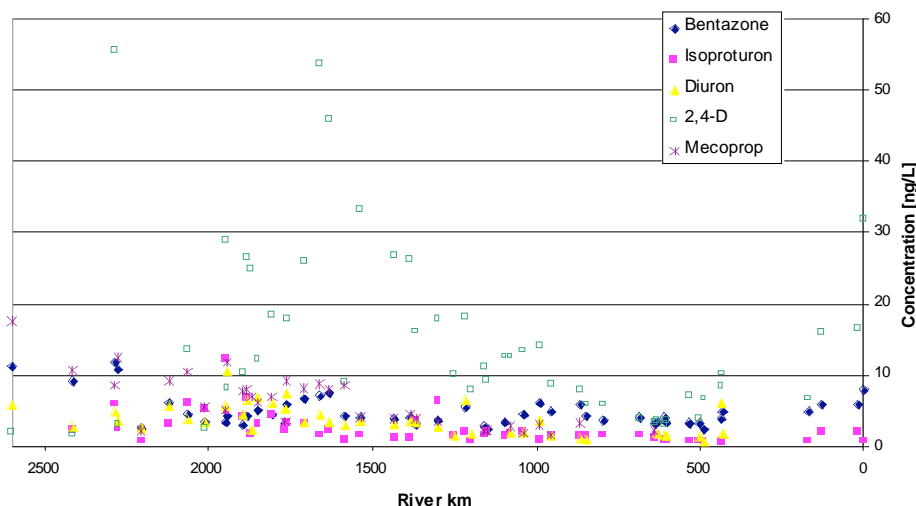


Figure 77: Selected pesticides in the Danube River

### 18.3.5 Estradiol hormones

In the Danube River the concentration levels of estradiol and ethinylestradiol were in all cases below the LOD (5 ng/l). Estrone could be detected in some samples in the area of Budapest and downstream of the Velika Morava confluence at concentrations around 1-2 ng/l. Moreover, 2 ng/l of estrone were detected in the Russenski Lom, and by far the highest measurement was taken in the Arges tributary (71 ng/l).

### 18.3.6 Nonylphenol

Nonylphenol (NP) concentrations are only reported for levels > 50 ng/l (due to the problematic blank) and they have only been detected in a small number of samples. The highest NP levels were found in the tributaries, the Arges (1300 ng/l in JDS84 and 1400 ng/l in AR2), the Timok (500 ng/l) and the Velika Morava (260 ng/l). The highest nonylphenol concentrations in the Danube River were found in Donji Milanovac (JDS61), at 240 ng/l, and in Vilkovo (JDS93), at 180 ng/l. There is however a suspicion that these two findings in the Lower Danube might well be caused by a blank contamination (of the bottles).

### 18.3.7 Benzotriazoles

Benzotriazole (130-300 ng/l) and tolyltriazole (62-130 ng/l) were only analysed in selected sites and were found at all of them. They are persistent chemicals used e.g. as corrosion inhibitors in dishwashers.

## 18.4    Conclusions

The analytical results for the polar compounds analysed (pharmaceuticals, pesticides, perfluorinated acids (PFOS/PFOA) and phenolic endocrine disrupting compounds) are in good agreement with other big European rivers such as the rivers Rhine, Elbe or Po.
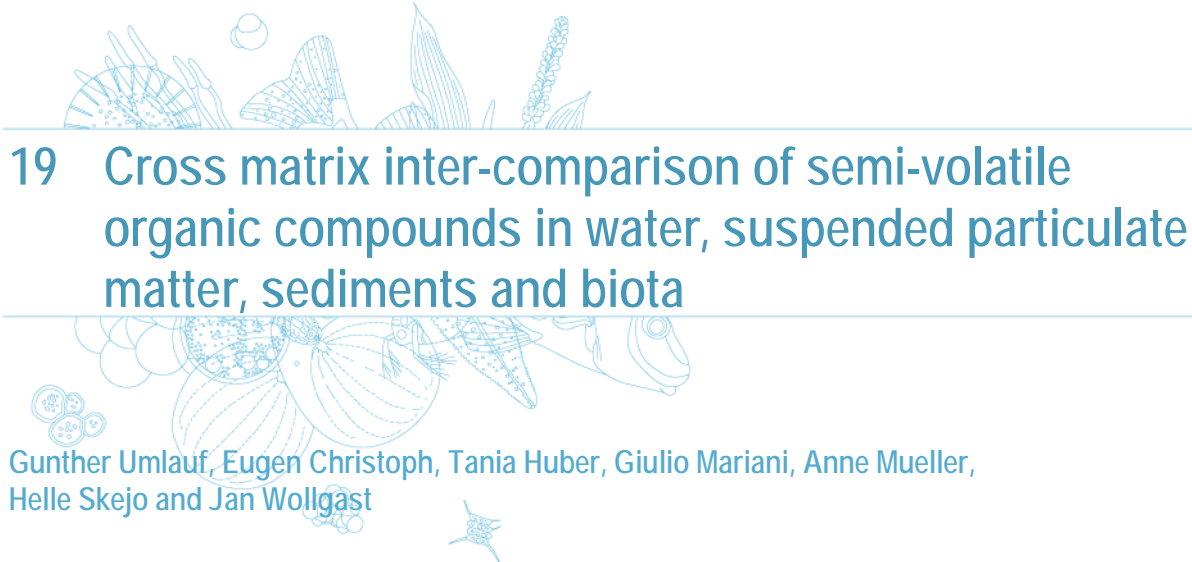
The levels for the priority compounds of the WFD (atrazine, simazine, isoproturon and diuron) were below the EQS values in the Danube and the tributaries, in all cases except for nonylphenol. The nonylphenol EQS of 0.3 µg/l was exceeded in the Timok (JDS66) and Arges rivers (JDS84).

For the other "emerging" compounds analysed, no limit values exist for surface waters.

The most relevant polar compounds identified in the Danube River Basin in terms of frequency of detection, persistency and concentrations were benzotriazoles, 2,4-D and carbamazepine.

## 18.5    References

LOOS, R., WOLLGAST, J., HUBER, T., HANKE, G. (2007): Polar herbicides, pharmaceuticals, perfluorooctansulfonate (PFOS), perfluorooctanoate (PFOA), nonylphenol and its carboxylates and ethoxylates in surface and tap waters around Lake Maggiore in Northern Italy, Anal. Bioanal. Chem. 387, 1469-1478.

LOOS, R., LOCORO, G., HUBER, T., WOLLGAST, J., CHRISTOPH, E.H., DE JAGER, A., GAWLIK, B.M., HANKE, G., UMLAUF, G., ZALDÍVAR, J.-M. (2008): Analysis of perfluorooctanoate (PFOA) and other perfluorinated compounds (PFCs) in the River Po watershed in N-Italy, Chemosphere 71, 306-313.

# 19 Cross matrix inter-comparison of semi-volatile organic compounds in water, suspended particulate matter, sediments and biota

Gunther Umlauf, Eugen Christoph, Tania Huber, Giulio Mariani, Anne Mueller, Helle Skejo and Jan Wollgast

## 19.1   Introduction

The target compounds of the cross-matrix programme were Polychlorinated Dibenzo-p-dioxins and Dibenzofurans (PCDD/Fs), Polychlorinated Biphenyls (PCBs), Polybrominated Diphenylethers (PBDEs), Organochlorine Pesticides (OCPs) and Polycyclic Aromatic Hydrocarbons (PAHs), all of them Semivolatile Organic Compound (SOC) classes which, depending on their lipophilicity and persistence, tend to accumulate in sediments and biota. In the aqueous phase, SOCs distribute between the dissolved phase and Suspended Particulate Matter (SPM), depending on their Octanol/Water partition coefficient (Ko/w) and the amount and type of SPM available. The transport within the water column is often associated with the hydraulic remobilisation of sediments and the subsequent transport and re-sedimentation of SPM.

The scope of the cross-matrix sampling was to obtain spatially overlapping data in sediment, SPM, water and biota from corresponding sites, which would allow an insight into the interactions between the relevant compartments. Results from 23 sites are reported, covering the Danube River over a distance of 2600 km from Germany to the Black Sea.

The main objective of JDS2 was to produce comparable and reliable information on the water quality of the whole Danube and many of its main tributaries. Regarding the chemical status, an important objective was to obtain a dataset for priority pollutants according to the daughter directive of the Water Framework Directive (WFD).

Among the SOCs reported in this study, the following substances are included in the List of Priority Substances of the WFD, with Environmental Quality Standards (EQS) established (Common Position, Annex I, 2007): Flame retardants such as PBDEs; OCPs such as Endosulfan, Hexachlorobenzene (HCB), Hexachlorocyclohexane (HCH), DDT and its metabolites; Cyclodiene pesticides (Aldrin, Dieldrin, Endrin, Isodrin) and the PAHs such as naphthalene, anthracene, fluoranthene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(ghi)perylene, benzo(k)fluoranthene and indeno(123-cd)pyrene.

### 19.1.1 Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs consist of condensed aromatic rings and do not contain heteroatoms or substituents. PAHs occur in oil, coal and tar deposits, and are produced as unintentional by-products of incomplete combustion processes. As a pollutant, they are of concern because some compounds have been identified as carcinogenic, mutagenic, and teratogenic.  PAHs are lipophilic, meaning they mix more easily with oil than water. The larger compounds are less water-soluble and less volatile.  As a result of these properties, PAHs in the environment are found primarily in soil and sediment.

16 EPA priority PAHs plus benzo(e)pyrene and benzo(j)fluoranthene were analysed in water, SPM and sediments.

### 19.1.2 Organochlorine Pesticides (OCPs)

Organochlorine pesticides are man-made organic chemicals. DDT was the first to be used on a large scale. DDT, Aldrin, Dieldrin, Endrin, and Lindane have been extensively used and are therefore found widely distributed in the environment, together with their persistent metabolites. A number of OCPs are listed under the Stockholm Convention on POPs (persistent organic pollutants).

Some OCPs have a low water solubility and a high $K_{o/w}$. They tend to be persistent and absorb to suspended solids and sediments. Due to their persistence and lipophilicity, they bio-accumulate. OCPs are highly toxic (including endocrine disruption) to aquatic organisms and mammals. 29 OCPs were analysed in water, SPM, sediments and biota.

### 19.1.3 Polychlorinated Dibenzo–p–Dioxins and Dibenzofurans (PCDD/Fs) and Polychlorinated Biphenyls (PCBs)

PCDD/Fs are unintentional by-products of poor combustion and a variety of chemical processes involving chlorine and organic compounds. The $Cl_4 – Cl_8$ substituted congeners having Cl at the 2,3,7,8 positions are considered as being toxic.

PCBs are - in contrast to the PCDD/Fs - intentionally produced chemicals with a broad spectrum of industrial applications such as dielectric fluids, paints, hydraulic oils etc. However, some of the $CL_4 – Cl_7$ substituted PCBs have a structural similarity with PCDD/Fs. In particular the non-ortho Cl-substituted  PCBs can display a co-planar geometry and show a structure and a toxicity comparable to the PCDD/Fs. Mono-ortho Cl-substituted PCBs still show similar "dioxin-like" toxic effects but at a lower intensity. The toxic responses of PCDD/Fs and dioxin like (DL)-PCBs include dermal toxicity, immunotoxicity, carcinogenicity and adverse effects on reproduction, development and endocrine functions.

Due to their similar behaviour and toxicological endpoints, PCDD/Fs and DL-PCBs are often evaluated and reported together. Both compound classes are included in a toxicity evaluation scheme that sums up the toxicity of the individual congeners of both classes (17 PCDD/Fs and 12 DL-PCBs) expressed as toxicity equivalents (TEQs) of the 2,3,7,8 tetrachloro dibenzo-p-dioxin (TCDD).

To this end, the World Health Organisation (WHO) agreed on 2,3,7,8-TCDD Toxicity Equivalency Factors (TEFs) for  the 17 PCDD/Fs and 12 DL-PCBs (WHO-TEQ), (Van den Berg et al., 1998).

Both PCDD/Fs and DL-PCBs are classified as POPs and are listed among the 12 POPs of the Stockholm Convention. Due to their hydrophobic nature and resistance towards metabolism they accumulate in fatty tissues of animals and humans.

17 PCDD/Fs and 12 DL-PCBs included in the TEQ scheme and the 6 indicator PCBs (EC-6) PCB 28, 52, 101, 138, 153 180 were analysed in water, SPM, sediments and biota. For the 2,3,7,8-substituted PCDD/Fs a "safe sediment value" of 20 ng/kg I-TEQ has been proposed (Evers et al., 1996).

### 19.1.4 Polybrominated Diphenylethers (PBDEs)

Polybrominated diphenylethers are used as flame retardants in polymers, especially in plastics for electrical and electronic products. Worldwide, only three types of Polybrominated diphenylether mixtures are commercially used: decabromodiphenylether (c-Deca BDE), octabromodiphenylether (c-Octa BDE) and pentabromodiphenylether  (c-Penta BDE.). The AA-EQS for the commercial pentabromodiphenylether (c-PentaBDE) mixture in inland waters is 0.5 ng/l for the $\sum$ of BDE 28, 47, 99, 100, 153 and 154. The fate and distribution dynamics of PBDEs in the environment is similar to that of PCDD/Fs and PCBs, they have low water solubilities and tend to bio-accumulate. In contrast to the 12 POPs subject to the Stockholm Convention, PBDEs show rising trends in the environment including human tissue. Although the toxicological endpoints of PBDEs are not entirely evaluated, their structural similarity to PCDD/Fs and PCBs suggests similar toxicological endpoints. 18 PBDE isomers that reflect the 3 commercial PBDE mixtures were analysed in water, SPM, sediments, and biota.

## 19.2    Methods

Sediment samples were obtained from 23 sites, 14 of which were sampled at both sides of the river. At some of these sites mussel samples were also taken. SPM samples were collected in the vicinity of the 23 sediment sampling sites with a centrifuge and dissolved phase samples were collected in parallel with a large volume Filter/XAD system (Olivella, 2006) during cruising. Mussel samples (*Anadonta anatina, Sinanodonta woodiana, Unio pictorum and Unio tumidus*) were taken on 24 sites that were only partially identical with the 23 sites selected for the inter matrix comparison.

The analysis of all compounds in <u>solid samples</u> was done using isotope dilution and GC/MS techniques, starting from one extract (Soxhlet, hexane/acetone 220:30) where all labelled internal standards were added prior to extraction. For the further analysis of SPM, sediments and biota, 10% of the extract was separated to execute the clean-up (Alumina/Florisil) followed by the analyses of PAHs (GC/LRMS) and OCPs (GC/HRMS). In the remaining 90% of the extract PCDD/Fs, PCBs and PBDEs were subject to a more intensive clean up (Acidic Silica, Basic Alumina and Active Carbon) and analysed using GC/HRMS.

For the analyses in the dissolved phase, <u>water samples</u> were collected on XAD 2 sorbent. Extraction was done with Accelerated Solvent Extraction from 100 ml cells containing 50 g of adsorbent.  First methanol, then n-hexane was used. Water was added to the combined extracts and the analytes were liquid/liquid-extracted by n-hexane.  PAHs, PCBs and PBDEs were analysed without clean-up in order to reduce the blank contribution. They were analysed respectively with GC/LRMS and GC/HRMS.  10% of the extract from the water samples was separated to execute the clean-up for OCPs (Alumina/Florisil) and analysis by GC/HRMS. The remaining 90% of the extract was then subjected to further clean up for PCDD/Fs (Acidic Silica, Basic Alumina and Active Carbon) and analysis by GC/HRMS. All concentrations for solids are reported on a dry weight basis.

## 19.3    Results

### 19.3.1 PAHs

PAHs were quantified in all samples. Most sediment and SPM samples display moderate ∑ PAH concentrations in a range of 250-750 µg/kg with extreme values of up to 2600 µg/kg for SPM, with concentrations in the SPM being slightly higher than in the sediments. For comparison, in the German stretch of the River Elbe, typical values for ∑ 16 EPA PAHs in SPM and SPM derived sediments are one order of magnitude higher and maximum levels range up to 50 mg/kg (ARGE Elbe, 2006). In  the delta of the River Seine the average concentrations of ∑PAH were around the maximum concentrations measured in sediments during JDS2 (Chailleaud et al., 2007).

In the water column, PAHs were more associated with SPM, with average (dissolved and SPM) concentrations of ∑PAH around 17 ng/l, and a maximum of 35 ng/l, which is at the lower end of typical findings in the River Elbe (ARGE Elbe, 2006).

Among the PAHs that were quantified in sediments and SPM, the most abundant compounds were fluoranthene and pyrene. Lower boiling PAHs such as fluorene and phenanthrene have a higher water solubility and are more abundant in the dissolved phase, which results in a higher relative contribution to the pattern in the water column (dissolved phase + SPM). Consequently, the PAH pattern in water is dominated by fluorene, phenanthrene, fluoranthene and pyrene. In the water, a significant amount of PAHs is associated with SPM, in particular the higher boiling compounds.

For the ∑benzo(g,h,i)perylene and indeno(1,2,3-cd)pyrene, concentrations at most of the 23 sites were close to the EQS of 2 ng/l, which was slightly exceeded at  five sampling stations JDS 02 (2.4 ng/l), JDS 16 (3.1 ng/l), JDS 39 (2.2 ng/l), JDS 92 (2.5 ng/l) and JDS 95 (2.3 ng/l).

The concentrations of the other priority PAHs in the water samples of the 23 sites were at least one order of magnitude below the WFD AA-EQS values.

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| | PAHs | | | |
|---|---|---|---|---|
| | Sediment | SPM | SPM in water | Water (SPM+diss.phase) |
| | [µg/kg] | [µg/kg] | [ng/l] | [ng/l] |
| Average | 493 | 696 | 11 | 18 |
| Median | 407 | 590 | 12 | 17 |
| Minimum | 111 | 216 | 3.1 | 8.9 |
| Maximum | 1135 | 2665 | 23 | 33 |
| 25-percentile | 220 | 436 | 6.9 | 13 |
| 75-percentile | 712 | 787 | 15 | 22 |
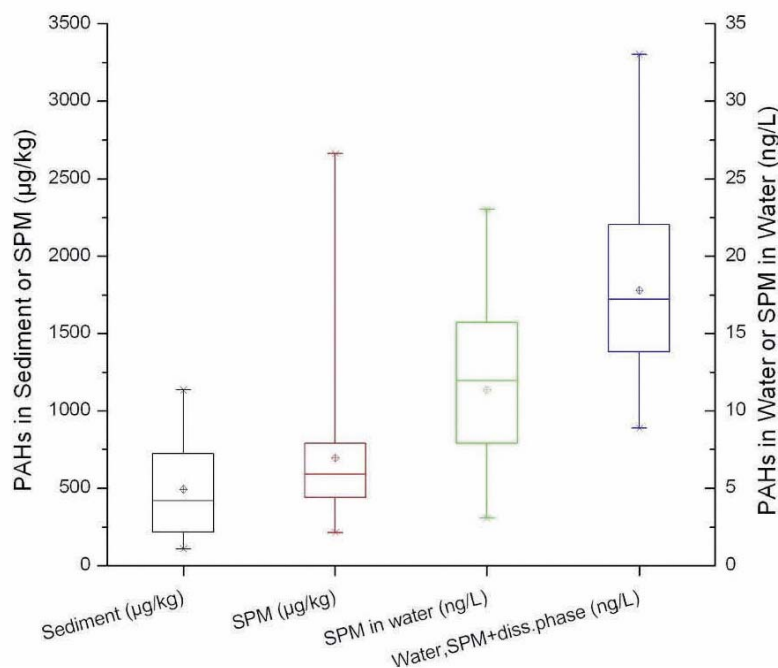


**Figure 78: PAH concentrations in all abiotic compartments**

Box-whiskers diagram: boxes represent the 25/75 percentiles with median and average (⊕);
the whiskers represent minimum and maximum values.

## 19.3.2 Organochlorine Pesticides (OCPs)

The concentrations of OCPs in the water phase were all below related EQS values, most of them by more than one to two orders of magnitude. Only the HCHs reached the same order of magnitude as the EQS in some isolated cases.

### 19.3.2.1 HCHs (∑ α-, β-, γ-, δ-HCH [2])

In the water column, HCHs were detected almost exclusively in the dissolved phase. For HCHs in water, both the AA-EQS is 0.02 µg/l and the MAC-EQS is 0,04 µg/l were not exceeded. The maximum of ∑HCHs in the water column was 0.011 µg/l at site JDS85 downstream of the Arges Tributary (RO).

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2 The group of HCHs includes 8 isomers. The EQS for HCH refers to α-, β-, γ-, and δ-HCH, the 4 major isomers present in the technical mixture. According to the Draft technical Guidance CMA the sum of α-, β-, γ-, and δ-HCH has to be reported.

| | Sum-HCH | | |
| --- | --- | --- | --- |
| | SPM | SPM in water | Water (SPM+diss.phase) |
| | [µg/kg] | [pg/l] | [pg/l] |
| Average | 0.77 | 23 | 2671 |
| Median | 0.42 | 5.1 | 793 |
| Minimum | 0.091 | 1.2 | 165 |
| Maximum | 2.3 | 105 | 11431 |
| 25-percentile | 0.26 | 2.4 | 391 |
| 75-percentile | 1.5 | 42 | 5255 |
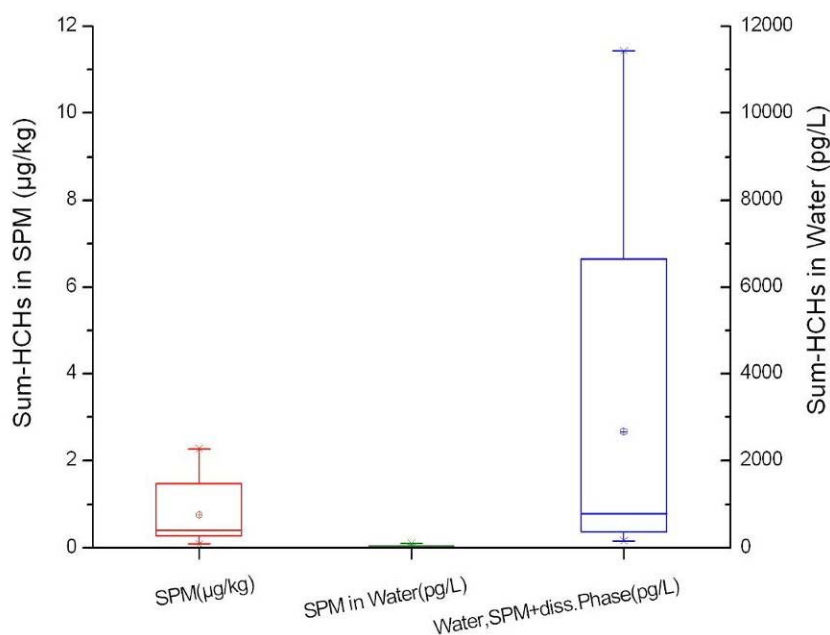


Figure 79: ∑HCHs concentrations in all abiotic compartments

The downstream profile displays a sharp increase of HCHs in the water column downstream of site JDS 58 (RS) towards the Black Sea. Upstream this site the ∑HCHs was dominated by γ-HCH, whereas downstream α- and β- HCH became abundant. The spatial distribution of the sites with high HCH concentrations is in accordance with the findings during the JDS1. The samples from the tributaries Drava, Sava and Velika Morava display slightly lower concentrations than the Danube itself.

### 19.3.2.2  Hexachlorobenzene
In the water column, HCB was detected both in SPM and the dissolved phase. The maximum value in the water column for HCB, detected at site JDS92 Reni (UA), was 0.11 ng/l, which is around 2 orders of magnitude below the respective AA-EQS of 10 ng/l and the MAC-EQS of 50 ng/l. The downstream profile of HCB is equilibrated and does not suggest particular emission maxima.

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| | HCB | | |
|---|---|---|---|
| | SPM | SPM in water | Water (SPM+diss.phase) |
| | [μg/kg] | [pg/l] | [pg/l] |
| Average | 1.0 | 25 | 59 |
| Median | 0.94 | 18 | 50 |
| Minimum | 0.33 | 1.8 | 20 |
| Maximum | 2.5 | 74 | 114 |
| 25-percentile | 0.51 | 6.1 | 39 |
| 75-percentile | 1.3 | 38 | 78 |
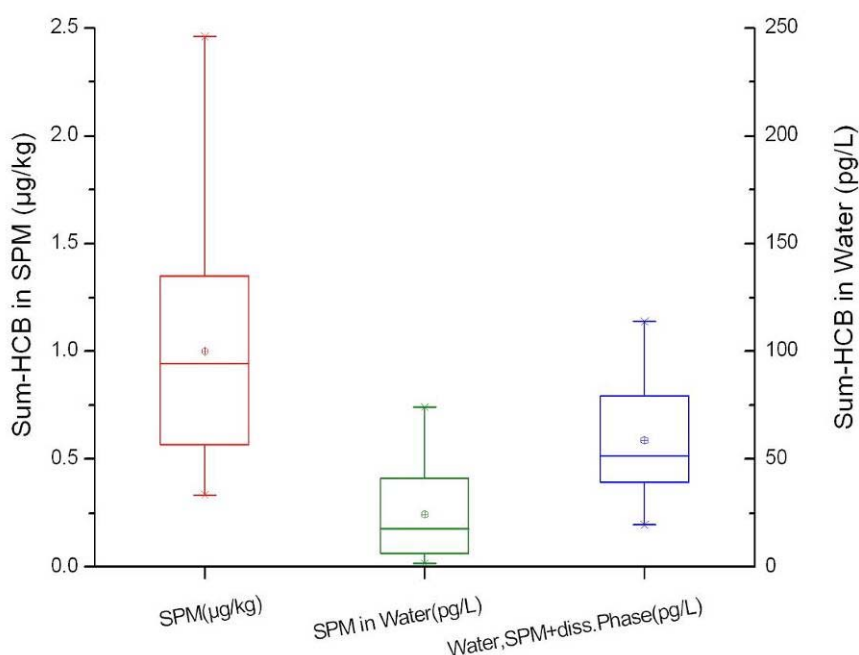


Figure 80: HCB concentrations in all abiotic compartments

### 19.3.2.3  DDT
∑p,p'-DDT, p,p'-DDE, p,p'-DDD, o,p-DDT

In the water column, DDT and its metabolites were detected both in the dissolved phase and in SPM.
The maximum concentration of ∑p,p'-DDT, p,p'-DDE, p,p'-DDD, o,p-DDT  in the water column was
around 1.2 ng/l at sites JDS92 and 95 (RO), which is more than one order of magnitude below the AA-
EQS of 25 ng/l. This maximum corresponds with high DDT concentrations in the SPM detected
during JDS1. The downstream profile in water suggests a constant increase of DDT and its metabolites
towards the Black Sea.

| | Sum-DDT and metabolites (WFD) | | |
|---|---|---|---|
| | SPM | SPM in water | Water (SPM+diss.phase) |
| | [µg/kg] | [pg/l] | [pg/l] |
| Average | 4.4 | 134 | 211 |
| Median | 4.0 | 81 | 133 |
| Minimum | 0.63 | 4.6 | 38 |
| Maximum | 14 | 1005 | 1229 |
| 25-percentile | 3.0 | 27 | 76 |
| 75-percentile | 4.9 | 111 | 180 |
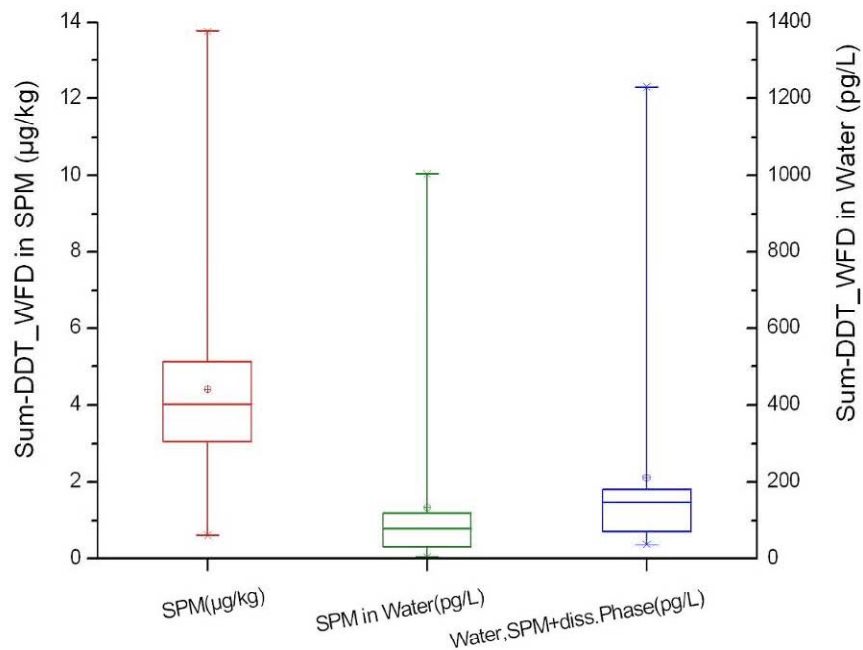


Figure 81: ∑DDT and metabolites concentrations in all abiotic compartments

For p,p'-DDT, the maximum level in the water column of 0.26 ng/l was detected in the Danube Delta, again more than one order of magnitude lower than the respective AA-EQS of 10 ng/l.

| | p,p'-DDT | | |
|---|---|---|---|
| | SPM | SPM in water | Water (SPM+diss.phase) |
| | [µg/kg] | [pg/l] | [pg/l] |
| Average | 1.4 | 36 | 47 |
| Median | 1.1 | 23 | 28 |
| Minimum | 0.13 | 1.7 | 6.0 |
| Maximum | 3.5 | 227 | 260 |
| 25-percentile | 0.58 | 7.2 | 14 |
| 75-percentile | 1.9 | 33 | 49 |

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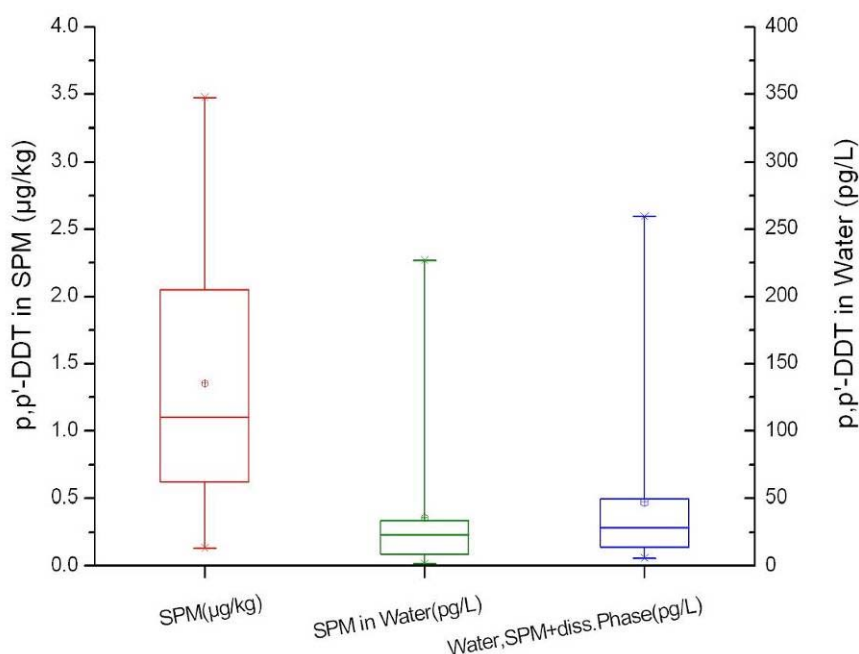


Figure 82: p,p'-DDT concentrations in all abiotic compartments

### 19.3.2.4 ∑ Aldrin, Dieldrin, Endrin and Isodrin

In the water column ∑ Aldrin, Dieldrin, Endrin and Isodrin were detected almost exclusively in the dissolved phase. Endrin could be quantified in all dissolved phase samples. For Aldrin, 14 sites were below the dissolved-phase LOD of 1.1 pg/l. For Endrin, 6 sites were below the LOD of 3.4 pg/l and Isodrin was detected in none of the sites (LOD of 6.1 pg/l). Within the sites with quantifiable amounts of ∑ Cyclodiene, Endrin concentrations were always dominant. For statistical evaluation only quantified concentration data are included.

Even when calculating upper bound concentrations in water, the ∑ Aldrin, Dieldrin, Endrin, Isodrin remain more than 2 orders of magnitude below the respective AA-EQS of 10 ng/l.

The downstream profile in water displays no particular trend.

| | Sum-Cyclodiene | | |
| --- | --- | --- | --- |
| | SPM (µg/kg) | SPM in Water (pg/l) | Water,SPM+diss.phase (pg/l) |
| Average | 0.086 | 1.9 | 23 |
| Median | 0.079 | 0.98 | 25 |
| Min | 0 | 0 | 1.8 |
| Max | 0.18 | 5.6 | 38 |
| 25-Percentile | 0.058 | 0.64 | 19 |
| 75-percentile | 0.12 | 2.7 | 27 |
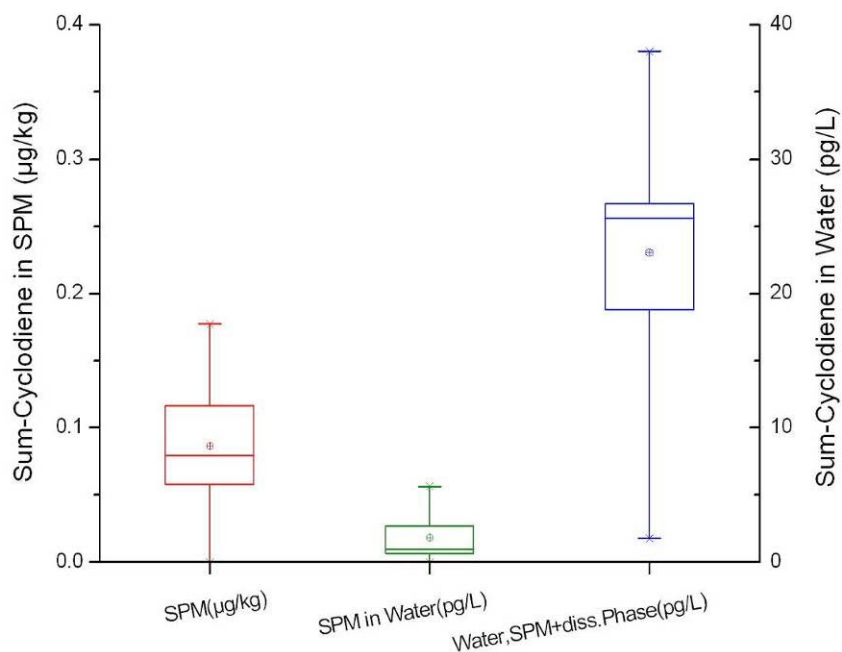
Figure 83: ∑ Aldrin, Dieldrin, Endrin and Isodrin concentrations in all abiotic compartments

### 19.3.2.5  ∑ α, β-Endosulfan

Due to very low concentration levels, a series of sites displayed non-detectable concentrations. The respective LODs were included in the data reported here to obtain upper bound concentrations, which however, can be considered as a worst case scenario. In the water column ∑ α,β-Endosulfan were detected to a large extent in the dissolved phase. An isolated (upperbound) maximum concentration (caused by one high value in SPM at site JDS56) of around 0.042 ng/l was detected.  However, this outlier is more than 2 orders of magnitude below the respective AA-EQS of 5 ng/l and the MAC-EQS of 10 ng/l.

The downstream profile suggests a slightly decreasing trend towards the Black Sea.

| | Sum-Endosulfanes upperbound (incl LOD) | | |
|---|---|---|---|
| | SPM | SPM in water | Water (SPM+diss.phase) |
| | [µg/kg] | [pg/l] | [pg/l] |
| Average | 0.58 | 4.6 | 12 |
| Median | 0.068 | 1.5 | 10 |
| Minimum | 0.031 | 0.30 | 4.0 |
| Maximum | 8.8 | 34 | 42 |
| 25-percentile | 0.053 | 0.84 | 8.6 |
| 75-percentile | 0.13 | 4.3 | 11 |

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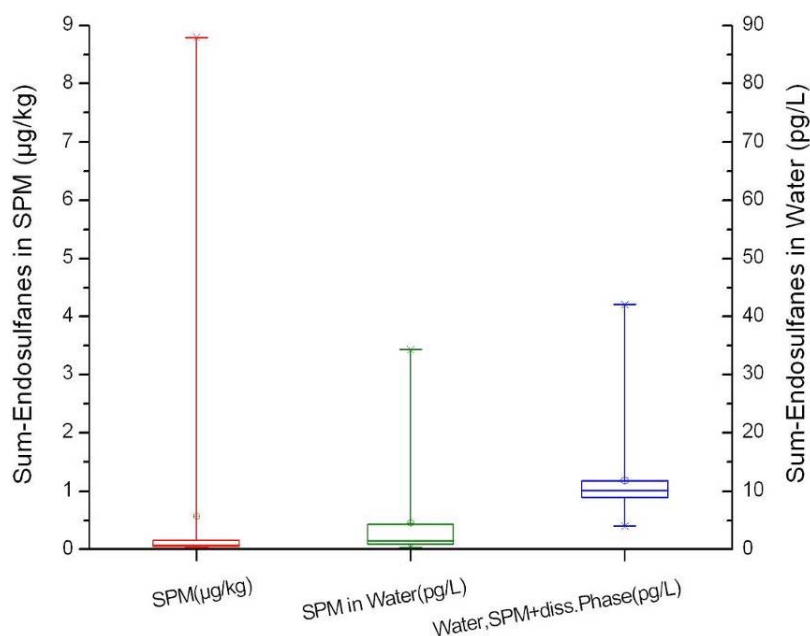


Figure 84: $\alpha$, $\beta$ -Endosulfan concentrations in all abiotic compartments

### 19.3.3 Indicator Polychlorinated Biphenyls (PCBs EC-6)

The average concentration of the EC6 PCBs in sediment was 4.3 µg/kg with a maximum of 46 µg/kg at JDS85 (RO), followed by site JDS53 (RS), with a clear impact from the Arges tributary. SPM samples display lower median/average concentrations of 3.1/2.1 µg/kg, also with a lower maximum of 9.1 µg/kg at JDS2 (GER). These data fit into the lower end of concentration ranges observed in the River Elbe (ARGE Elbe, 2006). In the Seine estuary, typical PCB contents in SPM are one order of magnitude higher (Chailleaud et al., 2007).

The PCB pattern in the sediments shows the typical "aged" environmental fingerprint with PCB 138, 153 and 180 being more abundant, with a dominance of PCB153. Sediments from the River Elbe (ARGE Elbe, 2006) and the River Seine (Chailleaud et al., 2007) show a similar distribution. The overall picture of the downstream concentration profile of EC-6 PCBs in sediments suggests some distinct historic inputs (because the distinction is not visible in the SPM data) form the left-hand side. The tributaries Drava, Sava and Velika Morava (JDS42, 51 and 56 respectively) show low concentrations compared to Danube sediments and indicate a diluting effect from those tributaries entering the Danube from the right-hand side.

The downstream profile in SPM is more equilibrated when compared to the sediments. An influence of the Arges tributary (JDS 53) and downstream of Pancevo (JDS 85) is not visible in the SPM samples, which supports the historic character of the sediment contamination of these sites. The higher PCB concentrations in SPM appear in the upper stretch of the Danube. After the Iron Gate constantly lower concentrations were observed, which suggests an efficient removal of PCB contaminated SPM in the Iron Gate reservoir through sedimentation.

None of the individual EC-6 PCBs exceeded the German chemical quality standard of 20 µg/kg for sediments. The lower PCB concentration in SPM suggests decreasing PCB emissions into the watershed.

In mussels, the $\sum$EC6 PCB concentrations were about 10 times higher than in the SPM, with a pattern similar to SPM, except PCB 28, showing a lower abundance in the mussels.

| | Sums of PCBs EC-6 | | | | |
| --- | --- | --- | --- | --- | --- |
| | Sediment | Mussels | SPM | SPM in water | Water (SPM+diss.phase) |
| | [µg/kg] | [µg/kg] | [µg/kg] | [pg/l] | [pg/l] |
| Average | 6.4 | 29 | 4.6 | 80 | 165 |
| Median | 4.3 | 25 | 3.6 | 68 | 158 |
| Minimum | 1.5 | 11 | 1.9 | 29 | 87 |
| Maximum | 46 | 116 | 9.9 | 200 | 268 |
| 25-percentile | 3.0 | 17 | 2.2 | 50 | 125 |
| 75-percentile | 6.3 | 34 | 6.4 | 90 | 186 |
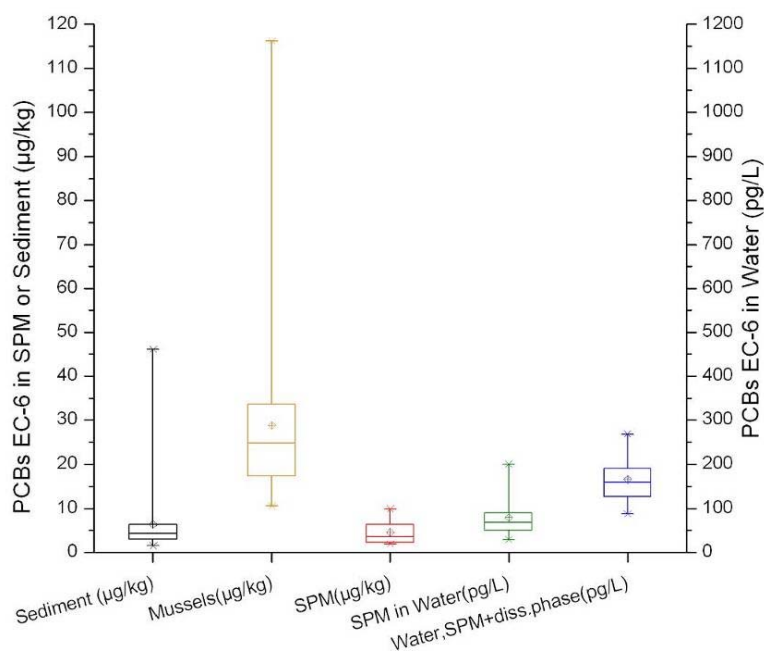


Figure 85: PCB EC-6 concentrations in all compartments

### 19.3.4 PCDD/Fs and DL-PCBs

#### 19.3.4.1 PCDD/Fs

PCDD/Fs were quantified in all samples. Most sediment samples display moderate TEQs with an average value of 2.8 ng/kg WHO TEQ, with an isolated maximum level of 21 ng/kg WHO TEQ (21 ng/kg I-TEQ) at site JDS53 (left hand side downstream of Pancevo (RS)).  This was the only site where the "safe sediment level" of 20 ng/kg I-TEQ (Ianuzzi et al., 1995) was exceeded slightly.

Similar levels in sediments were reported for the River Po showing PCDD/F concentrations between 1.3 and 13 ng/kg WHO TEQ (Fattore et al., 2002). For comparison: levels in sediments of the River Elbe are around 40-80 ng/kg WHO TEQ in the more industrialized stretches and around 5-10 ng/kg WHO TEQ along stretches with diffuse inputs (Stachel et al., 2005; Umlauf et al., 2004, 2005).

Concentrations in SPM were slightly lower than in sediments with an average of 2.0 ng/kg WHO TEQ and a maximum of 8.2 ng/kg WHO TEQ at site JDS 45 (HU). Lower concentrations in the SPM when compared to sediments suggest that the overall dioxin emissions into the catchment are decreasing and that the contents in sediments are a memory from historic inputs rather than a recent signal.

The average pattern of 2,3,7,8-PCDD/Fs in sediments and SPM, dominated by OCDD and some minor contribution from HpCDD and OCDF, is typical for a profile altered by long range atmospheric

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transport/deposition. It can be found worldwide in background soils and sediments in the absence of the influence of direct industrial emissions.

In the water column, no PCDD/Fs were detected in the dissolved phase. LOD for PCDD/Fs on a WHO TEQ base was 0.039 pg/l in the dissolved phase, which is around the same as the average concentration in water associated with SPM. However, in the water phase, PCDD/Fs are predominantly associated with SPM, which means that the average value of 0.037 pg/l WHO TEQ derived from the quantification based on "SPM in water" should only fairly reflect the total concentration in the water column. The downstream concentration profile of PCDD/Fs in the sediments shows only a few extremes and in most cases no interpretable differences between left- and right-hand side samples, which suggests input mainly from diffuse sources.

Comparably high concentrations at site JDS2 point again to an input from the Altmuehl tributary as observed for PAHs above. Another site with somewhat higher PCDD/F concentrations on both sides of the Danube was at JDS39 (HU), which had displayed the highest PCP result during JDS1. Maximum TEQ concentrations in sediment of 21 ng/kg were detected at JDS53 left-hand side downstream of Pancevo (RS), a site that has shown a high abundance of EC6- and DL- PCBs as well. The 3 tributaries, the Drava, Sava and Velika Morava (JDS42, 51 and 56 respectively) show low levels both in sediments and SPM when compared to the Danube itself.

| | PCDD/Fs in WHO-TEQ 1998 | | | |
| --- | --- | --- | --- | --- |
| | Sediment | SPM | SPM in water | Water upperbound (SPM+LOD of diss.phase) |
| | [ng/kg] | [ng/kg] | [pg/l] | [pg/l] |
| Average | 2.8 | 2.0 | 0.037 | 0.077 |
| Median | 1.9 | 1.6 | 0.032 | 0.072 |
| Minimum | 0.97 | 0.83 | 0.0094 | 0.049 |
| Maximum | 21 | 8.2 | 0.17 | 0.21 |
| 25-percentile | 1.4 | 1.1 | 0.021 | 0.061 |
| 75-percentile | 3.3 | 2.4 | 0.041 | 0.081 |
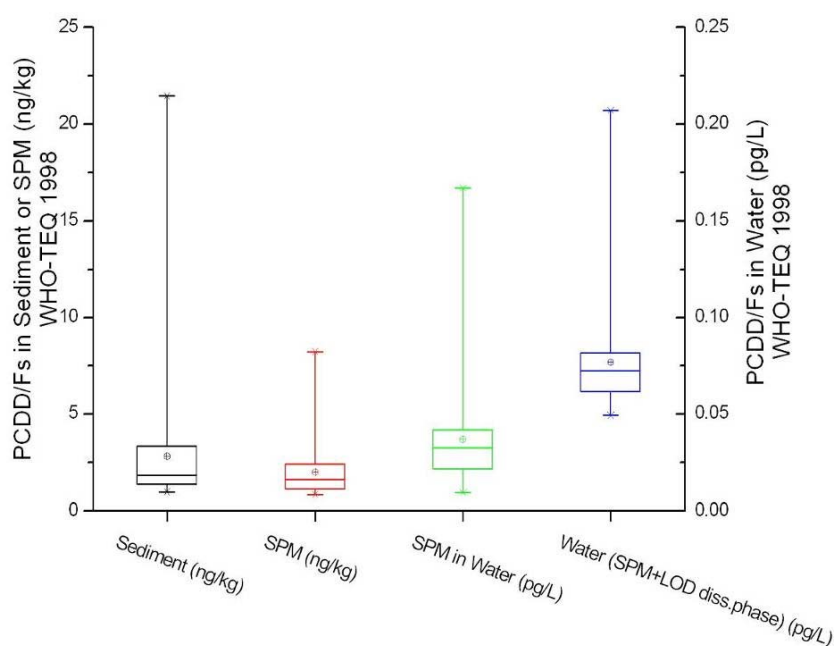


Figure 86: PCDD/F concentrations in all abiotic compartments

### 19.3.4.2 DL-PCBs

DL-PCBs were quantified in all samples. Most sediment samples display low TEQs with an average value of 0.6 ng/kg WHO TEQ; a maximum level of 2.6 ng/kg occurred at site JDS85 left hand side (downstream of the Arges tributary, RO) and two more distinctive spots at JDS53 (downstream of the Tamis tributary, RS) and JDS2 (downstream of the Altmuehl tributary, GER), both left-hand side. SPM samples displayed slightly lower values than had been seen before for PCDD/Fs, with highest concentration of 1.5 ng/kg WHO TEQ at site JDS2 downstream of the Altmuehl Tributary.

In the water column, DL-PCBs were detected in the SPM and dissolved phase.

The low overall contribution of PCBs of less than 20% to the combined (PCDD/F & DL-PCBs) WHO TEQ in SPM and sediments of the Danube is typical for surface waters without significant impact of industrial discharges and reflects the situation in atmospheric deposition to a greater extent.

| | DL-PCBs in WHO-TEQ 1998 | | | |
|---|---|---|---|---|
| | Sediment | SPM | SPM in water | Water (SPM+diss.phase) |
| | [ng/kg] | [ng/kg] | [pg/l] | [pg/l] |
| Average | 0.59 | 0.46 | 0.0091 | 0.013 |
| Median | 0.49 | 0.42 | 0.0081 | 0.012 |
| Minimum | 0.17 | 0.16 | 0.0033 | 0.0080 |
| Maximum | 2.6 | 1.5 | 0.021 | 0.025 |
| 25-percentile | 0.29 | 0.22 | 0.0061 | 0.011 |
| 75-percentile | 0.64 | 0.60 | 0.011 | 0.015 |
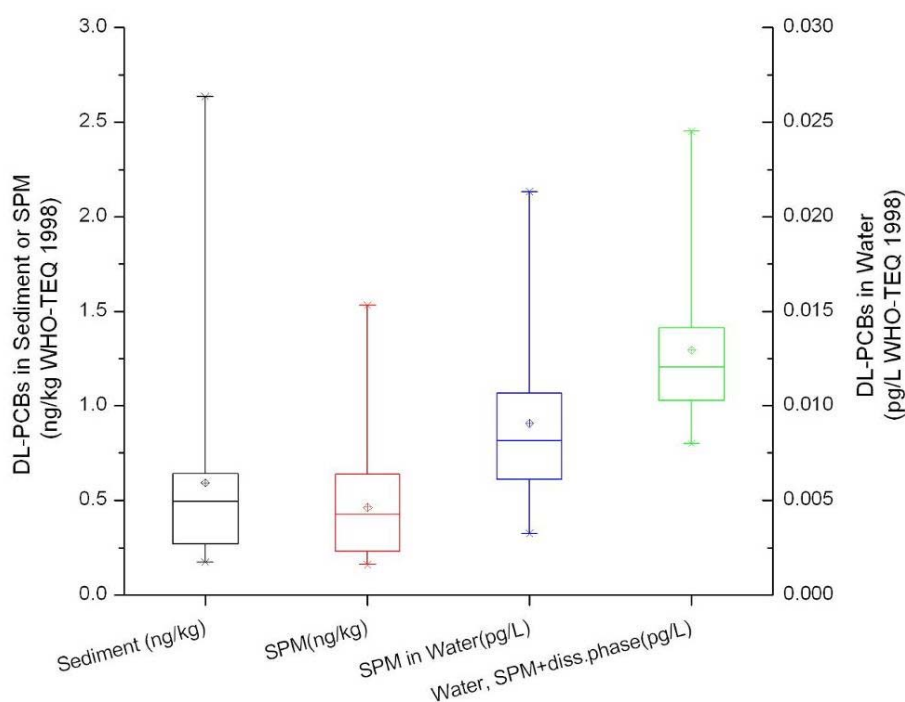


Figure 87: DL-PCB concentrations in all abiotic compartments

### 19.3.5 Polybrominated Diphenylethers (PBDEs)

PBDEs were quantified in all samples. Among the PBDEs measured in sediments, SPM and water samples, Deca-BDE dominated the pattern by far. In the downstream profile, ∑PBDEs in general

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displayed bigger gradients than PAHs and PCDD/Fs, which suggests a more recent emission history for this compound class.

The zone of comparably high PBDE concentrations in water, sediment and SPM appeared in the stretch between km 1560 (JDS35, HU) and km 1077 (JDS58, RS). The highest concentrations of ∑PBDEs in SPM and water were found at site JDS45 (HR); in sediments the maximum was slightly further upstream in the sediments of the Drava tributary JDS42 (HR). The downstream sediment data suggests that PBDEs are entering from the right-hand side of the catchment. PBDEs in sediments and in the water column show a clear impact from the Drava, Sava and Velika Morava tributaries all entering the River Danube from the right-hand side, which, for PAHs, PCBs and PCDD/Fs displayed a diluting effect.

### 19.3.5.1  Decabromodiphenylether (c-Deca BDE)  (∑BDE  206, 207, 208, 209)

Average concentrations of c-Deca BDE in SPM were 15 µg/kg with maximum levels of 56 µg/kg at site JDS45 (HR).  In the sediment samples, average and maximum concentrations were slightly lower than for SPM. These levels are one order of magnitude lower than in SPM collected in various rivers in the Netherlands, where De Boer et al., (2003) reported a median of 71 µg/kg and a range of  9-4600 µg/kg.

In water the average concentration of c-Deca BDE was 251 pg/l, the maximum was 1163 pg/l at site JDS45 (HR). In the water samples, c-Deca BDE was almost exclusively associated with SPM.

| | cDeca-BDE | | | |
|---|---|---|---|---|
| | Sediment | SPM | SPM in water | Water  (SPM+diss.phase) |
| | [µg/kg] | [µg/kg] | [pg/l] | [pg/l] |
| Average | 12 | 15 | 232 | 251 |
| Median | 5.6 | 7.6 | 162 | 180 |
| Minimum | 1.5 | 3.1 | 51 | 77 |
| Maximum | 51 | 56 | 1140 | 1163 |
| 25-percentile | 3.5 | 3.9 | 94 | 118 |
| 75-percentile | 18 | 26 | 224 | 228 |
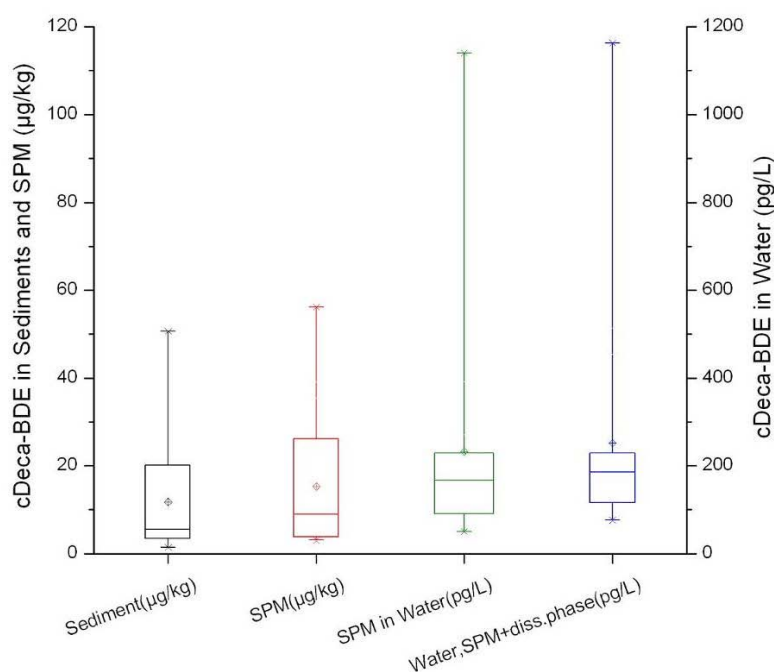


Figure 88: c-Deca BDE concentrations in all abiotic compartments

### 19.3.5.2 c-Octa BDE (∑of BDE 183, 196, 197, 203)

Average concentrations of c-Octa BDE in SPM were 0.17 µg/kg with maximum levels of 0.49 µg/kg at site JDS45 (HR). Sediments displayed almost identical values. In the water column, c-Octa BDE SPM is strongly associated with SPM.

| | cOcta-BDE | | | |
|---|---|---|---|---|
| | Sediment | SPM | SPM in water | Water  (SPM+diss.phase) |
| | [µg/kg] | [µg/kg] | [pg/l] | [pg/l] |
| Average | 0.17 | 0.17 | 2.6 | 3.3 |
| Median | 0.12 | 0.15 | 1.8 | 2.1 |
| Minimum | 0.027 | 0.039 | 0.97 | 1.3 |
| Maximum | 0.42 | 0.49 | 10 | 10 |
| 25-percentile | 0.069 | 0.057 | 1.6 | 1.9 |
| 75-percentile | 0.26 | 0.26 | 3.2 | 4.7 |
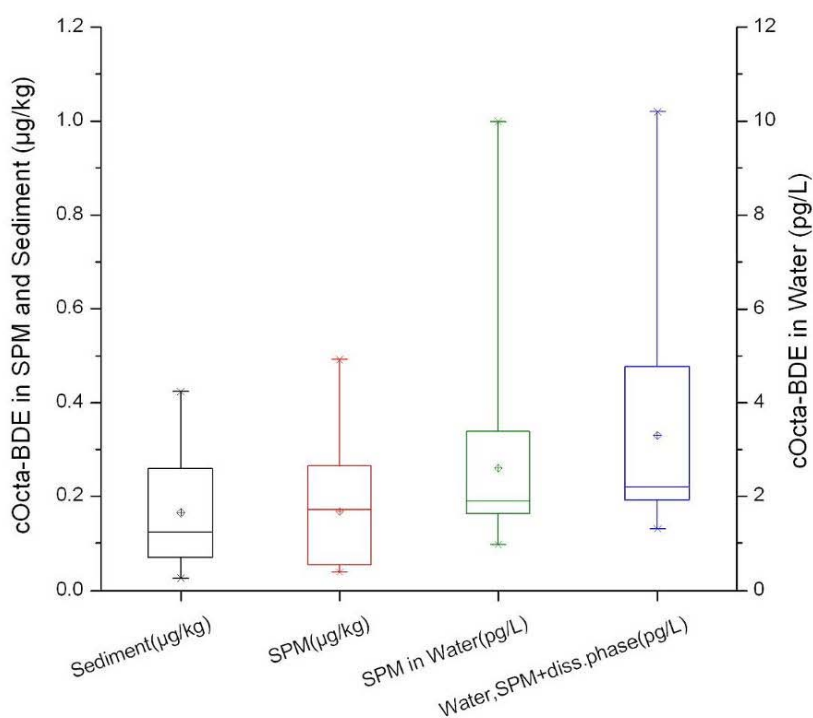


Figure 89: c-Octa BDE concentrations in all abiotic compartments

### 19.3.5.3  c-Penta BDE (∑ of BDE 28, 47, 99, 100, 153 and 154)

Average c-Penta BDE concentrations in SPM were at 0.60 µg/kg with a maximum level of 1.8 µg/kg. In the water column, c-Penta BDE was mainly associated with the dissolved phase. Interestingly, c-Penta BDE was more associated with the dissolved phase when compared with PAHs and PCDD/Fs having comparable Ko/w values, which suggests impact from products and process effluents rather then from atmospheric sources. Average c-Penta BDE concentrations in water (dissolved phase + SPM) were 57 pg/l with a maximum level of 121 pg/l, which is still fairly below the EQS of 500 pg/l.

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| | cPenta-BDE | | | |
|---|---|---|---|---|
| | Sediment | SPM | SPM in water | Water (SPM+diss.phase) |
| | [μg/kg] | [μg/kg] | [pg/l] | [pg/l] |
| Average | 0.48 | 0.60 | 9.0 | 57 |
| Median | 0.43 | 0.54 | 7.5 | 51 |
| Minimum | 0.20 | 0.12 | 2.8 | 25 |
| Maximum | 1.2 | 1.8 | 36 | 121 |
| 25-percentile | 0.30 | 0.17 | 5.0 | 43 |
| 75-percentile | 0.60 | 0.80 | 10 | 64 |
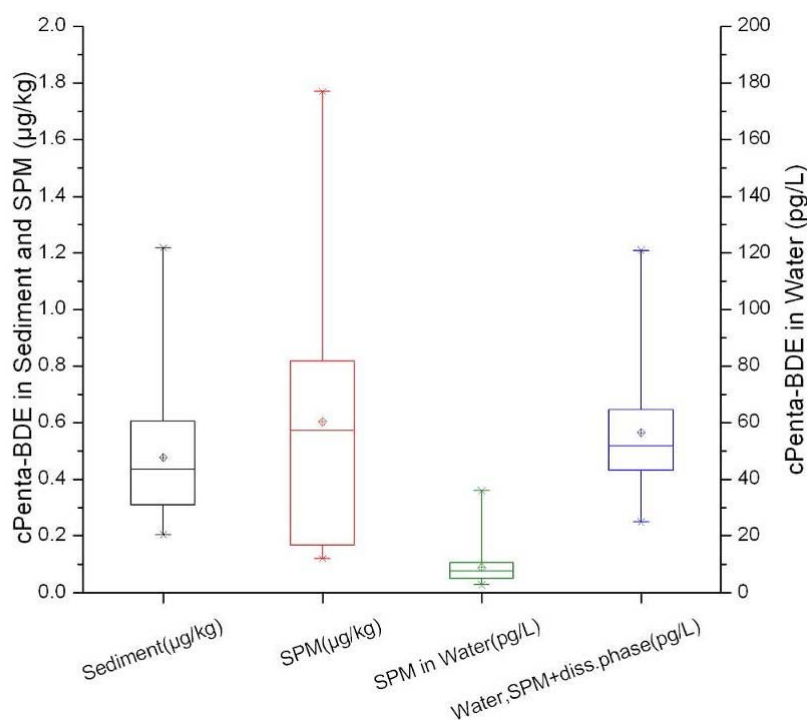


Figure 90: c-Penta BDE concentrations in all abiotic compartments

## 19.4    Conclusions

From the available data of the 23 sites analysed, it appears that the chemical status of the River Danube is good for most of the following compound classes:

–   PAHs: at all 23 sites, most of the PAH levels in water samples were far below the WFD AA-EQS values and values in sediments were about one order of magnitude lower than typically found in the River Elbe.

–   OCPs: most compounds in the water column were 2-3 orders of magnitude below the EQS and only HCH displayed some isolated maxima in the Lower Danube, which were, however, still a factor of 4 below the MAC-EQS. Preliminary results in the SPM and dissolved phase water samples suggest that concentrations in water are all below WFD EQS for inland waters.

–   PCDD/Fs and Dioxin-like PCBs: more than one order of magnitude lower in all compartments compared to the River Elbe and only one site exceeded slightly the "safe sediment value" for PCDD/Fs.

–   EC-6 PCBs: samples did not exceeding the related German quality standards in sediment.

– PBDEs: concentrations in SPM were an order of magnitude lower than in Dutch rivers for c-Deca BDE and where c-Penta BDE was around a factor of 5 below the EQS value in all water samples.

Only for the ∑benzo(g,h,i)perylene and indeno(1,2,3-cd)pyrene, were concentrations at most sites close to the EQS of 2 ng/l. In five sites the EQS was exceeded, namely at sampling stations JDS2, 16, 39, 92 and 95.

The concentration profiles downstream in the Danube suggest that PAHs and PCDD/Fs arise from diffuse sources, whereas PBDEs and PCBs display distinct zones of contamination. This fits into the picture of PAHs and PCDD/Fs as combustion by-products being dispersed mainly into the atmosphere, whereas "intentionally produced industrial chemicals" such as PCBs and PBDEs arise from punctual emissions through industrial and urban effluents. Among the OCPs in water, DDT and metabolites, as well as HCHs, displayed rising concentrations towards the Black Sea. HCB and the Cyclodiene pesticides displayed no spatial trend and Endosulfan concentrations decreased downstream the Danube.

The comparison of left and right-hand side sediment data suggests a diffuse emission from both sides of the catchment for PAHs.  PCDD/Fs and PCBs show some distinct signals from the left-hand side and PBDEs are emitted from the right-hand side of the catchment. Only PBDEs show a clear impact from the Drava, Sava and Velika Morava tributaries all entering River Danube from the right-hand side, whereas for the other compound classes reported here, these tributaries displayed a diluting effect.

However, the memory contained in the sediments is scarcely reflected by the data in the water column data, where (except for PBDEs, the most recent class of chemicals investigated in this study) the spatial gradients are less pronounced and maxima appear often at different sites. This underlines the historic character of the findings in the sediments. In order to assess the current situation of pollutant releases into the River Danube, and to localize their sources, temporally resolved water column data from the whole watershed are needed.
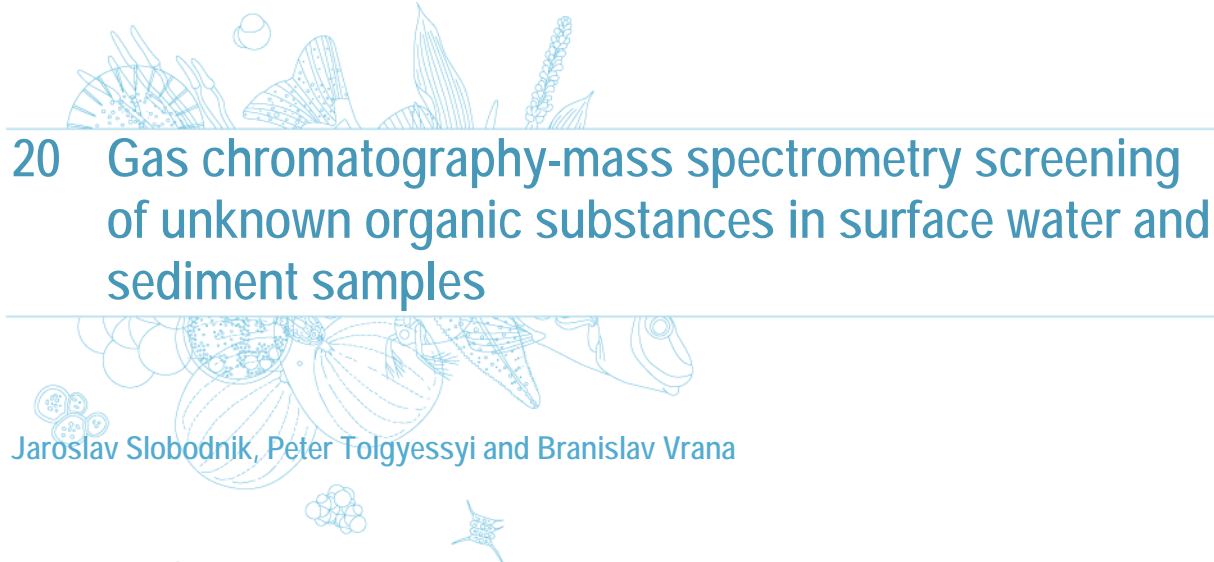
## 19.5    References
ARGE Elbe: Gewaesserguetebericht der Elbe 2006 (2006): http://www.arge-elbe.de/wge/Download/Berichte/06Guetebericht.pdf

CHAILLEAUD K., FORGET-LERAY, J., SOUISSI, S., HILDE, D., LEMENACH, K., BUDZINSKI, H. (2007), Chemosphere, in press, Seasonal variations of hydrophobic organic contaminant concentrations in the water-column of the Seine Estuary and their transfer to planktonic species. Part 1: PCBs and PAHs.

COMMON POISITION adopted by the Council with view to the adoption of a Directive of the European Parliament and of the Council on environmental quality standards in the field of water policy and amending Directives 82/176/EEC, 83/513/EEC. 84/156/EEC, 84/491/EEC, 86/280//EEC and 2000/60/EEC.ANNEX I, Brussels, 29.11.2007

DE BOER, J., WESTER, P.G., VAN DER HORST, A., LEONARDS, P.E.G. (2003): Polybrominated diphenyl ethers in influents, suspended particulate matter, sediments, sewage treatment plant and effluents and biota from the Netherlands: Environmental Pollution 122, 63-74.

EVERS, E.H.G., LAANE, R.W.P.M., GROENEVELD, G.J.J. AND OLIE, K., 1996. Levels, temporal trends and risk of dioxins and related compounds in the Dutch aquatic environment. *Organohalogen Compounds* 28, 117–122.

FATTORE, E., VIGANO, L., MARIANI, G., GUZZI, A., BENFENATI, E., FANELLI, R. (2002): Polychlorinated dibenzo-p-dioxins and dibenzofurans in River Po sediments. Chemosphere 71, 1156-1161

IANNUZZI, T.J., BONNEVIE, N.L. AND WENNING, R.J., 1995. An evaluation of current methods for developing sediment quality guidelines for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Arch. Environ. Contam. Toxicol.* 28, pp. 366–377.

OLIVELLA, M.A. (2006): Isolation and analysis of  polycyclic aromatic hydrocarbons from natural water using accelerated solvent extraction followed by gas chromatography-mass spectrometry
Talanta 69, 267-275.

STACHEL, B.,  JANTZEN, E.,  KNOTH, W., KRÜGER, F.,  LEPOM , P., OETKEN, M.,  REINCKE, H.,
SAWAL, G., SCHWARTZ, S.,  UHLIG, S. (2005):The Elbe Flood in August 2002—Organic Contaminants in
Sediment Samples Taken After the Flood Event.  J. Environ. Sci. Health A 40 (2), 265-287

UMLAUF, G., BIDOGLIO, G., CHRISTOPH, E., KAMPHEUS, J., KRUEGER, F., LANDMANN, D.,
SCHULZ, A.J., SCHWARTZ, R., SEVERIN, K., STACHEL, B., STEHR, D. (2005): The Situation of PCDD/Fs
and Dioxin-like PCBs after the Flooding of River Elbe and Mulde in 2002.
Acta Hydrochimica et Hydrobiologia 33, Issue 5 ; Special Issue: Displacement of Pollutants during the River
Elbe Flood in August 2002,  pp 543-554

UMLAUF, G., CHRISTOPH, E., BIDOGLIO, G. (2004): Dioxin- und Quecksilberanalysen an ausgewaehlten
Proben aus den Ueberschwemmungsgebieten. In: *Geller, W., Ockenfeld, K., Boehme, M., Knoechel, A. (Hrsg):
Schadstoffbelastung nach dem Elbe-Hochwasser 2002.*
*Endbericht des Ad-hocVerbundprojekts BMBF Foerderkennzeichen PTJ 033049.* ISBN: 3-00-0136615-0 (2004).
Download: http://www.ufz.de/data/HWEnd1333.pdf

VAN DEN BERG, M., L. BIRNBAUM, ET AL. (1998):  Toxic equivalency factors (TEFs) for PCBs, PCDDs,
PCDFs for humans and wildlife. Environmental Health Perspectives 106 (12): 775-792.

## 19.6    Acknowledgements

# 20 Gas chromatography-mass spectrometry screening of unknown organic substances in surface water and sediment samples

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## 20.1    Introduction

According to Annex V of the Water Framework Directive (WFD), EU member states are obliged to include "*other pollutants*" discharged in significant quantities into a water body (specific to an individual river basin), into the classification of overall ecological status. Once identified, these pollutants must be included in monitoring schemes and their environmental quality standards should be derived. At present, only four metals, arsenic (As), chromium (Cr), copper (Cu) and zinc (Zn), are listed as other pollutants specific to the Danube Basin. Similar to other basins in Europe, the inclusion of additional organic pollutants into the list is hampered by the lack and quality of available data. In a typical monitoring effort, most Danube countries focus on analysis of the 33 WFD Priority substances (PS; Dangerous Substances Directive, 2006) determining the chemical status of water bodies but overlooking the possible presence of tens of thousands of other substances, which could potentially enter the environment.

The main objective of the gas chromatography-mass spectrometry (GC-MS) screening of the JDS2 samples was therefore to identify and trace pollution trends of 'unknown' substances, which are not included (i) in the routine monitoring schemes of the Danube countries and (ii) among the JDS2 target parameters. Such pollutants are often termed as 'emerging substances' and may be candidates for future regulation, depending on research on their (eco)toxicity; potential health effects; public perception and monitoring data regarding their occurrence in the various environmental compartments. Emerging substances are of increasing concern to scientists, regulators and the public. They are not necessarily new chemicals and some of them have long been present in the environment, but their presence and significance are only now attracting closer attention. Personal care products, pharmaceuticals, fragrances, disinfection by-products, detergents, petrol additives, flame retardants and new types of pesticides are just some examples of the emerging substances frequently discussed today.

Mass spectra obtained from GC-MS screening in electron impact mode are widely accepted as unique fingerprints of individual organic compounds and can be compared against the existing databases (e.g. Wiley library contains more than 400,000 spectra). Despite the above, typically some 10 – 30% of compounds detected in an environmental sample stay unidentified. Here, a decision can be made to judge whether additional targeted research is needed to identify the detected unknown substances e.g. based on the overall ecotoxicity of the sample; frequency of occurrence and concentrations or evidence of biological impact in the vicinity of the sampling site(s).

Guidelines developed within the EU 6th Framework Programme research project NORMAN, dealing with emerging substances at the EU level (www.norman-network.net) were taken into account during evaluation of the results.

## 20.2    Methods

### 20.2.1 GC-MS screening of water samples

Water samples (100 ml) were placed in a 250 ml glass bottle and spiked with 10 µl (10 ng/µl) methanolic perdeuterated anthracene internal standard solution to give a concentration of 1 µg/l and

then extracted using stir bar sorptive extraction (SBSE) for 60 minutes at 600 rpm. After extraction, stir bar was removed from the sample, dried and placed in the liner of a TDU thermal desorption system (Gerstel, Mühlheim a.d. Ruhr, Germany). The GC-MS screening analysis was performed using Agilent 6890 gas chromatograph coupled to Agilent 5973 mass spectrometric detector (MSD; Agilent Technologies, Little Falls, DE, USA). The system was equipped with a TDU and a CIS4 programmed temperature vaporisation (PTV) injector system (Gerstel). Solvent venting thermal desorption was performed by programming TDU from 40 to 250ºC (5 minutes) at a rate of 12 ºC/s. The analytes were cryo-focused in the PTV at -30ºC with liquid $CO_2$ prior to injection. For splitless injection (5 minutes) the PTV was ramped from -30ºC to 250ºC (5 minutes) at a rate of 12 ºC/s. Capillary GC analysis was performed on a 30 m × 250 µm I.D., 0.25 µm $d_f$ HP-5MS column (Agilent Technologies). The oven was programmed from 70 ºC (2 minutes) at 25 ºC/min to 150 ºC, at 3 ºC/min to 200 ºC and finally at 8 ºC/min to 280 ºC (10 minutes). Helium was used as carrier gas. The head pressure was calculated using the retention locking (RTL) software so that chlorpyrifos methyl was eluting at a constant retention time of 16.596 minutes (Sandra et al. 2003, Sandra et al. 2003). The MSD was used in the scan mode (m/z 40–550) for all samples. Identification of compounds was performed using mass spectrum libraries Wiley 7n and NIST02, respectively.

### 20.2.2 GC-MS screening of sediment samples

The sediment sample (0.5 g) was weighed and placed into a1.5 ml micro test tube (Eppendorf AG, Hamburg, Germany) and spiked with 10 µl (10 µg/ml) methanolic perdeuterated anthracene internal standard solution to give a concentration of 0.2 µg/g. 1 ml of a solvent mixture methanol/dichloro-methane (9:1; v/v) was added and the mixture was sonicated for 2×15 minutes. After extraction, the mixture was centrifuged at 1000 rpm for 2 minutes and the supernatant was transferred to 100 ml Milli-Q water containing 10 ml methanol.  The resulting solution was extracted using SBSE for 60 minutes at 600 rpm. The GC-MS screening analysis was performed in the same way as for water samples.

It should be stressed that the GC-MS analyses of water and sediment samples were performed in the SCAN mode i.e. the mass ions created in the mass spectrometer were detected at the full spectral scale not to miss any information on created fragments. This operation mode, however, results in a limited sensitivity, which cannot be compared with the sensitivity of standard methods used for the determination of target organic compounds within the JDS2. Therefore, not all organic micropollutants determined by target analyses must have been necessarily detected by GC-MS.

## 20.3    Results

### 20.3.1 GC-MS screening of surface water samples

Altogether, 124 water samples from the Danube River and its tributaries were analysed by GC-MS and several organic compounds were identified in all but three samples (JDS12 and 69 on the Danube and JDS-PR2 on the Prut tributary). Based on the obtained spectral information, chemical structures of 158 analytes could be proposed (see Table 36 and Figure 91). An additional 43 compounds remained unidentified. For comparison, screening of 98 water samples in the JDS1 revealed the presence of 96 provisionally identified analytes. Here one should take into account that in the JDS1 water samples were prepared using liquid-liquid extraction into dichloromethane, whereas in the JDS2 a more advanced technique (SBSE, see Methods above) was applied. Both techniques concentrate hydrophobic compounds from water to an extraction solvent, and the extraction efficiency depends on the compound partition coefficient. Both methods have comparable performance characteristics; however, dichloromethane extraction enables detection of more volatile organic compounds (e.g. tetrachloroethene) and also more hydrophobic compounds that are usually adsorbed on the SPM (e.g. sterols). On the other hand, the SBSE technique is less laborious and showed that more compounds could be detected compared to the JDS1, despite the fact that the method allows only for extraction of organic substances dissolved in water.
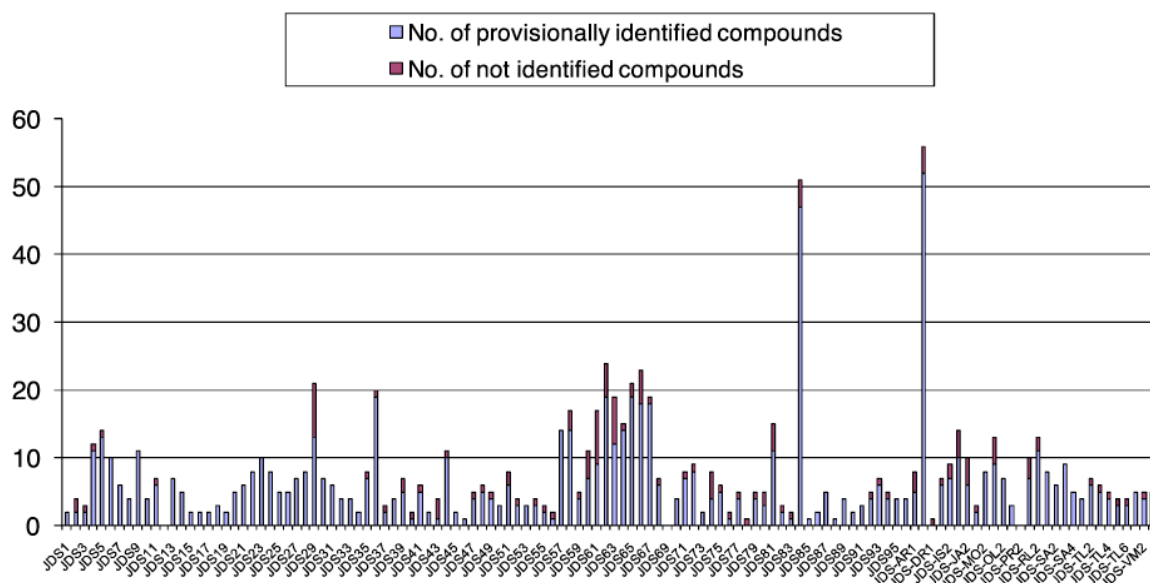
Figure 91: Number of compounds detected in the JDS2 surface water samples

In agreement with the results of the JDS1, phthalates and fatty acids belong to the most ubiquitous compounds detected. Phthalates are commonly used as plasticisers, industrial and lubricating oils, defoaming agents, cosmetics and insect repellents. Dibutyl phthalate, which is already on the list of 'other substances' for Slovakia and Finland (with provisional AA-EQS of 10 µg/l) was detected in 27 samples with highest estimated concentrations found at JDS22 (Iza/Szony) and JDS23 (Sturovo/Esztergom; cf. Figure 92. The significantly elevated concentrations detected in the river stretch between JDS20 (Komarno/Komarom) and JDS31 (downstream of Budapest) are a matter of concern and might be a topic for the follow-up study. Di(2-ethylhexyl) phthalate, which is on the list of WFD priority substances, was detected in 40 samples which confirms statements on its ubiquitous presence in the Danube Basin from target analyses (see Chapter 1). The most widespread representative of this group was isobutyl phthalate, present in 91 samples. Fatty acids enter the environment mainly from degradation of petroleum hydrocarbons and animal and vegetable fats, being often used as indicators of the efficiency of the treatment process in wastewater treatment plants.

In general, a significantly wider variety of esters of fatty acids and other acids, derivatives of benzene and polycyclic aromatic hydrocarbons (PAH) were detected in the JDS2 compared to the JDS1. A large number of derivatives of naphthalene and phenathrene were characteristic for JDS84 (Arges tributary), JDS-AR2 (Arges tributary downstream of Bucharest) and JDS-RL2 (Rusenski Lom tributary at Beli Lom, Pisanetz) sampling sites. Phenanthrene, detected in nine JDS2 samples,  is already included in the list of *other substances* for Slovakia with a provisional AA-EQS of 0.38 µg/l.

Alkylsubstituted benzenes represent typical degradation products of petroleum hydrocarbons coming mainly from oil pollution due to navigation and combustion of fuels. They were found in larger numbers and quantities in: the river stretch from river kilometre 1040 to 840 (Iron Gate reservoir (Golubac/Koronin) - Pristol/Novo Selo Harbour); at JDS36 (Paks, rkm 1533) and at JDS4, 5 and 6 sites (Deggendorf, Niederaltereich and Inn tributary).

A significant presence of personal care products**,** indicators of wastewater pollution or poor efficiency of wastewater treatment plants, was identified in most samples. Among the detected compounds were:

– Sun-screen agents: EHMC, drometrizole, acetophenone and benzophenone;

– Fragrances and musks: limonene, .alpha.-terpinene, junipene, longicyclene, isobornyl acetate, dihydro methyl jasmonate, dihydromyrcenol, menthol, galaxolide, 2,4,7,9-tetramethyl-5-decyne-4,7-diol and 1,4-dioxacycloheptadecane-5,17-dione (Musk T);

–   <u>Other cosmetic ingredients</u>: ethylene-, diethylene-, triethylene- and pentaethylene glycol monododecyl ethers, 2-hydroxybenzoic acid pentyl ester, dipropyleneglycol dibenzoate, bis(2-ethylhexyl) maleate, tributyl acetylcitrate, 2-ethyl-1-hexanol, 3,7-dimethyl-3-octanol, 2-(dodecyloxy) ethanol, 2-(1,1-dimethylethyl) cyclohexanol, 1-methyl-2-pyrrolidinone, acetylcedrene and 2,4-toluenediamine.

Galaxolide, which was also included among the JDS2 target determinands in SPM was found at the highest level at JDS84 and JDS-AR2 (both on the Arges tributary) indicating pollution by urban wastewater from Bucharest. It was also recorded, at ca. 10-fold lower concentration levels, at JDS31 and 35, reflecting pollution by wastewater from Budapest. The compound was also present in the tributaries, the Morava, Olt, Iskar and Russenski Lom. Galaxolide was detected at the JDS84 site (the Arges tributary) also during the JDS1 in 2001 at the same estimated concentration level of 0.4 µg/l as in 2007.

Other relevant groups of detected compounds included chlorinated compounds (e.g. 1,2-dichlorobenzene, bis(2-chloroisopropyl) ether), organophosphate flame retardants (OPFR), benzothiazoles (rubber accelerators in the tyre industry), nitrogen containing compounds (alkylnitrobenzenes, nitriles, amines) and a group of pesticides (hexachlorocyclohexane (alpha isomer), thiocarbamates).

Regarding the spatial distribution of identified compounds, ubiquitous, section-specific, site-specific and random occurrence could be distinguished. The highest number of organic compounds, including a wide variety of aromatic hydrocarbon derivatives and personal care products, was identified in samples from the Arges tributary (47 and 52 compounds in JDS84 and JDS-AR2, respectively).

A site-specific contamination by pesticides was detected in the Olt tributary in Romania. The identified compounds include the insecticide hexachlorocyclohexane (alpha isomer) and two thiocarbamate pesticide related compounds (bis(2-methylpropyl)-, S-ethyl ester and cyclohexylethyl-, S-ethyl ester of carbamothioic acid). Since these were the only samples from the JDS2 survey that contained detectable amounts of pesticides, one could assume that pesticides in general might not be applied in larger amounts in the given period of the year.

Compared to the JDS1 only one sterolic compound (androst-5,16-diene-3.beta.-ol) was identified and therefore no conclusions on the related faecal contamination could be made.

The WFD PS, nonylphenol, was detected in JDS-AR2 (Arges tributary upstream of Bucharest), which is in line with the findings of target analyses showing its highest concentration (3.28 µg/l) at the same site (see Chapter 1).

Benzothiazole, which is among the Slovak *Other pollutants* with provisional AA-EQS of 2 µg/l, was detected at thirteen JDS1 sites but only at one site (JDS13) during the JDS2 (Wildungsmauer). A related compound 2-methylthiobenzothiazole was found in samples from nine JDS2 sites in comparison to its previous occurrence at 31 JDS1 sites.

Tributyl phosphate, belonging to the group of OPFR, was present in most samples in the stretch from river kilometre 795 (JDS68 – Calafat) to the Black Sea, with the highest concentration at JDS84 (Arges tributary). In the upper part of the Danube, the compound was only present in samples from JDS37 (Sio tributary) and JDS39 (Hercegszanto). The majority of the OPFR have been on the market since the 1950s, used mainly as flame retardants in furniture, electronic devices and building products; however, little data exists about degradation and end-of-life issues, like deposition, mobility, long-term effects or bioaccumulation. The OPFR have come under intense environmental scrutiny, due to their acute toxicity to algae, invertebrates and fish, revealed in numerous environmental studies. The presence and toxic effects of the OPFR in the Danube River Basin certainly deserves serious attention and further investigation.
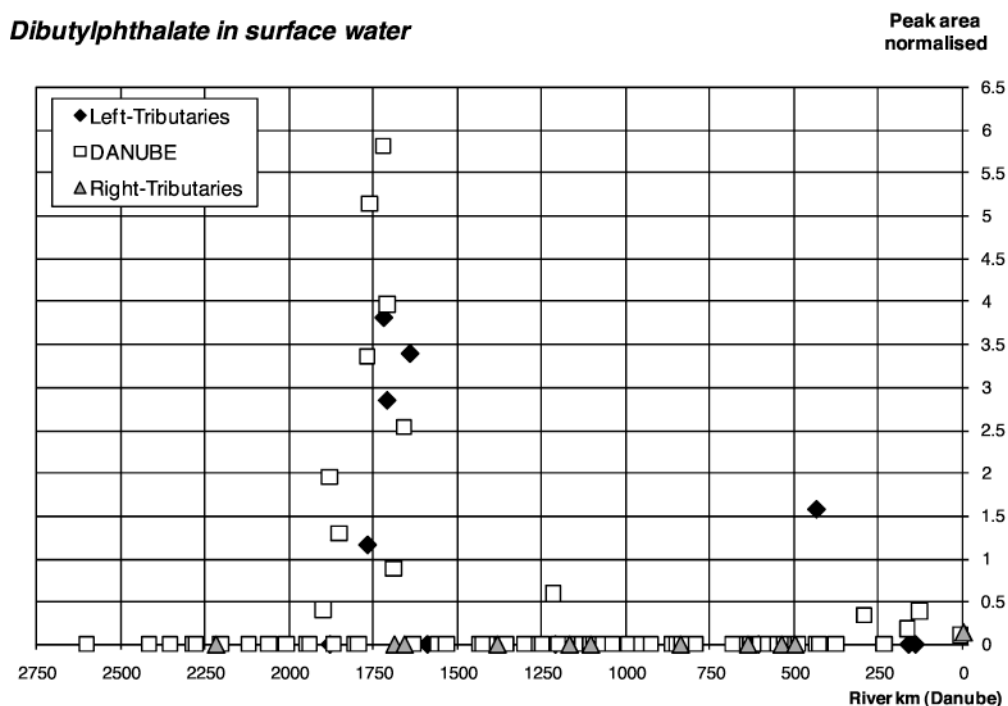
Figure 92: Occurrence profile of dibutyl phthalate in the JDS2 surface water samples

### 20.3.1.1  Semi-quantitative assessment

Even though there are no quantitative values of analytes identified by the GC-MS available, their concentrations were estimated using a semi-quantitative assessment. In the approach a relative abundance of an analyte is used, which is the ratio of its peak area and the peak area of the internal standard (anthracene-D10), both obtained from an extracted chromatogram at its base ion m/z. The obtained relative abundances allowed for a construction of the occurrence profiles of identified compounds. Complete information on the occurrence profiles of all detected compounds is presented in the full report on the CD-ROM.

### 20.3.1.2  Archivation of data on unidentified compounds

Despite using the advanced identification methodologies, ca. 10 – 30% of the detected compounds in each sample remained unidentified due to various interferences and/or missing spectra in the available libraries. In such cases the raw measurement data containing digital information of each mass spectrum were stored in a specifically developed GC-MS database of the ICPDR (next to the information on the provisionally identified compounds) in order to enable retrospective identification of an unknown compound in the future.

## Table 36: List of compounds provisionally identified in the surface water of the Danube River and its tributaries

| Compound | CAS no. | Compound | CAS no. |
|---|---|---|---|
| 2,6-Di(t-butyl)-4-hydroxybenzaldehyde | n/a | Diethylene glycol monododecyl ether | 3055-93-4 |
| .alpha.-iso-Methyl ionone | 127-51-5 | Dihydro methyl jasmonate | 24851-98-7 |
| .alpha.-Lindane (HCH) | 319-84-6 | Dihydromyrcenol | 53219-21-9 |
| .alpha.-Terpinene | 99-86-5 | Diisopropylnaphthalene | 38640-62-9 |
| 1,1'-Biphenyl, 4-methyl- | 644-08-6 | Dipropyleneglycol dibenzoate | n/a |
| 1,4-Dioxacycloheptadecane-5,17-dione | 105-95-3 | Dodecanal | 112-54-9 |
| 1-Decene | 872-05-9 | Dodecane | 112-40-3 |
| 1-Dodecanol | 112-53-8 | Dodecanoic acid | 143-07-7 |
| 1-Hexanol, 2-ethyl- | 104-76-7 | Dodecanoic acid, 1-methylethyl ester | 10233-13-3 |
| 1H-Indene, 2,3-dihydro- | 496-11-7 | Drometrizole | 2440-22-4 |
| 1H-Indene, 2,3-dihydro-4,7-dimethyl- | 6682-71-9 | Ethanol, 2-(dodecyloxy)- | 4536-30-5 |
| 1-Tetradecene | 1120-36-1 | Ethylene glycol monododecyl ether | 4536-30-5 |
| 1-Tridecene | 2437-56-1 | Fluorene | 86-73-7 |
| 2,4,7,9-Tetramethyl-5-decyne-4,7-diol | 126-86-3 | Galaxolide 1 and 2 | n/a |
| 2,4-Toluenediamine | 95-80-7 | Heptadecane | 629-78-7 |
| 2,6-Di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one | n/a | Heptanoic acid | 111-14-8 |
| 2,6-Diisocyanatotoluene | 91-08-7 | Hexadecane | 544-76-3 |
| 2,6-Diisopropylnaphthalene | 24157-81-1 | Hexadecanenitrile | 629-79-8 |
| 2,6-Di-tert-butyl-4-(dimethylaminomethyl)phenol | 88-27-7 | Hexadecanoic acid | 57-10-3 |
| 2-Benzimidazolinone, 5-methyl- | 5400-75-9 | Hexadecanoic acid, butyl ester | 111-06-8 |
| 2-Cyclopenten-1-one, 2-hydroxy-3-methyl- | 80-71-7 | Hexanedioic acid, diisooctyl ester | 1330-86-5 |
| 2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester | 5466-77-3 | Isobornyl acetate | 125-12-2 |
| 2-Pyrrolidinone, 1-methyl- | 872-50-4 | Isobutyl phthalate | 84-69-5 |
| 2-Pyrrolidinone, 1-methyl- | 872-50-4 | Junipene | 475-20-7 |
| 3-Ethylbenzophenone | 66067-43-4 | Limonene | 138-86-3 |
| 3-Octanol, 3,7-dimethyl- | 78-69-3 | Longicyclene | 1137-12-8 |
| 4,8,12-Tetradecatrienal, 5,9,13-trimethyl- | 66408-55-7 | Menthol | 1490-04-6 |
| 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione | n/a | Naphthalene | 91-20-3 |
| 7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione | n/a | Naphthalene, 1,2,3,4-tetrahydro-1,4-dimethyl- | 4175-54-6 |
| 9-Hexadecenoic acid | 2091-29-4 | Naphthalene, 1,2,3,4-tetrahydro-1-methyl- | 1559-81-5 |
| 9H-Fluorene, 2-methyl- | 1430-97-3 | Naphthalene, 1,2,3,4-tetrahydro-2-methyl- | 3877-19-8 |
| 9-Octadecenamide | 301-02-0 | Naphthalene, 1,2,3,4-tetrahydro-5-methyl- | 2809-64-5 |
| 9-Octadecenoic acid | 112-80-1 | Naphthalene, 1,2,3,4-tetrahydro-6-methyl- | 1680-51-9 |
| Acenaphthene | 83-32-9 | Naphthalene, 1,4,6-trimethyl- | 2131-42-2 |
| Acetophenone | 98-86-2 | Naphthalene, 1,4-dimethyl- | 571-58-4 |
| Acetylcedrene | n/a | Naphthalene, 1,6,7-trimethyl- | 2245-38-7 |
| Androst-5,16-diene-3.beta.-ol | 1224-94-8 | Naphthalene, 1-methyl- | 90-12-0 |
| Benzene, (1-butylheptyl)- | 4537-15-9 | Naphthalene, 2,3,6-trimethyl- | 829-26-5 |
| Benzene, (1-butylhexyl)- | 4537-11-5 | Naphthalene, 2,3-dimethyl- | 581-40-8 |
| Benzene, (1-butylnonyl)- | 4534-50-3 | Naphthalene, 2,6-dimethyl- | 581-42-0 |
| Benzene, (1-butyloctyl)- | 2719-63-3 | Naphthalene, 2-methyl- | 91-57-6 |
| Benzene, (1-ethyldecyl)- | 2400-00-2 | Nonadecanenitrile | 28623-46-3 |
| Benzene, (1-methyldecyl)- | 4536-88-3 | Nonanoic acid | 112-05-0 |
| Benzene, (1-methyldodecyl)- | 4534-53-6 | Nonylphenol | 25154-52-3 |
| Benzene, (1-methylundecyl)- | 2719-61-1 | Octadecane | 593-45-3 |

| Compound | CAS no. | Compound | CAS no. |
|---|---|---|---|
| Benzene, (1-pentylheptyl)- | 2719-62-2 | Octadecanoic acid | 57-11-4 |
| Benzene, (1-pentylhexyl)- | 4537-14-8 | Octadecanoic acid, butyl ester | 123-95-5 |
| Benzene, (1-propyldecyl)- | 4534-51-4 | Octanal, 2-(phenylmethylene)- | 101-86-0 |
| Benzene, (1-propylnonyl)- | 2719-64-4 | Octanoic acid | 124-07-2 |
| Benzene, (1-propyloctyl)- | 4536-86-1 | O-Diethoxybenzene | n/a |
| Benzene, 1,2,3-trimethyl- | 526-73-8 | Pentadecane | 629-62-9 |
| Benzene, 1,2,4,5-tetramethyl- | 95-93-2 | Pentadecanenitrile | 18300-91-9 |
| Benzene, 1,2-dichloro- | 95-50-1 | Pentadecanoic acid | 1002-84-2 |
| Benzene, 1,2-diethyl- | 135-01-3 | Pentaethylene glycol monododecyl ether | 3055-95-6 |
| Benzene, 1,3,5-trimethyl- | 108-67-8 | Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester | 1000140-77-5 |
| Benzene, 1,3-bis(1-methylethyl)- | 99-62-7 | Phenanthrene | 85-01-8 |
| Benzene, 1,4-bis(1-methylethyl)- | 100-18-5 | Phenanthrene, 1-methyl- | 832-69-9 |
| Benzene, 1-ethyl-2,3-dimethyl- | 933-98-2 | Phenanthrene, 2-methyl- | 2531-84-2 |
| Benzene, 1-ethyl-4-nitro- | 100-12-9 | Phenol, 2,4-bis(1,1-dimethylethyl)- | 96-76-4 |
| Benzene, 1-methyl-2-(1-methylethyl)- | 527-84-4 | Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl- | 128-37-0 |
| Benzene, 1-methyl-4-nitro- | 99-99-0 | Phthalic anhydride | 85-44-9 |
| Benzoic acid, 2-hydroxy-, pentyl ester | 2050-08-0 | Piperonyl butoxide | 51-03-6 |
| Benzophenone | 119-61-9 | Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester | 74367-34-3 |
| Benzothiazole | 95-16-9 | Squalene | 7683-64-9 |
| Benzothiazole, 2-(methylthio)- | 615-22-5 | Tetradecane | 629-59-4 |
| Bibenzyl | 103-29-7 | Tetradecanenitrile | 629-63-0 |
| Bis(2-chloroisopropyl) ether | 39638-32-9 | Tetradecanoic acid | 544-63-8 |
| Bis(2-ethylhexyl)  ether | n/a | Tetradecanoic acid, 12-methyl- | 5746-58-7 |
| Bis(2-ethylhexyl) maleate | 142-16-5 | Tetradecanoic acid, 1-methylethyl ester | 110-27-0 |
| Bis(2-ethylhexyl) phthalate | 117-81-7 | Tri(butoxyethyl) phosphate | 78-51-3 |
| Carbamothioic acid, bis(2-methylpropyl)-, S-ethyl ester | 2008-41-5 | Tributyl acetylcitrate | 77-90-7 |
| Carbamothioic acid, cyclohexylethyl-, S-ethyl ester | 1134-23-2 | Tributyl phosphate | 126-73-8 |
| Cyclododecane | 294-62-2 | Tridecane | 629-50-5 |
| Cyclohexanol, 2-(1,1-dimethylethyl)- | 13491-79-7 | Tridecanoic acid | 638-53-9 |
| Cyclohexanone, 4-(1,1-dimethylethyl)- | 98-53-3 | Triethylene glycol monododecyl ether | 3055-94-5 |
| Decane | 124-18-5 | Tri-n-butylamine | 102-82-9 |
| Decanoic acid | 334-48-5 | Triphenyl phosphate | 115-86-6 |
| Dibenzofuran | 132-64-9 | Undecane | 1120-21-4 |
| Dibutyl phthalate | 84-74-2 | | |
| Diethyl phthalate | 84-66-2 | | |

## 20.3.2 GC-MS screening of sediment samples

In total 28 sediment samples were analysed and 87 organic compounds with proposed chemical structures were identified in sediment extracts (for a list see Table 37), whereas 18 compounds remained unidentified. The sediment samples were taken from the 'top' 23 JDS2 sites (see Chapter 2 on survey preparation) and analysed as a mix of left and right side for 11 sampling sites (JDS7, 12, 22, 35, 39, 45, 47, 58, 83, 85, 89) and separately as left and right from the sites JDS16, 26, 53 and 92. Only one left or right sediment sample was available for analysis from the sites JDS2, 42, 51, 56, 76, 80, 86 and 95.

Among the most ubiquitous compounds found were polycyclic aromatic hydrocarbons (mainly phenanthrene, fluoranthene and pyrene) and their alkylderivatives (e.g. diisopropylnaphthalane), alkanes, cycloalkanes and aldehydes. Most of these substances originate from the chemical and

bacterial degradation of petroleum hydrocarbons, the probable source being oil pollution and fuel combustion.

Alarmingly, most of the sediments contained siloxanes, which are of increasing concern worldwide and present on the list of the emerging substances of NORMAN. Siloxanes belong to a group of substances used in a number of industrial applications and in consumer products such as additives in fuel, car polish, cleaners, anti-foamers and car waxes. Besides this, they are widely used in e.g. personal care and biomedical products. The wide-spread use of siloxanes, their broad application as well as their high volatility has raised concern for these compounds within various disciplines of environmental science. The results of monitoring programmes indicate that there is a general pollution of siloxanes in all matrices except soils. They seem to be emitted through diffuse pathways and enter the aquatic food chain. The use of siloxanes is extensive and it is possible that continued use will lead to increased environmental levels, eventually reaching effect concentrations.

An important large volume chemical in this group, decamethylcyclopentasiloxane (D5), is currently undergoing an in-depth risk assessment in the EU and may well soon be classified as a very persistent and bio-accumulating substance. This has already prompted some regulatory jurisdictions and industry to push development in this area.

The low molecular weight siloxanes: octamethylcyclotetrasiloxane, decamethyltetrasiloxane, D5, dodecamethylpentasiloxane, dodecamethylcyclohexasiloxane, tetradecamethylhexasiloxane and tetradecamethylcycloheptasiloxane were identified in the majority of samples. A pollution profile of the analysed sediments by the most abundant and ubiquitous D5 is shown in Figure 93. Similar to the findings of the JDS2, a separate study of siloxanes in various environmental (including sludge and biota) and food matrices in Sweden in 2004 showed that D5 was the dominating siloxane in most samples (Swedish National Screening Programme 2004). The results trigger the need to investigate the occurrence of siloxanes in the Danube River Basin on a more systematic basis.



Figure 93: Occurrence profile of decamethylcyclopentasiloxane (D5) in the JDS2 sediment samples

In general, most of the sediment contaminants showed diffuse pollution patterns. Site-specific contamination could only be observed at a few sampling locations e.g. JDS85 (downstream of the Arges/Oltenita). Here, the highest number of organic compounds in a single sample was detected (35 compounds) from which 31 analytes showed the highest estimated concentration values of individually detected compounds from among all samples. The contamination pattern matched that of

water samples taken from the Danube tributary, the Arges, in which *i.a.* galaxolide and numerous derivatives of naphthalene and phenanthrene were identified. This pattern indicates pollution by wastewater from Bucharest. Triphenyl phosphate (flame retardant on the NORMAN list of emerging substances) was found only at JDS16 in Bratislava (right bank).

A significantly smaller number of compounds was detected in the JDS2 compared to the AquaTerra Danube Survey from 2004, in which sediments from 30 sampling sites (from Vienna, Austria to Calafat, Romania) contained typically around 120 compounds. Similarly to the JDS2, the AquaTerra report states that a general trend of an increased number of detected compounds in the lower reach of the Danube was observed (typically over 300 detected compounds in each sample after the Sava confluence). In average, about 35% of detected compounds could not be provisionally identified in sediment samples. The GC-MS screening provided a lot of useful information on the design of a general pollution pattern for the Danube River e.g. indicating significant pollution impacts from either left or right bank sources. The detailed information on all detected compounds is presented in the full report on the CD-ROM.

## Table 37: List of compounds provisionally identified in the Danube River sediment samples

| Compound | CAS no. | Compound | CAS no. |
|---|---|---|---|
| 1,15-Hexadecadiene | 21964-51-2 | Hexanedioic acid, bis(2-ethylhexyl) ester | 103-23-1 |
| 1,1'-Biphenyl, bis(1-methylethyl)- | 69009-90-1 | Hexasiloxane, tetradecamethyl- | 107-52-8 |
| 1-Hexadecene | 629-73-2 | Naphthalene, 1,2(or 2,3)-diethyl- | 74710-00-2 |
| 1H-Indene, 2,3-dihydro-1,1,4,5,6-pentamethyl- | 16204-67-4 | Naphthalene, 1,2,3,4-tetramethyl- | 3031-15-0 |
| 1-Pentadecene | 13360-61-7 | Naphthalene, 1,4,5-trimethyl- | 2131-41-1 |
| 1S,cis-Calamenene | 483-77-2 | Naphthalene, 1,4,6-trimethyl- | 2131-42-2 |
| 2,6-Diisopropylnaphthalene | 24157-81-1 | Naphthalene, 1,6,7-trimethyl- | 2245-38-7 |
| 2-Decenal, (Z)- | 2497-25-8 | Naphthalene, 1,6-dimethyl-4-(1-methylethyl)- | 483-78-3 |
| 2-Undecenal | 2463-77-6 | Naphthalene, 1-methyl- | 90-12-0 |
| 3-Methyl-2-(3,7,11-trimethyldodecyl) furan | n/a | Naphthalene, 1-propyl- | 2765-18-6 |
| 4b,8-Dimethyl-2-isopropylphenanthrene, 4b,5,6,7,8,8a,9,10-octahydro- | n/a | Naphthalene, 2,3,6-trimethyl- | 829-26-5 |
| 7-Isopropyl-4-methylazulene | n/a | Naphthalene, 2,3-dimethyl- | 581-40-8 |
| 9-Octadecenoic acid | 112-80-1 | Naphthalene, 2,6-dimethyl- | 581-42-0 |
| Azulene, 1,4-dimethyl-7-(1-methylethyl)- | 489-84-9 | Naphthalene, 2,7-dimethyl- | 582-16-1 |
| Azulene, 7-ethyl-1,4-dimethyl- | 529-05-5 | Naphthalene, 2-methyl- | 91-57-6 |
| Benzene, 1,1'-(chloroethenylidene)bis- | 4541-89-3 | Octadecanoic acid | 57-11-4 |
| Benzene, 1-methyl-4-(1,2,2-trimethylcyclopentyl)- | 16982-00-6 | Pentadecanoic acid | 1002-84-2 |
| Bis(2-ethylhexyl) phthalate | 117-81-7 | Pentadecanoic acid, methyl ester | 7132-64-1 |
| Cholesta-3,5-diene | 747-90-0 | Pentasiloxane, dodecamethyl- | 141-63-9 |
| Cholestan-3-one, (5.beta.)- | 601-53-6 | Phenanthrene | 85-01-8 |
| Chrysene | 218-01-9 | Phenanthrene, 1-methyl- | 832-69-9 |
| Cyclododecane | 294-62-2 | Phenanthrene, 1-methyl-7-(1-methylethyl)- | 483-65-8 |
| Cycloheptasiloxane, tetradecamethyl- | 107-50-6 | Phenanthrene, 1-methyl-7-(1-methylethyl)- | 483-65-8 |
| Cyclohexasiloxane, dodecamethyl- | 540-97-6 | Phenanthrene, 2,3,5-trimethyl- | 3674-73-5 |
| Cyclopentadecane | 295-48-7 | Phenanthrene, 2,3-dimethyl- | 3674-65-5 |
| Cyclopentasiloxane, decamethyl- | 541-02-6 | Phenanthrene, 2,5-dimethyl- | 3674-66-6 |
| Cyclotetradecane | 295-17-0 | Phenanthrene, 2,7-dimethyl- | 1576-69-8 |
| Cyclotetrasiloxane, octamethyl- | 556-67-2 | Phenanthrene, 2-methyl- | 2531-84-2 |
| Decanal | 112-31-2 | Phenanthrene, 3,6-dimethyl- | 1576-67-6 |
| Decane | 124-18-5 | Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl- | 128-37-0 |
| Decanedioic acid, bis(2-ethylhexyl) ester | 122-62-3 | Phthalic anhydride | 85-44-9 |
| Dihydrocholesterol | 80-97-7 | Pyrene | 129-00-0 |
| Diisopropylnaphthalene | 38640-62-9 | Simonellite | n/a |

| Dodecanal | 112-54-9 | Squalene | 7683-64-9 |
|---|---|---|---|
| Dodecane | 112-40-3 | Tetradecanal | 124-25-4 |
| Dodecanoic acid | 143-07-7 | Tetradecanoic acid | 544-63-8 |
| Dodecanoic acid, methyl ester | 111-82-0 | Tetradecanoic acid, methyl ester | 124-10-7 |
| Fluoranthene | 206-44-0 | Tetrasiloxane, decamethyl- | 141-62-8 |
| Galaxolide 1 and 2 | n/a | Tridecanal | 10486-19-8 |
| Heptadecanoic acid | 506-12-7 | Tridecane | 629-50-5 |
| Hexadecanamide | 629-54-9 | Triphenyl phosphate | 115-86-6 |
| Hexadecanoic acid | 57-10-3 | Undecanal | 112-44-7 |
| Hexadecanoic acid, butyl ester | 111-06-8 | Undecane | 1120-21-4 |
| Hexadecanoic acid, methyl ester | 112-39-0 | | |

## 20.4    Conclusions

159 and 87 organic compounds were detected and provisionally identified by GC-MS screening analysis in the 124 JDS2 surface water and 28 sediment samples, respectively. Among the main groups of substances found were plasticisers, degradation products of petroleum hydrocarbons, fuel additives, personal care products and organophosphate flame retardants (all ubiquitous in the Danube River Basin). Some of the detected compounds, such as the plasticiser dibutyl phthalate, polyaromatic hydrocarbon phenanthrene and rubber accelerator benzothiazole, are already included in the national monitoring of some Danube countries as *'other pollutants'* with provisional EQS (e.g. Slovak Republic: 10, 0.38 and 2 µg/l, respectively).

Despite its lower sensitivity, GC-MS screening of unknown substances provided relevant complementary information to the analyses of target compounds. It also confirmed the findings of the other JDS2 laboratories in terms of identification of the most relevant diffuse and point pollution patterns in the Danube Basin (e.g. for nonylphenol, DEHP, galaxolide etc.). The most polluted part of the basin in terms of number of detected compounds and their concentrations was the Arges tributary, draining wastewater from the Bucharest area.

Only three pesticides were detected in the JDS2 samples in the Olt tributary, which indicates that sampling in August – September might not be representative for this group of compounds. Here, the deployment of passive samplers providing integrated information over a longer time period (months) would certainly be of more use in future investigations.

Many of the detected compounds from the groups of personal care products (e.g. siloxanes, sun-screen agents, fragrances and musks); plasticisers (phthalates) and flame retardants (organophosphates) are on the list of emerging substances of European relevance. Carefully selected representatives of the above groups of compounds may be considered as candidates for future monitoring on a basin-scale. Their toxic effects and widespread occurrence in Europe are under the scrutiny of the EU FP6 NORMAN project, which will start to operate as a permanent network from 2009, supporting the EC Chemical Monitoring Activity (CMA). Close cooperation with the NORMAN network in the coming years is recommended in order to harmonise strategies for deriving the compounds' EQSs; develop methodologies for their analysis; set up schemes for investigative monitoring and develop eventual measures for their removal. The remaining challenge is to identify compounds that remain unidentified due to their missing mass spectra in the currently available libraries.

## 20.5   References

10732/08 - CODEC 776 ENV 376 Proposal for a Directive of the European Parliament and of the Council on environmental quality standards in the field of water policy and amending Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and 2000/60/EC – Outcome of the European Parliament's second reading (Strasbourg, 16 to 19 June 2008)"

SANDRA P., TIENPONT B. and DAVID F. (2003): Stir bar sorptive extraction (TwisterTM) RTL-CGC-MS. A versatile method to monitor more than 400 pesticides in different matrices (water, beverages, fruits, vegetables, baby food), Research Institute for Chromatography, AppNote 2003/1, 1-15.

SANDRA, P., TIENPONT, B. and DAVID, F. (2003): Multi-residue screening of pesticides in vegetables, fruits and baby food by stir bar sorptive extraction-thermal desorption-capillary gas chromatography-mass spectrometry, Journal of Chromatography A, 2003, 1000:299-309.

Results from the Swedish National Screening Programme 2004, Subreport 4: Siloxanes; IVL Report B 1643 http://www.ivl.se/rapporter/pdf/B1643.pdf.

# 21  Radioactivity

Franz Josef Maringer, Andreas Baumgartner, Valeria Gruber, Claudia Seidel, Sylvia Weilner, Yuriy Nabyvanets, Volodimyr Kanyevets and Gennady Laptyev

## 21.1    Introduction

Highly elevated levels of artificial and/or natural radionuclide concentration in rivers lead to increased health risks for populations drinking the processed river water or consuming contaminated river fish. Additionally, the use of contaminated river water for irrigation can increase the health risk by consumption of the products produced on the irrigated areas. Therefore it is of high importance to periodically investigate the radioecological status of the Danube River ecosphere to asses the impacts of radionuclides on the health of the population living in the basin.

$^{137}$Cs activity concentration (half-life of 30 years) in Danube water and solid particles originated primarily from the nuclear power accident in Chernobyl (April-May 1986) and secondarily from atmospheric nuclear weapons testing during the 1950s and 1960s.

Major naturally occurring radioactive constituents of the earth's crust comprise the isotopes $^{238}$U (half-life ($T_{1/2}$) = 4.5 x 10$^9$ years) and $^{232}$Th ($T_{1/2}$ = 14.1 x 10$^9$ years). Created during the cosmic formation of the elements in a supernova and then building the earth's material, these radioisotopes can now be found in almost every type of rock and its weathering product, the soil, due to their extremely long physical half-life. The ubiquity of these radioisotopes also implies the presence of their decay products in rocks and soil such as $^{226}$Ra ($T_{1/2}$ = 1.6 x 10$^3$ years) and $^{228}$Ra ($T_{1/2}$ = 5.7 years) respectively.

$^{226}$Ra and $^{228}$Ra analysis of sediment allows for the identification of the geochemical background and the influence of the mining industry as well as the sediment sources. The influence of the main tributaries e.g. Drava, Tisa, Sava and Velika Morava on the radionuclide activity concentrations was also evaluated. Sediment cores taken in the Iron Gate reservoir were used to analyse sedimentation sequences. In the framework of the JDS2, levels of natural ($^{40}$K, $^{226}$Ra, $^{228}$Ra, $^{232}$Th) and artificial ($^{137}$Cs) radionuclide concentrations of 72 sediment samples, collected in the Danube and main tributaries, were analysed by radiometry.

## 21.2    Methods

### 21.2.1 University of Natural Ressources and Applied Life Scienece, Vienna (BOKU)

The radiometric analysis of the homogenised and frozen dried sediment samples (grain size fraction < 63 µm) was carried out by low-level gamma-spectrometry. By this method, which was carried out with three low-level Germanium detectors in a specially shielded laboratory (Low-level Counting Laboratory Arsenal, Vienna), the activity concentration of the gamma emitting radionuclides in the samples was analysed. The radiometric equipment consists of two coaxial low-level HP-Ge-detectors (22% and 34% rel. efficiency) detecting gamma energies between 100 to 2800 keV, and two low-level planar HP-Ge-detectors for gamma energies between 5 and 700 keV. Calibrations of the detectors were carried out by NIST-, IAEA-, and PTB-standard reference materials. The calculations of the radionuclide concentrations and data handling were done by Genie 2000® Spectroscopy System (Canberra Inc.) and software developed by the departmental research group (Microsoft Access®).

Due to relatively low radioactivity concentrations, long counting periods (24 to 72 hours) were necessary to obtain reasonable counting statistics for all radionuclides of interest.

### 21.2.2 Ukraine Research Hydrometeorology Institute Kiev (UHMI)

Samples were unfrozen over night (12 hours) at laboratory temperature (+20 to 22 °C) in the Department of Environmental Radiation Monitoring. Samples were then carefully mixed manually until the reached the consistence of so-called "dense sour cream". Aliquots of 90 g were taken from each sample for further treatment. The wet aliquots where placed on the top of a set of RETSCH sieves with different mesh sizes (3.15 mm, 1.0 mm, 0.250 mm, 0.125 and 0.050 mm) and washed out by distilled water for separation of fractions less than 50 µm. Homogenised and freeze-dried test samples of sediments (fractions <50 µm) were placed into polypropylenecontainers for gamma-spectrometry measurements. The Ortec Gamma-X HPGe Detector GMX40 (energy range 10 to 3000 keV) was used for gamma-spectrometry analysis of the sediment samples. Counting time was adjusted to allow for the majority of the samples to achieve peak area statistical uncertainty below 10% (combined uncertainty [±1 sigma] is expressed as the square root of the sum of variances of all conceivable sources). QA/QC procedures of gamma-spectrometry analysis were verified by in-between measurements of the Certified Reference Material (CRM – soil matrix) provided by the IAEA to the Laboratory for the WWO Proficiency Testing 2007.

### 21.3    Results

The regional distribution of soil contamination in the Danube Basin by artificial radioactivity, which mainly originates from the Chernobyl accident in May 1986, is shown in Figure 94. The highest contamination of soil was found in the upper part of the Danube Basin – especially in the Alpine environment in Austria.



**Cs-137 contamination of soil 1986 [kBq/m_]**
- < 2
- 3 - 10
- 11 - 40
- > 40
- no data available
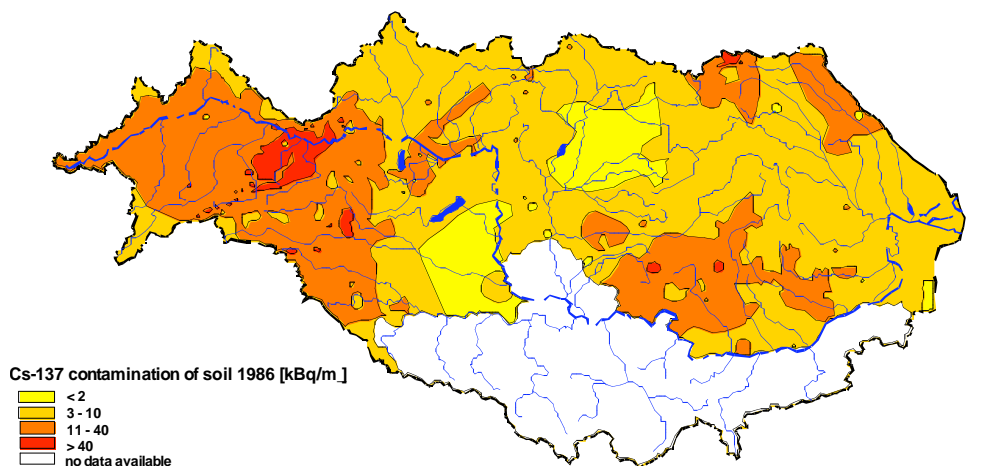
**Figure 94: Distribution of $^{137}$Cs activity contamination of the Danube River Basin after the Chernobyl accident, May 1986, BOKU, Vienna (*data source: UNSCEAR 2000 report*)**

The results of the radiometric analysis of sediment samples from the Danube and selected tributaries are given in Figure 95. The < 63 µm fraction was analysed in the BOKU laboratory, and the < 50 µm fraction in the UHMI laboratory. Good compliance between the results of both laboratories was observed. The slight deviations are caused by different sample material and grain size.
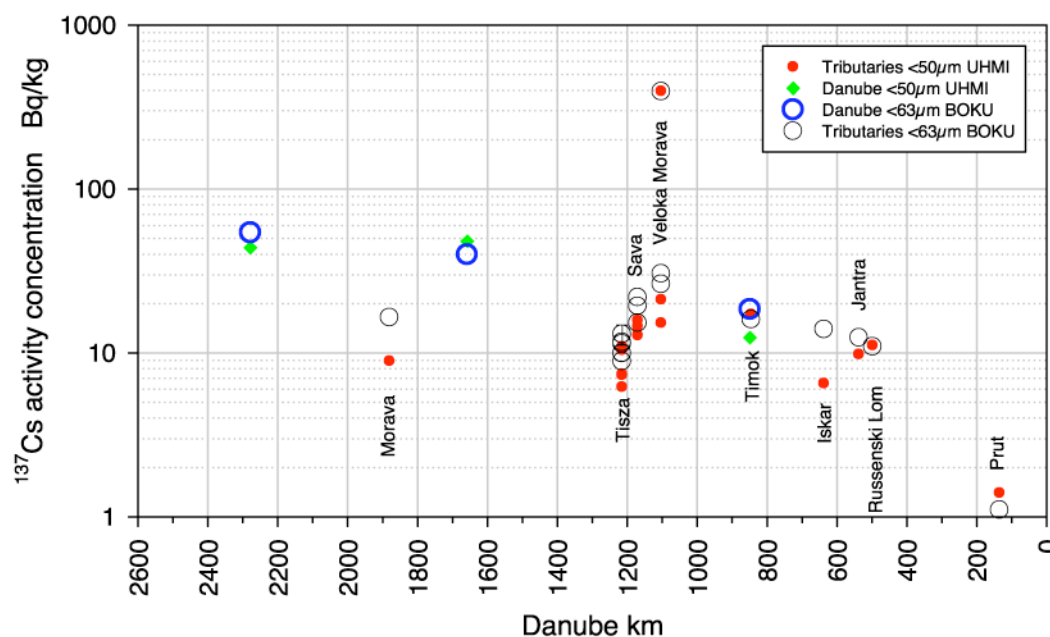
Figure 95: [137]Cs activity concentration of sediment samples (analysed by BOKU, Vienna, and UHMI, Kiev) - grain size fraction < 63 **µ**m and < 50 **µ**m respectively

Despite the given statistical limitations concerning the collected samples, an acceptable correlation between ^{137}Cs concentration and river km was found. This was the case primarily for the < 20 µm sediment fraction as the major part of the ^{137}Cs is tightly bound to this fine material. As shown in Figure 96, a clear and well-defined downstream decrease of ^{137}Cs levels in the < 20 µm sediment fraction can be observed. The reliability of these results is confirmed and supported by the results obtained from similar sampling campaigns on the Danube in 1988 by IAD (Internationale Arbeits- gemeinschaft Donauforschung) and in 2004 by the survey carried out within the EC FP7 Aquaterra project. The data from these studies also suggest a decrease of ^{137}Cs activity downstream, although higher variations were observed in 1988. In general, it can be said that the < 20 µm fraction exhibits the same pattern as the whole sample but due to higher ^{137}Cs level, the variation is lower. The decline of ^{137}Cs concentration in the top sediment layers is caused by reduced ^{137}Cs concentrations at the source of sediments; due to erosion the top layers of soil (where most of the ^{137}Cs was bound) were eroded into rivers, gradually reducing the fresh influx of [137]Cs.

The regional ^{137}Cs distribution in the < 20µm sediment fraction observed during JDS2 showed an increased  activity in the Upper Danube, being about 100 Bq/kg with a decrease downstream the Danube to about 10 Bq/kg in the delta (Figure 96). To compare it with the situation after the Chernobyl accident, during the 1988 IAD cruise ^{137}Cs activity concentration values between 1000 Bq/kg (Upper Danube) and 100 Bq/kg (delta region) were recorded.
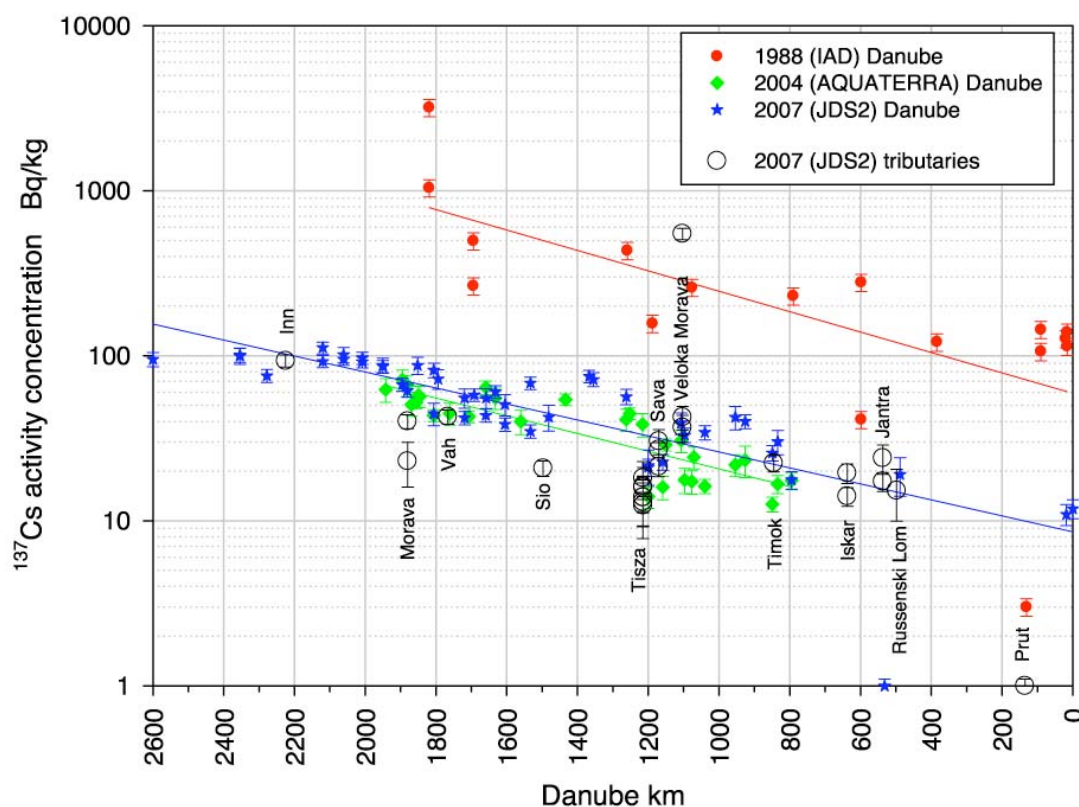
**Figure 96:** $^{137}$Cs activity concentration of sediment samples (normalised to grain size fraction < 20 μm) of JDS2 cruise (BOKU, Vienna) and two other surveys (AQUATERRA, 2004 and IAD, 1988)

From 1988 (IAD cruise) to 2004 (AQUATERRA cruise), the ecological half-life for $^{137}$Cs in sediments is about 4.6 years. From 2004 to 2007 almost no decrease in the $^{137}$Cs activity in sediment is observed. In some parts of the Danube even an increase of the $^{137}$Cs activity concentration from 2004 to 2007 was found (rkm 1400 – 1200, rkm 1000 – 800). Downstream transport of remobilised bottom sediment during flood events, as well as an increased soil erosion in contaminated areas of sub-basins, could explain this phenomenon. The impact of the climatic change in the Danube Basin may be the potential drivers of these effects.

The main radioactive representatives of the natural $^{238}$U and $^{232}$Th decay chain are $^{226}$Ra and $^{228}$Ra, respectively. In Table 38 the statistic parameters of the radioanalytical results from analyses of 72 sediment samples (Danube and tributaries, sediment grain size fraction < 63 μm) are given.

The impact of geochemical background on the natural radioisotope concentrations in sediments in several major sub-basins  (Tisa, Velika Morava, Timok and Jantra) are apparent in Figure 97. In general, a downstream decrease of $^{226}$Ra activity concentration and an increase of the $^{226}$Ra/$^{228}$Ra activity concentration ratio were found. The prevalent occurrence of $^{226}$Ra and $^{238}$U in upstream regions is a good indicator for the sand- and limestone dominated geological structure of the Upper Danube Basin. Further downstream the Alpine influence is apparently reduced in favour of a more regional influence.

## Table 38: Range of radium isotope activity concentrations and ratios in sediment samples (BOKU, Vienna)

| | $^{226}Ra$ Bq/kg | | $^{228}Ra$ Bq/kg | | $^{228}Ra$ / $^{226}Ra$ | |
|---|---|---|---|---|---|---|
| | Danube | Tributaries | Danube | Tributaries | Danube | Tributaries |
| Minimum | 43.5 | 31.0 | 15.0 | 24.6 | 0.5 | 0.6 |
| Mean | 64.3 | 57.9 | 51.9 | 52.8 | 0.8 | 1.0 |
| Maximum | 100.5 | 97.2 | 69.7 | 68.7 | 1.3 | 1.6 |
| Standard deviation | 12.0 | 17.1 | 11.5 | 9.3 | 0.2 | 0.2 |

Downstream of the Danube, a clear decrease of $^{226}Ra$ activity concentration and an increase of the $^{228}Ra/^{226}Ra$ activity concentration ratio can be observed in the analysed sediment samples (< 63 µm) (Figure 97). The ratio of $^{228}Ra/^{226}Ra$ is commonly used as an indicator of the source material, as $^{228}Ra$ is derived from $^{232}Th$, and $^{226}Ra$ is formed by the decay of $^{238}U$.
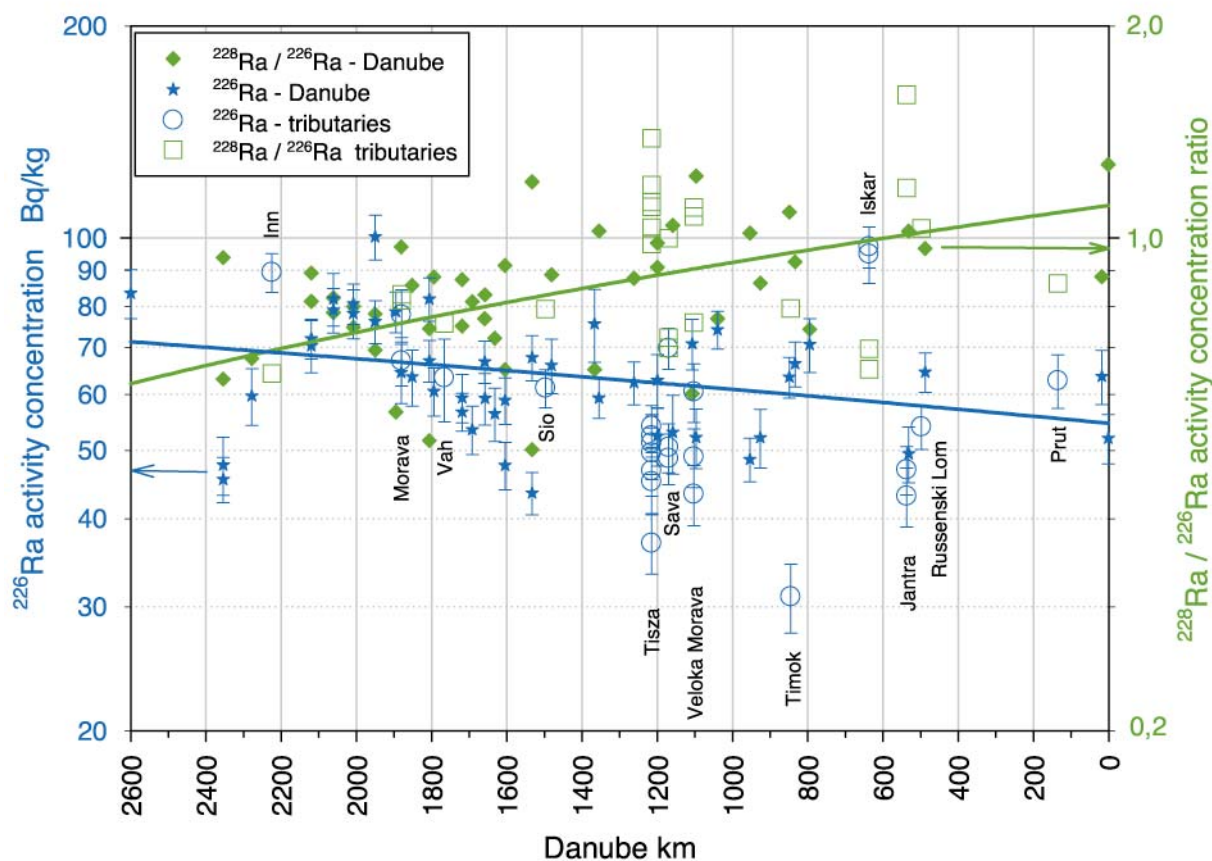


Figure 97: $^{226}Ra$ activity concentration of sediment samples (grain size fraction < 63 µm) and $^{228}Ra$ / $^{226}Ra$ activity concentration ratio – Danube and tributaries (BOKU, Vienna)

## 21.4    Conclusions

Overall a clear general decrease in the $^{137}$Cs activity concentration of Danube sediments by a factor of 10 (due to physical decay and a transfer of contaminated upper soil layers in the Danube catchment area) was observed between 1988 and 2007. Since 2004, a generally constant $^{137}$Cs level was detected along the Danube, except for the middle section where a slight increase was detected. This effect could be explained by the downstream transport of remobilised sediment and by locally increased $^{137}$Cs input by soil erosion. The observed radioecological behaviour is a good illustration of the impacts of climatic change causing seasonal and regional changes in contaminated soil erosion in the Danube catchment. There is a clear relationship between $^{137}$Cs concentrations in sediment and hydrological conditions in the Danube Basin.

Due to generally decreased artificial radioactivity levels (e.g. $^{137}$Cs) in the Danube River, no health risk for the population could be assumed. Similarly, natural radionuclide concentrations were generally found at average levels, even if significant variations of natural radionuclide activity concentrations were observed. It can be concluded that the Danube River was in a good radioecological status in 2007. However, locally elevated concentrations especially in the tributaries (e.g. Inn, Velika Morava; Figure 96), caused either by contaminated soil erosion (Chernobyl accident) or by emissions from industrial sites (mining activities increasing the natural radioactivity), must be mentioned as well.

# 22    Isotope survey of the Danube

Brent Newman, Wolfgang Papesch, Dieter Rank, Tomas Vitvar, Hana Hudcová,
Pradeep Aggarwal and Manfred Groening

## 22.1    Introduction

Isotope analysis was a new component of the JDS2. Due to their unique characteristics, isotopes provide a complementary value to other data collected during JDS2. The main objectives of the isotope survey were primarily hydrological, although there were also objectives related to water quality. Since there is only limited existing environmental isotope data from the Danube (especially from the Lower Danube), the JDS2 was a good opportunity to improve the database for environmental isotopes in this region. Samples were collected for analysis of stable isotopes of water ($\delta^2$H and $\delta^{18}$O) and tritium ($^3$H) at the official JDS sampling sites, as well as at some additional locations. A radon survey ($^{222}$Rn) was carried out using an on-board analyser. The main hydrological objectives were to use the isotope methods to better understand the impacts of tributary inputs and mixing in the Danube; the distribution of groundwater discharge and to provide a more complete baseline of isotope data for assessing future impacts within the Danube Basin. Isotopes can be good indicators of change because they are often sensitive to land-use or major hydro-climatic factors. In addition to being a hydrological tracer, $^3$H can also be viewed as a water quality parameter related to, for example, nuclear power plant discharges. This chapter describes basic methods and results from the isotope survey.

## 22.2    Methods

Grab samples were collected within the upper 1 m depth for all isotopes except $^{222}$Rn. Additional points near the left and right banks, above and below the confluences of eight major tributaries, were sampled for $\delta^2$H, $\delta^{18}$O and $^3$H to provide information on mixing with tributary waters. Samples were stored in plastic bottles and analysed in the Austrian Research Centre (ARC) in Seibersdorf or the IAEA Isotope Hydrology Laboratory in Vienna. Water stable isotopes ($\delta^2$H and $\delta^{18}$O) were measured using either isotope ratio mass spectroscopy (at ARC) or laser absorption spectroscopy (at IAEA). All results are reported as relative abundance ($\delta^2$H and $\delta^{18}$O, respectively) of the isotopes $^2$H and $^{18}$O in permil (‰) with respect to the international standard V-SMOW (Vienna-Standard Mean Ocean Water). Precisions of the $\delta^2$H and $\delta^{18}$O analyses are better than $\pm$ 1.0 ‰ and $\pm$ 0.1 ‰, respectively ($\delta^{18}$O precisions from laser-based analyses are better than $\pm$ 0.3 ‰). The $^3$H samples were enriched electrolytically and analysed using low-level liquid scintillation methods. Precisions of the $^3$H analyses are better than $\pm$ 1 Tritium Unit (T.U., 1 T.U. = 0,119 Bq/kg for water). A portable $^{222}$Rn detector and water analysis attachment (RAD-7 and RAD-AQUA, Durridge Corp.) were used to measure $^{222}$Rn directly on-board the Argus. The one sigma $^{222}$Rn uncertainties are typically better than 30 Bq/m$^3$. Analyses of the nitrogen isotope samples are not yet available.

## 22.3    Results

Heavy isotopes $^{18}$O and $^2$H are stable parts of the water molecule and important tracers of water movement. Their concentrations vary in the natural water cycle by isotopic fractionation (changes in the ratio of the rare to common isotope e.g. $^{18}$O/$^{16}$O) during evaporation and condensation processes.

As fractionation depends strongly on temperature, the isotopic composition of precipitation carries information about the origin and meteorological/climatic conditions of the precipitation input to a basin. The precipitation isotopic signal can be used as a natural label of water pathways once the precipitation arrives at the land surface and moves via evaporation, infiltration or runoff. In the river surveys, $^{18}$O and $^{2}$H are important indicators of how the river reacts on the precipitation input and the spatial and temporal impact of adjacent aquifers with different isotopic fingerprints. Results for $\delta^{18}$O from the official JDS2 stations are shown in Figure 98(results for $\delta^{2}$H are similar and not shown). The $\delta^{18}$O record exhibits three significant changes along the river (Figure 98). The first and largest change occurs after the confluence of the Upper Danube (higher $\delta^{18}$O value from a mainly lowland drainage area) with the Inn (lower $\delta^{18}$O value from a mainly alpine drainage area), reflecting the impact of the large Inn discharge. The second significant change is in the area of the Tisa and Sava confluences, where the tributaries have higher $^{18}$O contents than the Danube. The third change occurs in the Iron Gate area and is related to the extreme precipitation event in Central Europe during Sept. 5 – 7 (labelled 'high water influence' in Figure 98).
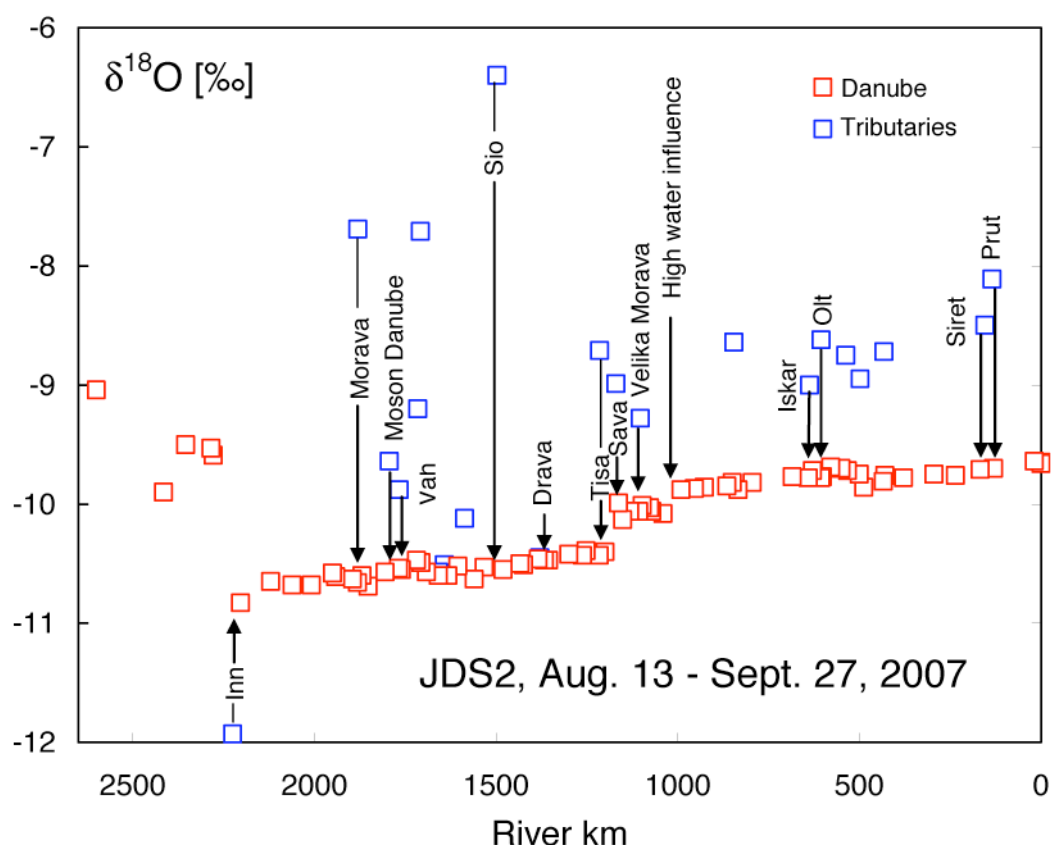


**Figure 98: $\delta^{18}$O along the Danube (Aug. 13 – Sept. 27, 2007)**

For a description of the $\delta^{18}$O measurements, see the Methods section.

According to the run-off data from the Lower Danube, all the $\delta^{18}$O values between the Iron Gate and the river mouth increased by 0.2 – 0.3 ‰ as a consequence of this precipitation event. The total increase of 1.2 ‰ in $\delta^{18}$O between the Inn confluence and the mouth of the Danube is mainly due to the decreasing influence alpine runoff contributions and a corresponding increase from lower elevation contributions.

The $\delta^2$H-$\delta^{18}$O diagram (Figure 99) shows that most values lie close to the Global Meteoric Water Line ($\delta^2$H = 8$\delta^{18}$O + 10) which suggests that surface water evaporation along the Danube river course is minor and may be neglected for the Danube and the majority of tributaries. Only the Sio carries water significantly influenced by evaporation because it contains the discharge of Lake Balaton water; but also the tributaries Ipoly, Morava and Prut show slightly pronounced evaporation effects. The presence of water influenced by evaporation in these tributaries is identified by the position of the isotopic signal below the Global Meteoric Water Line (Figure 99). When water undergoes evaporation, the residual water becomes progressively more enriched in $^{18}$O than in $^2$H, and the isotopic signal shifts from the meteoric water line. The higher deuterium excess values (d = $\delta^2$H – 8$\delta^{18}$O) in the upper sections of the Iskar, Jantra and Arges are probably due to local orographic (mountainous) conditions. Such a dependence of the deuterium excess on the orographic situation was found in the Austrian Alps, where mountain and valley precipitation differed significantly in deuterium excess as a consequence of re-evaporation processes (high d values in the mountains, low d values in the valleys, Rank & Papesch 2005).

The first isotope record for the Danube originates from a ship-based scientific excursion organised by the "Internationale Arbeitsgemeinschaft Donauforschung (IAD, International Association of Danube Research) in March 1988 (Rank et al. 1990). The comparison of IAD data with those from the JDS2 exhibits a significant increase in heavy isotope content during the last 20 years (Figure 100).
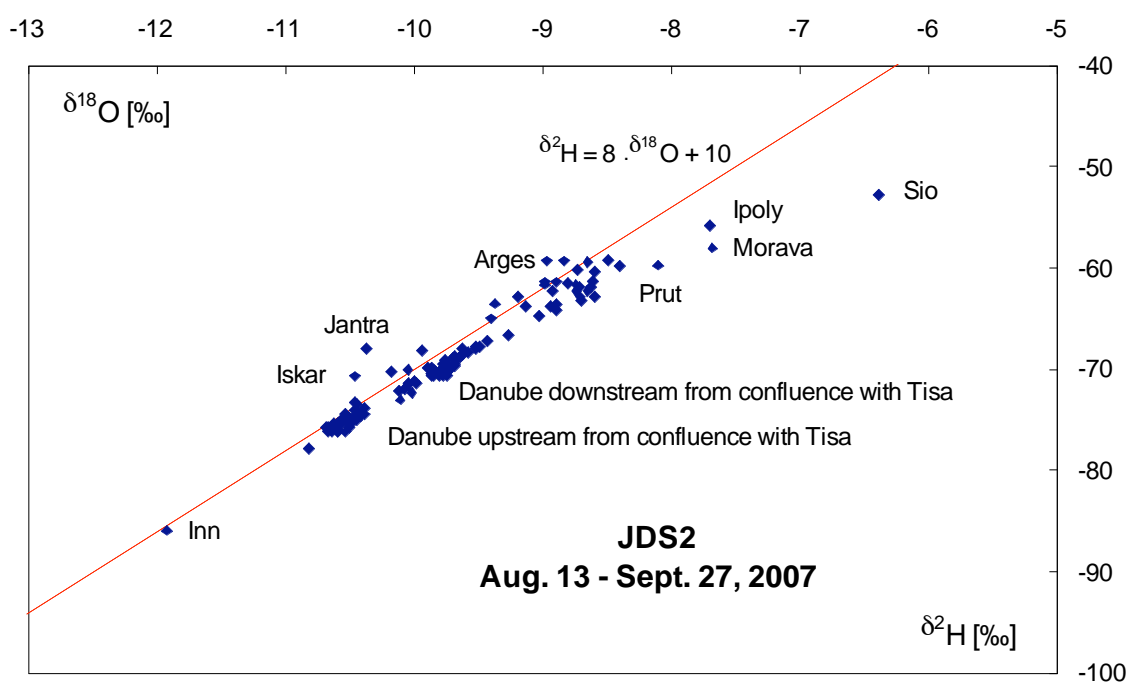


Figure 99: $\delta^2$H-$\delta^{18}$O diagram for river water samples from the Danube Basin

For a description of the $\delta^2$H and $\delta^{18}$O measurements see the Methods section.

This difference could partly be attributed to the influences of seasonal effects and individual precipitation events between the two sampling periods. However, it has also been shown that the increase of environmental temperatures over the last few decades has led to an increase of heavy isotope concentrations in precipitation in central Europe (Rank & Papesch 1996, Rank & Papesch 2005). This increase in precipitation isotope values is a consequence of the strong temperature dependence of isotopic fractionation during evaporation and condensation processes. Therefore, the higher isotope values in the JDS2 samples are also consistent with known climatically driven shifts in the isotope composition of precipitation that have occurred over the last few decades.
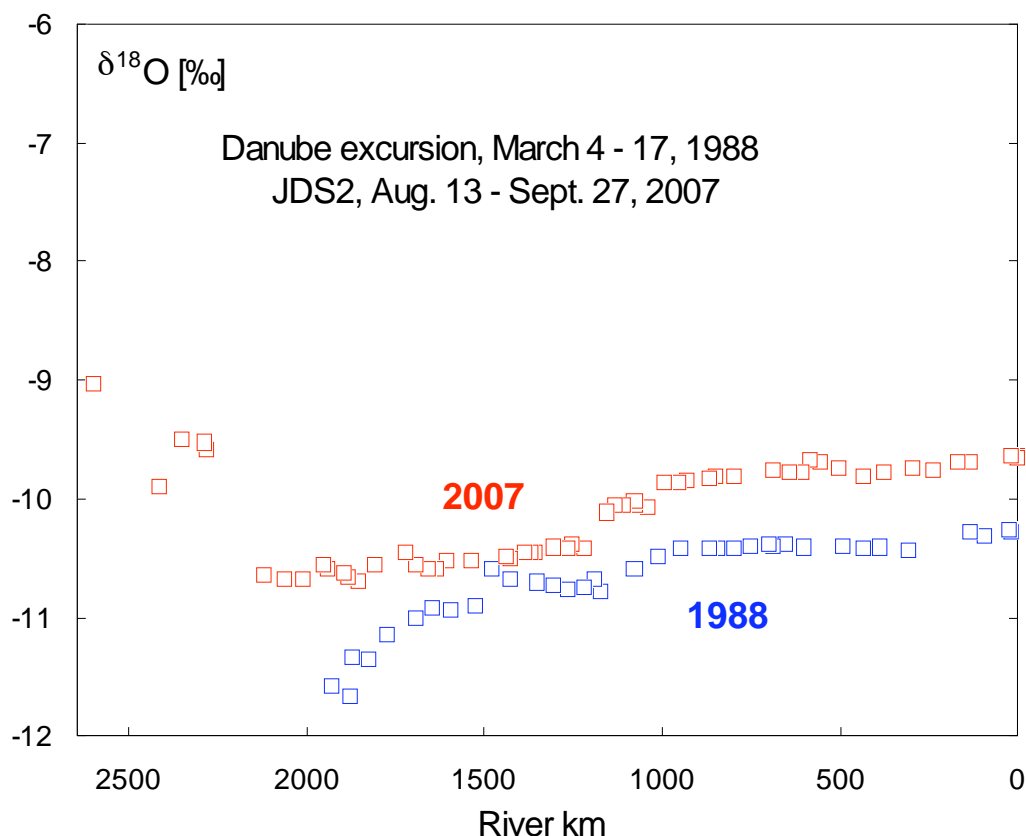
**Figure 100: Comparison of δ¹⁸O values along the Danube from the 1988 survey and JDS2 in 2007**

The natural production of tritium ($^3$H) by cosmic radiation in the upper atmosphere leads to a $^3$H concentration of about 10 TU in actual precipitation in Central Europe. Local $^3$H releases into the river system of the Danube Basin caused by human activities (nuclear power plants and nuclear industry) modify this 10 TU value. Tritium in rivers is therefore a valuable indicator of the river response on spatially and temporally well defined input. The evolution of $^3$H along the Danube is shown in Figure 101, which mainly reflects tributary $^3$H contributions and mixing within the Danube. The homogeneous and relatively low $^3$H signal of the Upper Danube area (e.g. around the Danube/Inn) is altered by temporally (pulse effects) and spatially variable (left-side or right-side tributary) contributions of elevated $^3$H inputs from nuclear powerplant discharges. (note that "elevated" is only used in a relative sense, even the highest values detected in the study are well below any health limits). Preliminary qualitative data analysis shows no influence on the Danube of slightly elevated 3H signal in Morava and that although the Vah contributes less than 10% of the total discharge of the Danube, the elevated 3H signal can be traced along the left Danube side below the Vah confluence. The Vah 3H signal on the right side of the river, however, shows minor to no influence on the Danube. Further downstream, the Sava (10% of the total Danube discharge) supplies water with lower $^3$H content than the Danube, and a small dilution effect appears to be evident below the confluence. From below the Sava confluence and further downstream, a relatively uniform $^3$H distribution is maintained with slight changes between values on the right and left sides of the Danube according to the incoming direction of the tributaries (e.g., the Velika Morava from the right, and Olt from the left). The uniform signal below the Sava might be related to the impact of the large September rain event mentioned earlier, as well as by potential infiltration and return groundwater flow through adjacent aquifers. Tritium values of the September precipitation in Austria that caused the elevated Danube discharges are around 10 T.U., which is not significantly different from Danube water prior to the large rainfall event.
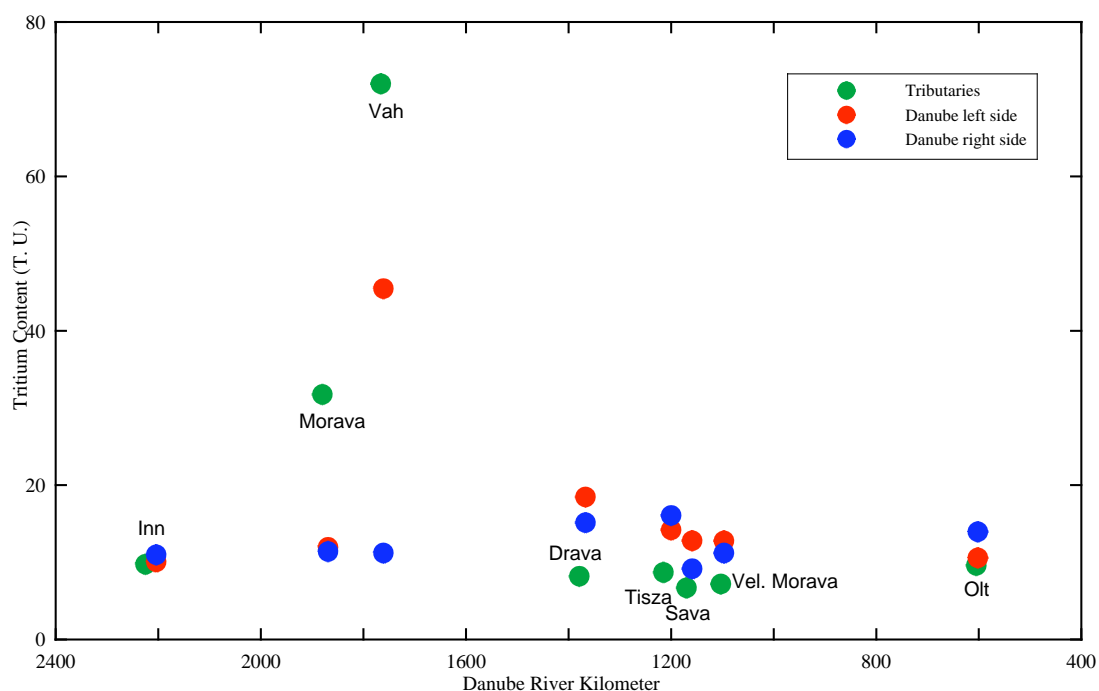
Figure 101: Tritium from JDS2 sampling points in selected tributaries (green), and on the left (red) and right (blue) sides of the Danube below the respective confluences

Note that the Morava, Vah, Tisza and Olt are left-side tributaries, whereas the Inn, Drava, Sava and Velika Morava are right-side tributaries.

The $^{222}$Rn data were collected to identify potential locations with significant groundwater inputs and also to examine mixing between the Danube and its tributaries. Although $^{222}$Rn in natural waters can be affected by geology and other factors, elevated $^{222}$Rn values have been shown to be an effective indicator of groundwater discharges in rivers and along coastal zones. It is an effective isotope approach because groundwater values tend to be much higher than those in rivers because surface waters release radon relatively quickly into the atmosphere and $^{222}$Rn production in rivers is much lower than in groundwater.

The $^{222}$Rn profile along the Danube has some interesting features and is shown in Figure 102. Overall, the values are low and the lowest values are effectively at the limit of detection as is typical for surface water. However, there are significant differences between some parts of the Danube and between the Danube and some tributaries. The overall trend is for higher radon concentrations in the Upper Danube, which suggests that this is the area where groundwater contributions to the river are the largest (although tributaries may still be major inputs as well). This interpretation is consistent with previous studies (e.g. Pawellek et al., 2002). Some of the tributaries (e.g. the Sava, Velika Morava and Siret) also have high $^{222}$Rn, which suggests they have groundwater inputs in the vicinity of the JDS2 sampling points. In terms of mixing, the Sava appears to have the largest impact on the Danube $^{222}$Rn values, although values drop off quickly until the Velika Morava and then decrease rapidly again. Although the Siret has relatively high $^{222}$Rn, its impact on the Danube appears to be minor and is within the measurement error. This lack of impact is probably related to the low discharge of the Siret relative to the Danube.
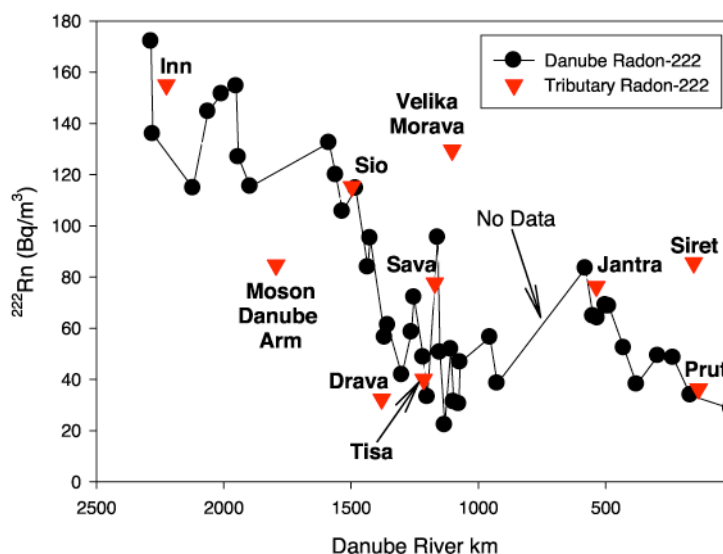
Figure 102: 222Radon in the Danube Basin

## 22.4    Conclusions

A variety of isotope data (i.e. $\delta^2H$, $\delta^{18}O$, $^3H$, and $^{222}Rn$) were collected during JDS2 to improve hydrological and geochemical characterisation of the Danube Basin. The results obtained support the conclusions from previous isotope work in the Danube Basin, which emphasised the dominant role of tributaries and in-channel mixing over direct groundwater inflows from aquifers along the Danube. The Inn has the largest tributary impact on $\delta^2H$ and $\delta^{18}O$ values in the Danube as a result of its large discharge and its more negative isotope composition. Downstream of the Inn confluence, tributary inputs from lower elevation areas to the Danube contribute only a small increase in isotope values. In addition, the $\delta^2H$ and $\delta^{18}O$ data do not indicate a strong evaporation process in the Danube, although water sources influenced by evaporation are significant in some tributaries such as the Sio.

The $^3H$ results show that the Váh and Morava tributaries are influenced by nuclear power plant discharges. The values detected during the JDS2 are however well below any health limits.

The $^{222}Rn$ data suggest that groundwater inputs to the river are largest in the Upper Danube, and that direct groundwater inputs are likely minor along the mid- to lower-Danube. The data also suggest that some tributaries such as the Sava and Velika Morava may have significant groundwater inputs near the JDS2 sampling sites within the tributaries. The isotope data reported here also provide an important environmental baseline needed for the implementation of the EU Groundwater Directive, more specifically in monitoring and conceptual assessment of river/groundwater interactions in the major transboundary aquifers in the Danube Basin.

## 22.5    References

PAWELLEK, FRAUENSTEIN, F. and VEIZER, J. (2002): Hydrochemistry and isotope geochmistry of the upper Danube River. Geochim. Cosmochim. Acta, 66, 3839-3854.

RANK, D., PAPESCH, W. (1996): Die Isotopenverhältnisse im Donauwasser als Indikatoren für Klimaschwankungen im Einzugsgebiet. – Limnologische Berichte der 31. Arbeitstagung der Internationalen Arbeitsgemeinschaft Donauforschung, Wissenschaftliche Referate, 1, 521-526, Göd/Vácrátót.

RANK, D., PAPESCH, W. (2005): Isotopic composition of precipitation in Austria in relation to air circulation patterns and climate.  In: Isotopic composition of precipitation in the Mediterreanean Basin in relation to air circulation patterns and climate. IAEA-TECDOC-1453, 19-36, IAEA, Vienna.

RANK, D., PAPESCH, W., RAJNER, V. (1990): Tritium($^3H$)- und Sauerstoff-18($^{18}O$)-Gehalt des Donauwassers zur Zeit der Internationalen Donaubereisung 1988. - In: Ergebnisse der Internationalen Donauexpedition 1988. Ed.: Internationale Arbeitsgemeinschaft Donauforschung, Wien, 307-312.

# 23 Comments on ecological status

Franz Wagner and Franz Lamprecht

## 23.1 Introduction

Assessment of *ecological status* has to be done exclusively by EU member states for their national water bodies and was not a primary objective of the JDS2. However, for all Danube states there are various open issues within the assessment process for ecological status. The JDS2 could support these national efforts with data and experiences concerning sampling and assessment methods.

It attempts to outline the open questions in the ecological status assessment for the Danube and to highlight the potential contribution of the JDS2 to solving some of these issues. In the future intercalibration will guarantee the comparability of the various national assessment systems along the Danube and the outcome of the JDS2 could also support this process.

## 23.2 Assessment of *ecological status* according to the Water Framework Directice

The assessment process for the *ecological status* of water bodies is defined in the Water Framework Directive (WFD). The CIS-Guidance document "Overall Approach to the Classification of Ecological Status and Ecological Potential" (CIS 2003) gives guidance on the implementation of the requirements of the Directive. Figure 103 provides an overview.

The assessment process uses results from the Biological Quality Elements (BQE); hydromorphological conditions; physico-chemical conditions and specific pollutants (i.e. those pollutants that are not regulated by the EU but by member states at the national level). For rivers, the relevant biological quality elements are fish, benthic invertebrate fauna, phytoplankton and macrophytes/phytobenthos (the latter two form one quality element together). The *ecological status* for each BQE is assessed in five status classes: high (1), good (2), moderate (3), poor (4) and bad (5).

The *overall ecological status* is also assessed in five status classes (Figure 103) by taking the worst result from the BQE – thus called the "*worst case approach*". This ensures the comprehensive coverage of deviations from the reference conditions (conditions undisturbed by humans) as particular BQE indicate the different impacts of the various pressures on the ecosystem.

Additionally *supportive quality elements* are taken into account: for 'high' status, the physico-chemical conditions and hydromorphological conditions have to meet 'high' status; for 'good' status, the specific pollutants have to meet 'good' status. Regarding 'good' status, the physico-chemical conditions do not overrule the BQE (in the case that the biology shows a 'good' status and physico-chemical conditions a 'bad' status) but are an indication of the potential need for further adaptation of the assessment system (for more details see CIS 2003).

Crucial aspects for meeting the requirements of the WFD are that monitoring data for the assessment is generated with WFD–compliant sampling methods; that the assessment method is compliant with the WFD and that the assessment is done for whole water bodies.
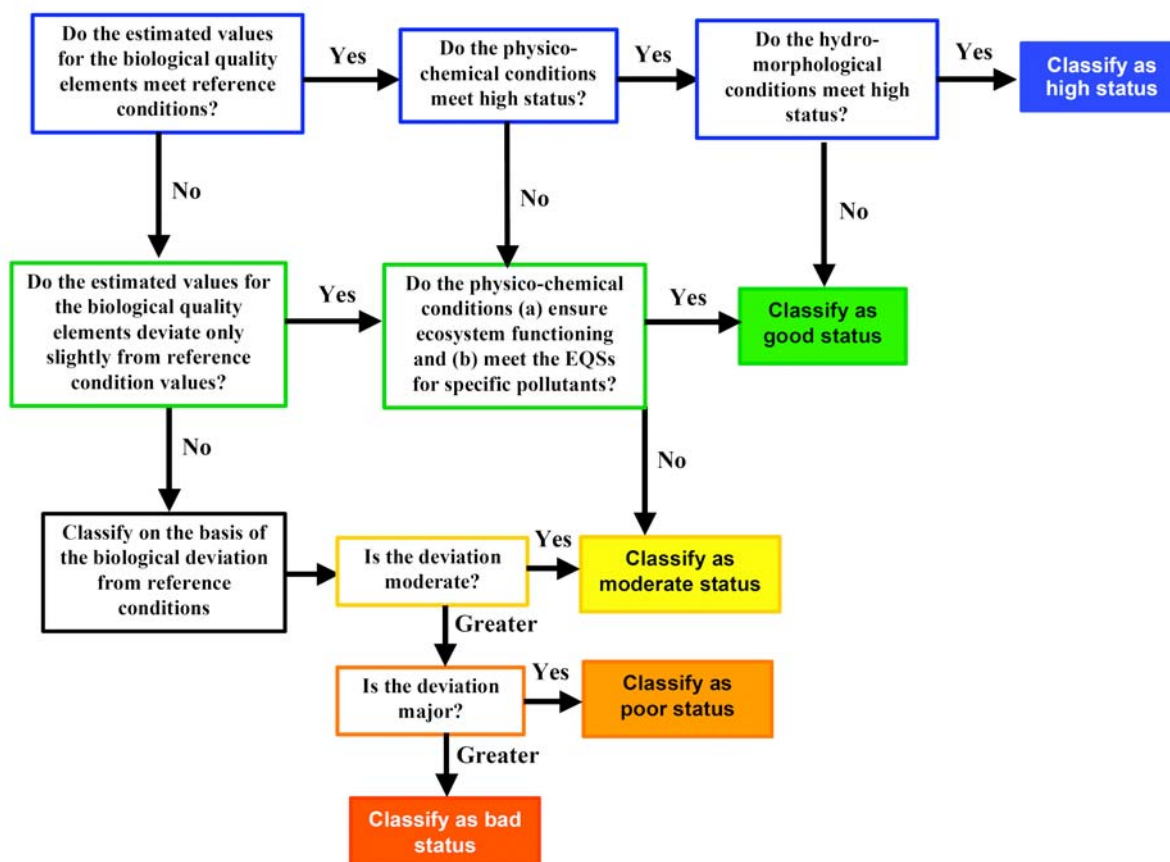
Figure 103: *Ecological status* classification using biological, hydromorphological and physico-chemical quality elements according to normative definitions in Annex V, 1.2. of the WFD (taken from: CIS 2003)

## 23.3    Strengths and limitations of the JDS2 data

The outcome of the JDS2 is an extensive data-set covering the whole Danube and comprising a comprehensive set of variables that is partially beyond the scope of the WFD. The data was generated with uniform methods and represents comparable results for the total Danube.

This report presents a first and condensed analysis and interpretation of the JDS2 data; however there is high potential for further research activities, especially method development, which will lead to a better understanding of the Danube ecosystem. The JDS2 will be of significance, particularly for the Danube intercalibration process, as this new data-set will bring forward work on typology, reference conditions and scientific tools for comparison of national assessment methods.

Despite the high scientific value of the data there are several limitations regarding the assessment of *ecological status* according to the WFD – caused by the requirements of the WFD. Due to these reasons it was not possible for the JDS2 scientists to make definite statements concerning the ecological status of the Danube water bodies:

– **Ecological status assessment is a task of EU member states**. The methods used in JDS2 for the whole length of the Danube may differ for various reasons from national methods used for monitoring and ecological status assessment. Additionally, at present the international intercalibration process is not completed.

– **At present national type-specific assessment methods are not available for some countries**. The lack of knowledge concerning type-specific reference conditions is a major problem in assessment systems for all large rivers in Europe. As a result, in agreement with the Monitoring and Assessment Expert Group of the ICPDR, available methods and expert decisions on reference conditions were used to enable at least a first "*indication of status assessment*".

– **Status assessment (according to the WFD) has to be done for water bodies**. The JDS2 investigated individual sites and not water bodies. For a final assessment of the ecological status of all water bodies, it is probable that an insufficient number of representative sampling sites are available – this decision is up to the member states.

– Some methodical limitations apply to the JDS2 data (JDS 2 data was generated by methods approaching the methodological requirements of the national WFD assessment methods). The limitations are caused by the requirements of the WFD and their national implementation in the member states:

– The sampling season of the JDS2 may differ from the requirements of the national WFD sampling methods and the JDS2 survey can offer only an indication of the status for this particular time. National WFD-compliant methods may require sampling at a different time of year or even several sampling replicates throughout the year.

– The JDS2 sampling sites may not be representative of the water bodies.

– The sampling conditions during JDS2 may have biased the result (e.g. discharge).

– In some cases an insufficient number of replicates per site may be available for a scientific sound assessment. Thus results from JDS2 and national monitoring may differ – this is an effect of natural variability.

Considering these limitations and existing problems with national methods, a final assessment of *ecological status* is not possible within the framework of the JDS2. However it was not the objective of the JDS2 to replace the national assessment of ecological status. Rather the challenge for the JDS2 researchers was to make statements and suggestions for an *indication of ecological status* to support member states in their national assessment process.

*In summary, precaution should be taken when interpreting differences between JDS2 and national monitoring results. Only the national status assessment is valid and officially reported by member states to the European Commission.*

## 23.4    Indication of ecological status of JDS2 sites
This chapter provides an overview of quality elements that are relevant for the WFD-compliant assessment of *ecological status*. The significance of *indication of ecological status* given here is somehow limited due to the constraints described above. Nevertheless the assessment process is demonstrated and a snapshot of the situation in the River Danube at the JDS2 survey is given. In addition, the approaches used for sampling and assessment methods should be taken as impulse for further advancement in the future. Further information, references and more details can be found in the previous chapters for particular quality elements.

### 23.4.1 Biological quality elements (BQE)
Generally the assessment of status based on BQE in the Danube is difficult due to the difficult sampling procedure for large lowland rivers. In addition, information on reference conditions is often lacking in large rivers. The experts involved in the JDS2 agreed on assessment methods that are in some case a compromise and express the state of development. However the proposals here are meant

to stimulate further innovation of sampling and assessment methods and the on-going discussion in the intercalibration process.

### 23.4.1.1  Benthic invertebrate fauna - macrozoobenthos

Two sampling approaches were used and lead to differing statements concerning the *indication of ecological status*. These differences are due to the sampling techniques but also due to spatially separated sampling spots in the river.

Sampling from the ship with the *Air-lift sampler* and *Multicorer* is a quantitative sampling method that characterises the river bottom of the Danube. It is a highly standardised sampling method that allows the sampling of the largest and most significant habitat, especially of large rivers, but it omits the typical near-shore habitats. *Kick & Sweep sampling* and *Dredging* are semi-quantitative methods and the former is restricted to the near-bank zone that can be reached by wading. It allows the assessment of habitats near the shoreline up to a depth of about 70 cm and usually leads to under-representation of the typical bottom sediments that are sampled with the Air-lift sampler, the Multicorer or the Dredge.

All the sampling methods have certain restrictions and it is up to the member states to decide which sampling procedure is more appropriate in the national context. A combination of methods used during the JDS2 would be preferable but would require consideration of varying sampling efficiency between the methods and varying reference conditions between channel and bank zone. These aspects of standardisation within a country are quite important and therefore member states may choose different solutions. However, to further support this decision process, more analysis of the JDS2 data concerning sampling methods for benthic invertebrates is in progress (see also the full report on macrozoobenthos on the CD-ROM).

National assessment methods for the benthic invertebrate fauna in the Danube are missing in many countries. The JDS2 experts propose a 2-module system consisting of a *multi-metric index* (MMI) and a *saprobic index* (SI). The MMI covers general hydromorphological degradation indicated by the benthic invertebrate fauna. The SI indicates organic pollution and also responds to changes in heterotrophic degradation due to impoundments. Air-lift and multi-habitat samples were used for calculating metrics and saprobic indices as they represent quantitative and area-related approaches compliant to the requirements of the WFD. In this report, only the SI is used for the *indication of ecological status* as the application of the MMI needs more (international) discussion concerning reference conditions and boundary values. However the 2-module system is a proposal for national systems and for the international intercalibration process.

For the *indication of ecological status* available national SI systems were used for German, Austrian and Slovakian sections; downstream of Slovakia the Romanian SI was used. This data gives an impression of saprobic conditions in the Danube; however it may not be appropriate at all sites as national adaptations to the assessment system would be needed in some countries (including revision in terms of section type-specificity). In addition, it is important to notice that this *indication of ecological status* is based on the SI only, which is not compliant with the WFD.

In many cases Neozoa dominate the fauna. This represents a problem for assessment of *ecological status*. Most of them indicate ß-mesosaprobic water quality according to the national classification systems. Due to their dominance this usually leads to the assessment of 'good' ecological status. On the other hand excluding Neozoa from the SI calculation may result in unreliable assessments due to the low number of remaining species. Thus, a scientific adaptation of the saprobic index calculation to cover the new species composition would be necessary (critical scientific review of the SI values for the Neozoa species).

### 23.4.1.2  Phytoplankton

The phytoplankton data collected during JDS2 was not sufficient to assess ecological status because at least four sampling dates per year would be required to cover the annual variability of the river ecosystem. Therefore, only the *chlorophyll-a* results were used to give an indication of possible

eutrophication in the Danube using the German system. The data collected (Table 41) gives an impression of the conditions during the cruise of the JDS2.

In addition, the applicability of phytoplankton for *ecological status* assessment in rivers is still discussed in some countries, especially in the upstream region of the Danube where phytoplankton occurs only in impoundments or immediately below due to drift.

### 23.4.1.3  Macrophytes and phytobenthos

In the WFD, Phytobenthos forms a BQE in combination with Macrophytes. In this report the two groups were separately sampled and evaluated.

#### Phytobenthos

The assessment of phytobenthos was based on diatoms. The Pollution Sensitivity Index (IPS) in combination with reference values and class boundaries adopted in the Slovak classification system was used to get an *indication of the ecological status* of the sites. The right and left banks of the Danube were sampled and analysed separately at most sites; in Table 41 the *indication of ecological status* for the right and left bank side are separated with a slash. The results should be viewed critically as the reference conditions may need national adaptations. However, the *indication of ecological status* gives an impression of the situation concerning eutrophication and pollution and shows clearly the negative influence of some tributaries to the Danube.

#### Macrophytes

For the assessment the Austrian national method was used in combination with new type-specific reference conditions that have been derived from historical data sources. At some sites the number of species was insufficient for the calculation of *ecological status* when strictly following the methodological instructions. Nevertheless, wherever it was possible, the experts calculated the *ecological status* for macrophytes (for more details see the full report on macrophytes on the CD-ROM). Where the identification of reference conditions was not possible, the sites were excluded from the *indication of ecological status*.

The results demonstrate that impoundments have a significant negative effect on the macrophyte community but that in free-flowing sections of the channel, the macrophytes have the potential for an indication of 'good' *ecological status.*

### 23.4.1.4  Fish fauna

The JDS2 carried out the first fish survey covering the complete length of the Danube. The fish data will therefore be of great importance for the intercalibration process of the Danube countries and for further method development.

At present none of the existing indices is fully applicable for assessment of the Danube. Thus for the *indication of ecological status* using the JDS2 fish data the experts used two different assessment methods. The *European Fish Index* (EFI) was developed within a European wide cooperation of fish experts and is sensitive mainly to water quality pressures but has limitations in detecting hydromorphological pressures (such as canalisation and migration barriers). Thus in addition, the *Austrian Fish Index* (FIA) was calculated which focuses on an indication of hydromorphological pressures.

For an indication of total fish *ecological status* for every site, the worst result from these two methods was used (worst case approach) to cover both pressure aspects. The *indication of ecological status* shows the negative impacts of hydromorphological alterations in the upper stretches of the Danube and water quality problems in the middle and lower stretches.

During JDS2, samples were taken at each site during one day due to the time schedule. As fish migrate to different habitats during the year some species might not be caught within a certain part (site) of the river at any one time. This means that the JDS2 results are not representative for the national assessment of *ecological status.*

### 23.4.2 Hydromorphological conditions

The hydromorphological survey revealed that the Lower Danube is in a better situation than the upper reaches where the river channel was regulated and modified a long time ago. At no site was the channel morphology close to conditions necessary for reaching 'high' *ecological status* but at several sites banks and floodplains showed very good conditions. In summary, the hydromorphological conditions show that there is no site with sufficiently good conditions to reach '*high' ecological status.*

### 23.4.3 Chemical and physico-chemical conditions

#### 23.4.3.1  General conditions: physico-chemical parameters (including nutrients)

The measurements of general physico-chemical parameters give a snapshot of the conditions that occurred during JDS2. Classification systems usually require the analysis of time series data (e.g. 12 measurements per year) in order to address natural variability (e.g. due to season, discharge or even daily fluctuations). Despite the fact that only single values were obtained, the results were classified into three status classes (high – good – moderate) in order to give an indication of the water quality encountered during the survey.

To date, only a few type-specific assessment systems exist in the Danube River Basin. Austrian boundary values (BMLFUW 2008) were used to demonstrate the use of this quality element. However the explanatory ability of this assessment outside Austria is limited.

The boundary between the indication of 'good' and 'moderate' status was exceeded  in 15 out of 96 JDS2 sites; and at 14 sites the biological quality elements indicated a 'moderate' or worse status (Table 39).

#### 23.4.3.2  Specific pollutants (pollutants regulated on a national level)

Official Environmental Quality Standards (EQS) are still missing in most Danube countries. The Environmental Quality Standards of Austria (BMLFUW 2006) and Slovakia (unpublished) have been used to get a first impression and to demonstrate the assessment.

By combining the results from the two national EQS results in a "worst case approach", the results show that two sites are failing to meet 'good' status due to bisphenol A (one in the Danube and one in a tributary); one tributary site is failing to meet 'good' status due to zinc and three Danube sites are failing to achieve 'good' status due to copper.

For further discussion of these results, uncertainties arising from variable conditions along the whole Danube (e.g. variable natural background concentration for some substances) should be taken into consideration and would need an adaptation of the EQS values in some countries. In addition, only one measurement was made per site, which is not sufficient for a statistically sound assessment of chemical conditions. Due to these reasons more specific information on the sampling sites is not given here. For more detailed information please see the chapters on chemical analysis of the JDS2 data.

### 23.4.4 Overview on the *ecological status* of the JDS2 sites

The results on the *indication of ecological status* for all quality elements are presented in Table 39 and in Figure 104 to Figure 107, showing the distribution of sampling sites along the Danube. Hydromorphological conditions are relevant for 'high' ecological status only – as there is no site with such conditions the hydromorphological classification is not presented here (for more details see the chapter on hydromorphology). Regarding the fish assessment, it should be considered that the sampling sites were in some cases not identical to sampling sites for the other quality elements (due to methodological reasons). However in all possible cases the fish sampling sites were assigned.

#### 23.4.4.1  Indication of overall ecological status

An *indication of the overall ecological status* is not given in this report because the particular components of the ecological status (as described in section 23.2) are already afflicted with various

sources of uncertainty. An indication of *overall ecological status* would compound these uncertainties and result in implausible results for some sites, making an interpretation difficult.

However, a WFD-compliant assessment of the *indication of overall ecological status* would be executed as described in section 23.2. As there are no sites with hydromorphological conditions that meet the 'high' status, there would be no site with an overall indication of 'high' ecological status. The data for specific pollutants and phytoplankton could not be included in the overall *indication of ecological status* for the reasons described above. Thus for demonstration purposes and further plausibility checks, the overall ecological status could be calculated by taking the worst result from the quality elements Phytobenthos, Macrophytes, Fish and Benthic invertebrates for each site.

**Table 39:** *Indication of ecological status* for the biological quality elements and general chemical and physical parameters

| JDS2 sampling site | Country | Station | Indication of *ecological status* for the biological quality elements | | | | | General chemical and physical parameters |
|---|---|---|---|---|---|---|---|---|
| | | | Benthic invertebrates* | Phyto-plankton | Phyto-benthos | Macro-phytes | Fish | |
| JDS1 | DE | Upstream Iller | 2 | 1 | 1 | 2 | | 2 |
| JDS2 | DE | Kelheim | 2 | 1 | 2 | 2 | 2 | 2 |
| JDS3 | DE | Geisling | 2 | 1 | 4 | 3 | | 2 |
| JDS4 | DE | Deggendorf | 2 | 1 | 3 | 2 | | 1 |
| JDS5 | DE | Niederalteich | 2 | 1 | 3 | 2 | 2 | 1 |
| JDS6 | DE, AT | Inn | | 1 | 3 | 4 | 5 | 1 |
| JDS7 | DE, AT | Jochenstein | 3 | 1 | 1 | 2 | 4 | 1 |
| JDS8 | AT | Upstream dam Abwinden-Asten | 2 | 1 | 1 | 3 | 4 | 1 |
| JDS9 | AT | Upstream dam Ybbs-Persenbeug | 2 | 1 | 1 | 1 | 5 | 1 |
| JDS10 | AT | Oberloiben | 2 | 1 | 1 | 1 | 4 | 1 |
| JDS11 | AT | Upstream dam Greifenstein | 3 | 1 | 1 | 3 | | 1 |
| JDS12 | AT | Klosterneuburg | 1 | 1 | 3 | 4 | | 1 |
| JDS13 | AT | Wildungsmauer | 1 | 1 | 3 | 3 | 2 | 1 |
| JDS14 | AT | Upstream Morava (Hainburg) | 1 | 1 | 3 | 2 | | 1 |
| JDS15 | AT, SK | Morava | | 2 | 3 | 2 | 2 | 3 |
| JDS16 | SK | Bratislava | 2 | 1 | 3 | 3 | 3 | 1 |
| JDS17 | SK, HU | Gabcikovo reservoir | 2 | 1 | 1 | 3 | 5 | 1 |
| JDS18 | SK, HU | Medvedov/Medve | 2 | 1 | 3 | 2 | 5 | 1 |
| JDS19 | HU | Moson Danube Arm | 4 | 2 | | 2 | 3 | 2 |
| JDS20 | SK, HU | Komarno/Komarom | 2 | 1 | 3 | 2 | | 1 |
| JDS21 | SK | Vah | | 1 | 3 | 2 | 2 | 1 |
| JDS22 | SK, HU | Iza/Szony | 2 | 1 | 3 | 3 | | 3 |
| JDS23 | SK, HU | Sturovo/Esztergom | 2 | 1 | 3 | 2 | | 1 |
| JDS24 | SK | Hron | | 1 | 3 | 2 | 2 | 3 |
| JDS25 | SK, HU | Ipoly | | 1 | 5 | 2 | 3 | 3 |
| JDS26 | HU | Szob | 2 | 1 | 3 | 2 | 3 | 1 |
| JDS27 | HU | Upstream end of Szentendre Island | 2 | 1 | 2 | 2 | | 1 |
| JDS28 | HU | Upstream end of Szentendre Island | 2 | 1 | 3 | 2 | | 1 |
| JDS29 | HU | Budapest upstream | 2 | 1 | 2 | 2 | | 1 |

| JDS2 sampling site | Country | Station | Indication of *ecological status* for the biological quality elements | | | | | General chemical and physical parameters |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | | Benthic invertebrates* | Phyto-plankton | Phyto-benthos | Macro-phytes | Fish | |
| JDS30 | HU | Budapest (old Danube) end of S.arm | 2 | 1 | 3 | 2 | 4 | 1 |
| JDS31 | HU | Rackeve-Soroksar Danube Arm - start | 2 | 1 | 2 | 2 | | 1 |
| JDS32 | HU | Budapest downstream | 1 | 1 | 3 | 2 | 2 | 1 |
| JDS33 | HU | Adony/Lórév | 2 | 1 | 3 | 2 | | 1 |
| JDS34 | HU | Rackeve-Soroksar Danube Arm - end | 2 | 1 | 3 | 2 | | 3 |
| JDS35 | HU | Dunafoldvar | 2 | 1 | 2 | 2 | | 1 |
| JDS36 | HU | Paks | 2 | 1 | 3 | 2 | | 1 |
| JDS37 | HU | Sio | | 4 | 4 | | | 3 |
| JDS38 | HU | Baja | 2 | 2 | 2 | 2 | | 1 |
| JDS39 | HU | Hercegszanto | 2 | 2 | 2 | 2 | 2 | 1 |
| JDS40 | HR, RS | Batina | 2 | 3 | 3 | 2 | 3 | 1 |
| JDS41 | HR, RS | Upstream Drava | 2 | 3 | 3 | | 3 | 1 |
| JDS42 | HR | Drava (rkm 1.4) | | 2 | 2 | | | 1 |
| JDS43 | HR, RS | Downstream Drava (Erdut/Bogojevo) | 2 | 2 | 3 | | | 1 |
| JDS44 | HR, RS | Dalj | 2 | 2 | 3 | 2 | | 1 |
| JDS45 | HR, RS | Ilok/Backa Palanka | 2 | 3 | 3 | 2 | 3 | 1 |
| JDS46 | RS | Upstream Novi-Sad | 2 | 3 | 3 | 2 | | 1 |
| JDS47 | RS | Downstream Novi-Sad | 2 | 3 | 3 | 2 | 3 | 1 |
| JDS48 | RS | Upstream Tisa (Stari Slankamen) | 2 | 3 | 4 | 2 | | 1 |
| JDS49 | RS | Tisa (rkm 1.0) | | 1 | 3 | 2 | | 3 |
| JDS50 | RS | Downstream Tisa/Upstream Sava (Belegis) | 2 | 3 | 3 | 2 | 3 | 3 |
| JDS51 | RS | Sava | | 1 | 3 | 2 | | 1 |
| JDS52 | RS | Upstream Pancevo/Downstream Sava | 2 | 2 | 3 | 2 | 3 | 1 |
| JDS53 | RS | Downstream Pancevo | 4 | 2 | 3 | 2 | | 1 |
| JDS54 | RS | Grocka | 2 | 2 | 4 | 2 | 3 | 1 |
| JDS55 | RS | Upstream Velika Morava | 2 | 1 | 4 | 2 | | 1 |
| JDS56 | RS | Velika Morava | | 4 | 4 | 2 | | 3 |
| JDS57 | RS | Downstream Velika Morava | 2 | 1 | 3 | 2 | 3 | 1 |
| JDS58 | RS | Starapalanka – Ram | 3 | 1 | 4 | 2 | | 1 |
| JDS59 | RS, RO | Banatska Palanka/Bazias | 2 | 1 | 3 | 2 | | 3 |
| JDS60 | RS, RO | Iron Gate reservoir (Golubac/Koronin) | 3 | 1 | 2 | 2 | 5 | 1 |
| JDS61 | RS, RO | Donji Milanovac | 3 | 1 | 2 | 2 | | 1 |
| JDS62 | RS, RO | Irongate reservoir (Tekija/Orsova) | 3 | 1 | 2 | 2 | | 1 |
| JDS63 | RS, RO | Vrbica/Simijan | 3 | 1 | 3 | 2 | 3 | 3 |
| JDS64 | RS, RO | Iron Gate II | 2 | 1 | 3 | 2 | 4 | 1 |
| JDS65 | RS, RO | Upstream Timok | 2 | 1 | 2 | 2 | 3 | 1 |

| JDS2 sampling site | Country | Station | Indication of *ecological status* for the biological quality elements | | | | | General chemical and physical parameters |
|---|---|---|---|---|---|---|---|---|
| | | | Benthic invertebrates* | Phyto-plankton | Phyto-benthos | Macro-phytes | Fish | |
| | | (Rudujevac/Gruia) | | | | | | |
| JDS66 | RS, BG | Timok | | 3 | 4 | | | 3 |
| JDS67 | RO, BG | Pristol/Novo Selo Harbour | 2 | 1 | 3 | 2 | | 1 |
| JDS68 | RO, BG | Calafat | 2 | 1 | 3 | 2 | 4 | 1 |
| JDS69 | BG, RO | Downstream Kozloduy | 2 | 1 | 2 | 2 | 4 | 1 |
| JDS70 | BG, RO | Upstream Iskar (Bajkal) | 2 | 1 | 1 | 2 | | 1 |
| JDS71 | BG | Iskar (rkm 0.3) | | 1 | | 2 | | 3 |
| JDS72 | BG, RO | Downstream Iskar | 1 | 1 | | 2 | 4 | 1 |
| JDS73 | RO, BG | Upstream Olt | 2 | 1 | 3 | 2 | | 1 |
| JDS74 | RO | Olt | | 1 | | 2 | | 1 |
| JDS75 | RO, BG | Downstream Olt | 1 | 1 | 2 | 2 | 4 | 1 |
| JDS76 | RO, BG | Downstream Turnu-Magurele/Nikopol | 1 | 1 | 2 | 2 | | 1 |
| JDS77 | RO, BG | Downstream Zimnicea/Svishtov | 2 | 1 | 3 | 2 | 5 | 1 |
| JDS78 | BG | Jantra (rkm 1.0) | | 1 | | 2 | | 2 |
| JDS79 | RO, BG | Downstream Jantra | 2 | 1 | 1 | 2 | | 1 |
| JDS80 | BG, RO | Upstream Ruse | 2 | 1 | 3 | 2 | | 1 |
| JDS81 | BG | Russenski Lom | | 1 | | | | 3 |
| JDS82 | BG, RO | Downstream Ruse/Giurgiu | 1 | 1 | 2 | 2 | 3 | 1 |
| JDS83 | RO, BG | Upstream Arges | 2 | 1 | 3 | 2 | 4 | 1 |
| JDS84 | RO | Arges | | 5 | | | | 3 |
| JDS85 | RO, BG | Downstream Arges, Oltenita | 1 | 1 | 2 | 2 | | 1 |
| JDS86 | RO, BG | Chiciu/Silistra | 3 | 1 | 1 | 2 | 4 | 1 |
| JDS87 | RO | Upstream Cernavoda | 2 | 2 | 3 | 2 | 3 | 1 |
| JDS88 | RO | Giurgeni | 4 | 1 | | 3 | | 1 |
| JDS89 | RO | Braila | 2 | 2 | 3 | 2 | 3 | 1 |
| JDS90 | RO | Siret | | 1 | | 3 | | 1 |
| JDS91 | RO, MD | Prut | | 1 | | | 3 | 1 |
| JDS92 | RO, UA | Reni | 2 | 1 | 3 | 2 | | 1 |
| JDS93 | RO, UA | Vilkova - Chilia arm/Kilia arm | 2 | 1 | 4 | 2 | 3 | 1 |
| JDS94 | UA | Bystroe canal | 2 | 1 | 2 | 3 | 2 | 1 |
| JDS95 | RO | Sulina - Sulina arm | 2 | 1 | 3 | | 3 | 1 |
| JDS96 | RO | Sf.Gheorghe - Sf.Gheorghe arm | 2 | 1 | | | 4 | 1 |

* For tributaries an indication of the ecological status for Macrozoobenthos is not possible due to lack of reference conditions.

Key for *indication of ecological status*: 1: high; 2: good; 3: moderate; 4: poor; 5: bad.
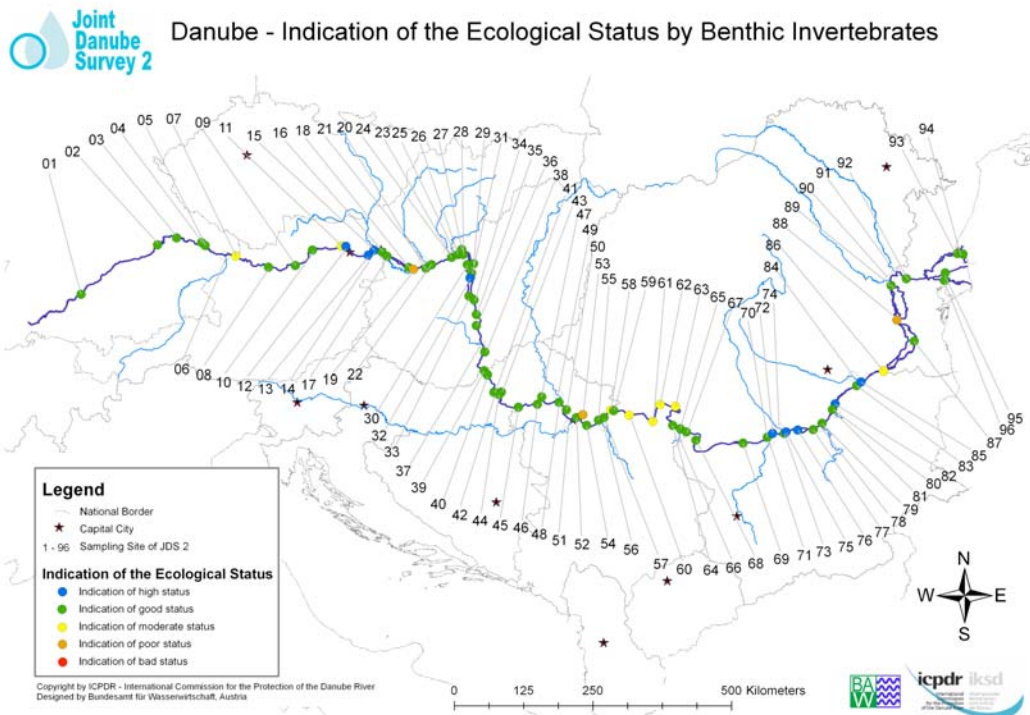
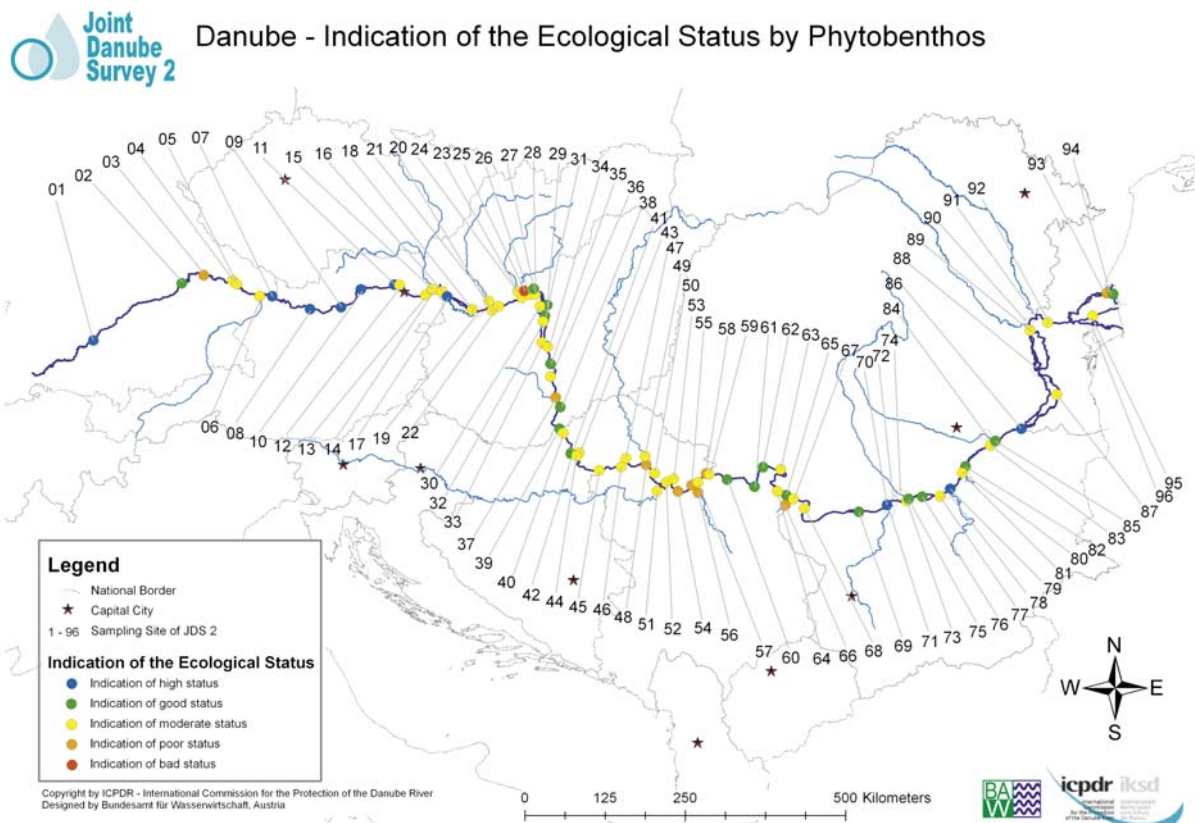Figure 104: *Indication of ecological status* with biological quality element Macrozoobenthos



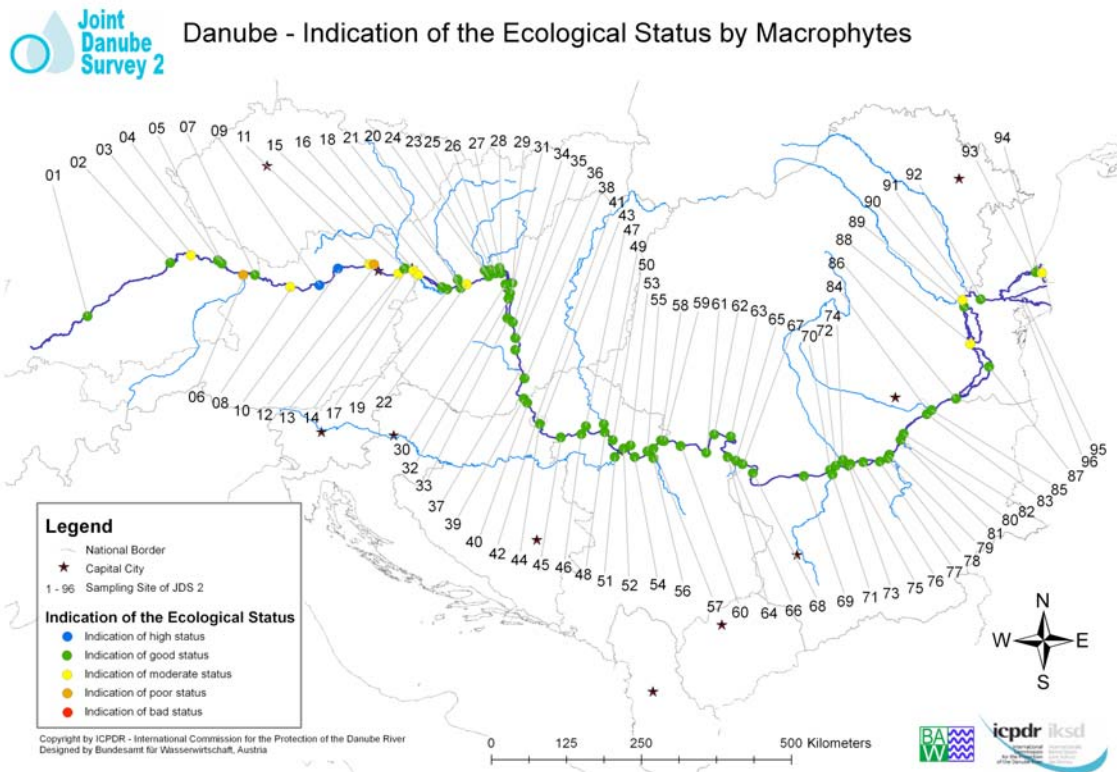Figure 105: *Indication of ecological status* with the biological quality element Phytobenthos

Figure 106: *Indication of ecological status* with the biological quality element Macrophytes.
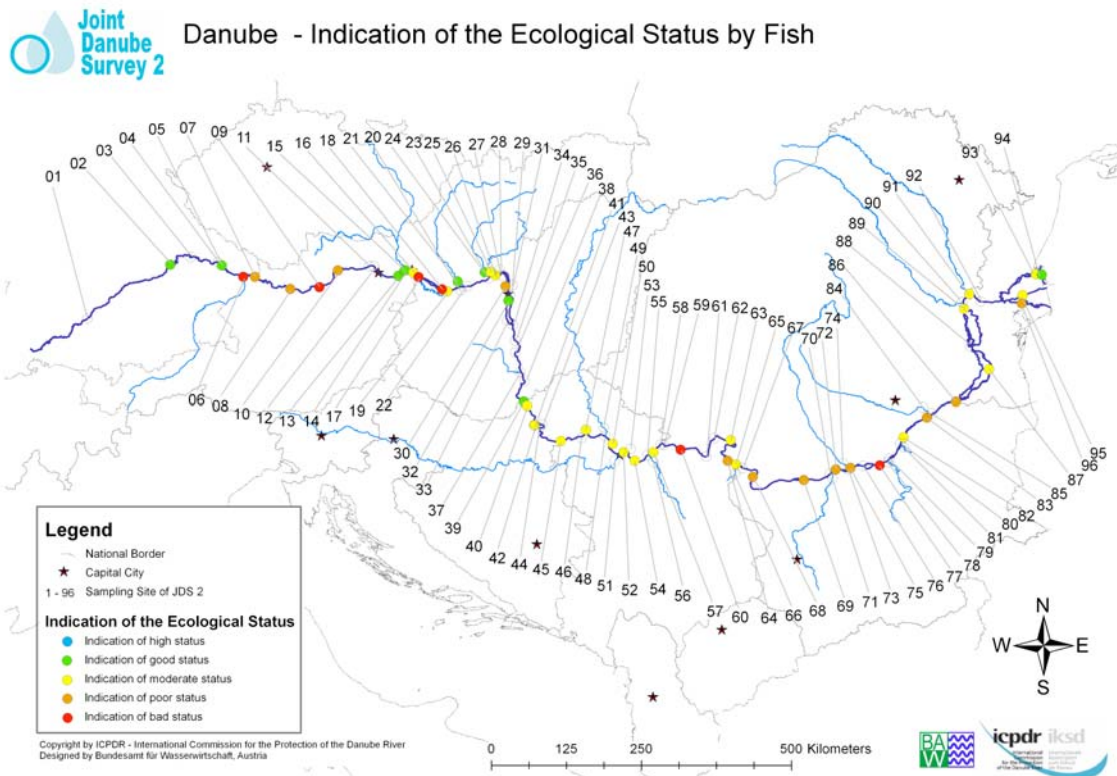


Figure 107: *Indication of ecological status* with the biological quality element Fish.

## 23.4.4.2  Interpretation of the results

Although there are many limitations to the conclusions on the *indication of ecological status,* some general trends are visible such as the impacts of hydromorphological alterations, impoundments and the pollution input from tributaries. Often the results for phytobenthos and/or fish show the worst assessment. More detailed analysis is needed to fully understand the reasons for these results. In many cases, it is not clear if these are due to weaknesses of the methodologies applied or due to existing pressures that are not yet fully understood.

In the case of the fish results, the combination of the two indices (EFI and FIA, see section 23.4.1.4) shows the effects of the hydromorphological alterations that are predominant especially in the upper region of the Danube as well as the pollution that is more dominant in the lower stretches of the Danube.

In the case of phytobenthos, the *ecological status* assessment indicates the impact of nutrients and changed flow characteristics (such as impoundments), which also alter the impact of nutrients on the ecosystem. Remarkably the phytobenthos often displays a moderate or worse indication of *ecological status* even though the nutrient level does not exceed quality standards. This may be an effect of the fact that phytobenthos is a long-term indicator and integrates pressures over a longer period (few weeks) whereas the chemical results are giving a snapshot of the situation on the sampling date. Other explanations would be that the *ecological status* assessment method is not type-specific, or that these differences are a result of short-term effects due to high water levels in the Upper Danube or specific low water conditions encountered in the Lower Danube. It would require more sampling dates for a conclusion.

In any case the limitations of methods involved in the JDS2 for assessment of *ecological status* should always be considered.

## 23.5     References

BMLFUW (2008): Leitfaden zur typspezifischen Bewertung der allgemein physikalisch-chemischen Parameter in Fließgewässern gemäß WRRL. Download: www.lebensministerium.at

CIS-Common Implementation Strategy, 2003: Overall approach to the classification of ecological status and ecological potential (Ecostat). Download at: http://circa.europa.eu/Public/irc/env/wfd/library

BMLFUW (2006): QZVO Chemie OG: Qualitätszielverordnung Chemie Oberflächengewässer - Verordnung des Bundesministers für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft über die Festlegung des Zielzustandes für Oberflächengewässer- Download: www.ris.bka.gv.at

# 24  Comments on *chemical status*

Igor Liška

One of the major goals of the survey was to obtain homogeneous information on the occurrence of all Water Framework Directive (WFD) priority substances (with the exception of $C_{10}$-$C_{13}$ chlorinated paraffins due to methodological problems) in the Danube and its major tributaries. The results obtained through such a monitoring activity provide a comprehensive snapshot on pollution of the basin by the priority substances and give an indication of the chemical status at each sampling site. According to the EU WFD, a formal assessment of the chemical status of water bodies is the responsibility of the EU member states. The JDS2 results, however, give national experts and managers of national monitoring schemes a good opportunity to compare their results with JDS2 data.

The preliminary indicative assessment of *chemical status* using JDS2 results also highlights the problems in the Danube River Basin concerning availability of sufficiently sensitive analytical methods with satisfactory limits of quantification and uncertainty levels. Thus the content of this chapter has to be considered primarily as a supportive action for implementation of the WFD, including the proposed daughter directives (Dangerous Substances Directive, QA/QC Directive), at the national level in the Danube River Basin.

## 24.1  Assessment of *chemical status* according to the WFD

According to the EU WFD, 'good' surface water *chemical status* means the chemical status required to meet the environmental objectives for surface waters established in Article 4(1)(a), that is the chemical status achieved by a body of surface water in which concentrations of pollutants do not exceed the environmental quality standards (EQS) established in Annex IX and under Article 16(7), and under other relevant Community legislation setting environmental quality standards at Community level. As a first step of this strategy under the WFD, a list of priority substances was adopted (Decision 2455/2001/EC) identifying 33 substances of priority concern at the Community level.

A new proposal for a directive of the European Parliament and of the Council on Environmental quality standards in the field of water policy and amending Directive 2000/60/EC (Dangerous Substances Directive) aims to ensure a high level of protection against risks to or via the aquatic environment stemming from these 33 priority substances and certain other pollutants, by setting EQS. For compliance checking, an average value of 12 measurements within one year has to be used.

As regards presentation of monitoring results and classification of chemical status, WFD states that where a body of water achieves compliance with all EQS established in Annex IX, Article 16 and under other relevant Community legislation setting EQS, it shall be recorded as achieving 'good' *chemical status*. If not, the body shall be recorded as failing to achieve good *chemical status*.

Member states shall provide a map for each river basin district illustrating chemical status for each body of water, colour-coded (in accordance with the second column of the table set out below) to reflect the chemical status classification of the body of water:

| Chemical status classification | Colour code |
|---|---|
| Good | Blue |
| Failing to achieve good | Red |

## 24.2     Strengths and limitations of the JDS2 chemical data

One of the outcomes of the JDS2 with respect to WFD implementation is an indication of non-compliance with the definition of a 'good' *chemical status*, which is based on a data-set of priority substances covering the Danube River and its major tributaries. For each WFD priority substance, the data were generated within a single laboratory and thus represent comparable results for the whole Danube. For some quality elements and for a limited number of sampling stations (23) there are two parallel data-sets available because the cross matrix inter-comparison of semi-volatile organic compounds (in water, suspended particulate matter, sediments and biota) carried out by the EC JRC (Joint Research Centre) also covered some of the parameters of the target analyses of priority substances performed by other JDS2 laboratories. In such situations the worse of the two results obtained was used for the indication of *chemical status*.

As regards target analyses of priority substances, for most of them the limits of quantification (LOQ) were below or at the level of the EQS. For some compounds however, the LOQ were above the EQS, so that an assessment of WFD-compliance was not possible.

Despite the high scientific value of the data there are several limitations regarding the assessment of the *chemical status* according to the WFD:

–   ***Chemical status* assessment is formally a task of the EU member states**. Final status assessment is an exclusive competence of a member state.

–   **Status assessment (according to WFD) has to be done for water bodies**. The JDS2 investigated individual sites and not water bodies – for a final assessment of the chemical status of all water bodies an insufficient number of sampling sites was available.

–   **Some methodical limitations apply to the JDS2 data**. EQS for priority substances are defined for an average value of 12 measurements within one year. JDS2 provided a single data value from August/September which definitely cannot be representative for the whole period of one year (e.g. pesticide application takes place at only certain periods of the year, thus an appropriate sampling plan has to be designed).

Considering these limitations, a final assessment of *chemical status* of water bodies is not possible within the framework of the JDS2. Anyhow, it was not the objective of the JDS2 to replace the national assessment of *chemical status*. The primary goal of JDS2 in this respect was to provide an *indication of the chemical status at the given sampling sites* for supporting the member states in their national assessment process.

*In general this means that the above mentioned limitations should be taken into account when interpreting any potential differences between JDS2 data and national monitoring results. Only the national status assessment is the official one to be reported by the member states to the European Commission.*

## 24.3     Indication of the *chemical status* of the JDS2 sites

This chapter provides an overview of priority substances that are relevant for the WFD-compliant assessment of *chemical status* indicating exceedance of EQS.

According to Annex 4 of the WFD, surveillance monitoring of priority substances must be done on a monthly basis for the period of one year.

The draft Dangerous Substances Directive defines the application of EQS in the following way:

–   "For any given surface water body, applying the EQS-AA ("EQS-annual average") means that, for each representative monitoring point within the water body, the arithmetic mean of the concentrations measured at different times during the year does not exceed the standard";

– "For any given surface water body, applying the EQS-MAC ("EQS-maximum allowable concentration") means that the measured concentration at any representative monitoring point within the water body does not exceed the standard".

For about half of the priority substances, the EQS-MAC is marked as "not applicable". In these cases the EQS-AA values are considered protective against short-term pollution peaks in continuous discharges since they are significantly lower than the values derived on the basis of acute toxicity.

For JDS2 one single water sample was taken at each sampling site. For this reason the assessment of the chemical status cannot be done in-line with the WFD requirements and the following procedure was applied:

– An *indication of the chemical status* is given for each sampling site (not water body);

– For compliance checking, the proposed EQS-AA for inland waters is used as the value recorded during the survey can be considered as an approximate representative of commonly occurring values rather than the maximum value of the year. This assumption is naturally not valid for seasonally affected occurrence of substances but selection of AA rather than MAC also represents a kind of worst-case approach.

In the case that there were two results from different laboratories available for a particular site, the worse of the two results was used in the final evaluation. Table 40 shows the sampling sites where EQS are exceeded as well as the corresponding parameters.

## Table 40 Sampling sites and parameters exceeding EQS for WFD priority substances

| Sampling station | Location | Parameter/s > EQS |
|---|---|---|
| JDS2 | Kelheim | ∑ benzo(g,h,i)perylene & indeno(1,2,3-cd)pyrene |
| JDS10 | Oberloiben | 1,2,4-trichlorobenzene |
| JDS13 | Wildungsmauer | DEHP |
| JDS14 | Upstream Morava (Hainburg) | DEHP |
| JDS16 | Bratislava | ∑benzo(g,h,i)perylene & indeno(1,2,3-cd)pyrene |
| JDS17 | Gabcikovo reservoir | DEHP |
| JDS26 | Szob | tributyltin |
| JDS32 | Budapest downstream | mercury |
| JDS33 | Adony/Lórév | mercury |
| JDS34 | Rackeve-Soroksar Danube Arm - end | DEHP |
| JDS35 | Dunafoldvar | DEHP, tributyltin |
| JDS36 | Paks | DEHP |
| JDS38 | Baja | DEHP |
| JDS39 | Herczegszanto | DEHP, ∑benzo(g,h,i)perylene & indeno(1,2,3-cd)pyrene |
| JDS40 | Batina | DEHP |
| JDS41 | Upstream Drava | DEHP |
| JDS42 | Drava (rkm 1.4) | DEHP |
| JDS43 | Downstream Drava (Erdut/Bogojevo) | DEHP |
| JDS44 | Dalj | DEHP |
| JDS45 | Ilok/Backa Palanka | DEHP, tributyltin |
| JDS46 | Upstream Novi-Sad | DEHP |
| JDS47 | Downstream Novi-Sad | DEHP |
| JDS49 | Tisa (rkm 1.0) | DEHP |
| JDS50 | Downstream Tisa/Upstream Sava (Belegis) | DEHP |
| JDS51 | Sava (rkm 7.0) | DEHP |
| JDS52 | Upstream Pancevo/Downstream Sava | DEHP |
| JDS53 | Downstream Pancevo | DEHP |

| Sampling station | Location | Parameter/s > EQS |
|---|---|---|
| JDS54 | Grocka | DEHP |
| JDS55 | Upstream Velika Morava | DEHP |
| JDS56 | Velika Morava | benzo(g,h,i)perylene |
| JDS57 | Downstream Velika Morava | DEHP |
| JDS58 | Starapalanka – Ram | DEHP |
| JDS59 | Banatska Palanka/Bazias | DEHP |
| JDS60 | Irongate reservoir (Golubac/Koronin) | DEHP |
| JDS61 | Donji Milanovac | DEHP |
| JDS62 | Irongate reservoir (Tekija/Orsova) | DEHP |
| JDS65 | Upstream Timok (Rudujevac/Gruia) | DEHP |
| JDS66 | Timok (rkm 0.2) | DEHP, nonylphenol, nickel |
| JDS68 | Calafat | DEHP |
| JDS69 | Downstream Kozloduy | DEHP |
| JDS72 | Downstream Iskar | DEHP |
| JDS73 | Upstream Olt | DEHP |
| JDS74 | Olt (rkm 0.4) | DEHP |
| JDS76 | Downstream Turnu-Magurele/Nikopol | tributyltin |
| JDS79 | Downstream Jantra | DEHP |
| JDS80 | Upstream Ruse | tributyltin |
| JDS81 | Russenski Lom | nonylphenol, DEHP |
| JDS82 | Downstream Ruse/Giurgiu | DEHP |
| JDS83 | Upstream Arges | tributyltin |
| JDS84 | Arges | nonylphenol, DEHP |
| JDS92 | Reni | tributyltin, ∑benzo(g,h,i)perylene & indeno(1,2,3-cd)pyrene |
| JDS94 | Bystroe canal | DEHP |
| JDS95 | Sulina - Sulina arm | tributyltin, ∑benzo(g,h,i)perylene & indeno(1,2,3-cd)pyrene |
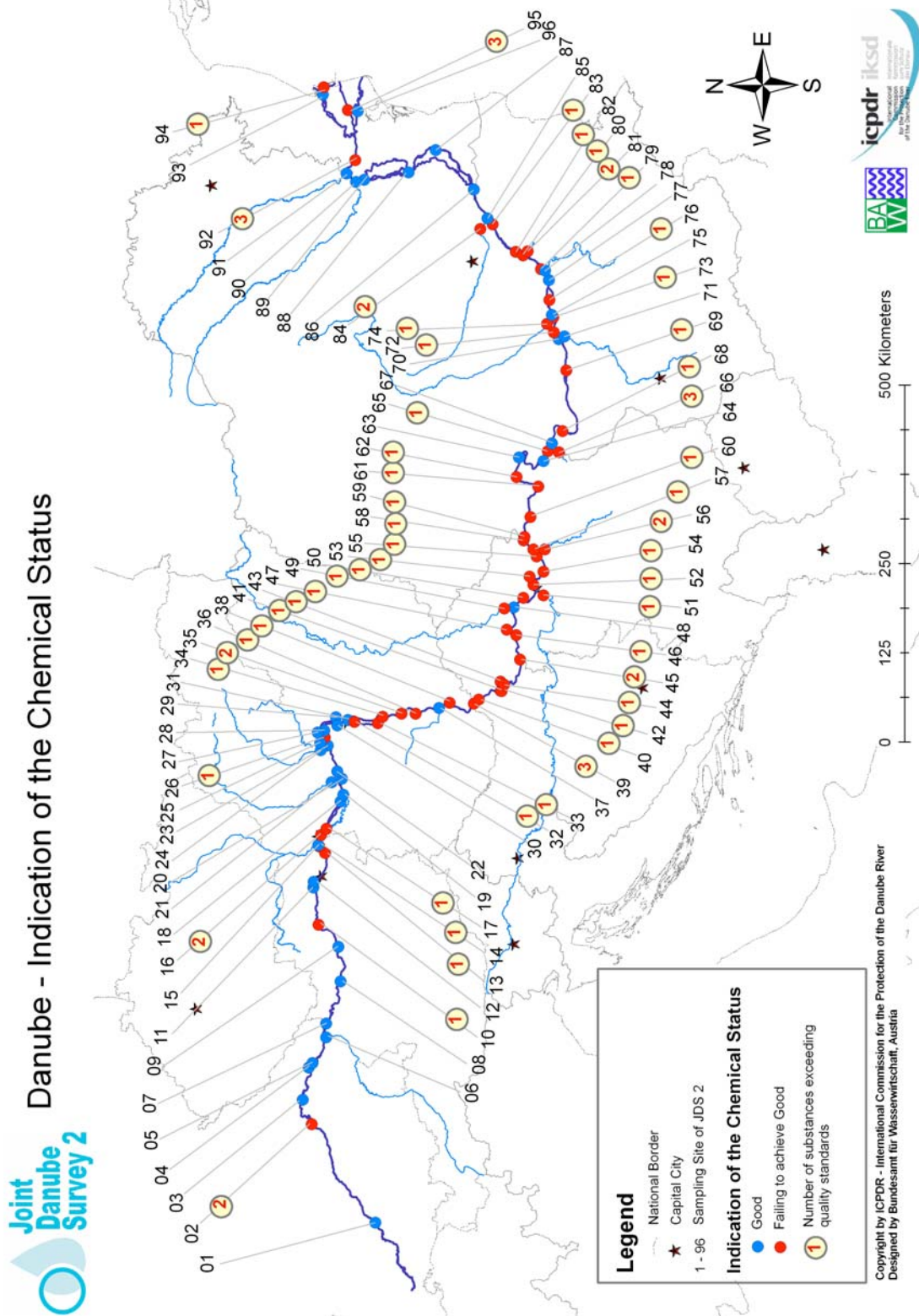
Figure 108: Indication of the *chemical status* of the JDS2 sampling sites

The target analyses of priority substances showed that at 53 out of the 96 JDS2 sites on the Danube and the mouths of the major tributaries (approx. 55% of the sites) the concentration levels of one or more WFD relevant substances exceeded the EQS in water.

The formal reasons why the chemical status assessment has only an indication value were explained above; an example is discussed below that clearly demonstrates that *chemical status* assessment requires a thorough monitoring of water bodies through the period of one year (at least).

The analyses done by the EC JRC (see Chapter 1) showed that the levels of the sum of benzo(g,h,i)perylene & indeno(1,2,3-cd)pyrene exceeded EQS at five sites (as is indicated in Table 40). A closer look at the results of the PAH analyses reveals that at several other sites the sum of benzo(g,h,i)perylene & indeno(1,2,3-cd)pyrene was very close to the EQS value and, given the uncertainty of an analytical method operating at the ng/l level, there is an obvious potential probability of non-compliance at more than just five JDS2 sites in the case that results from the whole year be collected (considering that ubiquitous PAHs have no specific seasonal pattern).
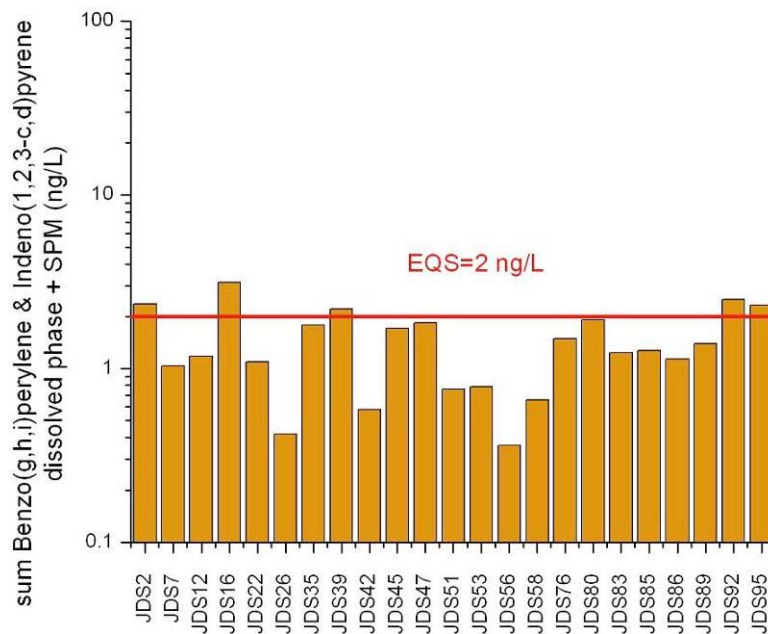


Figure 109: ∑Benzo(g,h,i)perylene and indeno(1,2,3-cd)pyrene in water (EC JRC)

## 24.4    Conclusion

Summarising the results in Table, DEHP is the most problematic priority substance in the Danube and its main tributaries, followed by the PAHs, tributyltin and nonylphenol.

The analyses of priority substances however revealed another "significant issue" which is the availability of sufficiently sensitive analytical methods capable of providing accurate and precise results at the trace level. Considering the results in Table 40, it must be taken into account that for some data the uncertainty level was too high due to high limits of quantification (LOQ) and for some compounds even the LOQ was above the EQS, so that an assessment of WFD-compliance was not possible. These outcomes underline the necessity of the future implementation of the planned EU QA/QC Directive.

# 25 Lessons learned

Igor Liška, Franz Wagner and Jaroslav Slobodnik

## 25.1 Introduction

The JDS2 has moved our knowledge base about the status of the Danube River a big step forward, providing a complex overview of an aquatic ecosystem of a large river. However, the value of the survey is not only in the enormous amount of data that was collected, analysed and evaluated, but also in the new findings and experience gained concerning sampling strategies and techniques, analytical methods applied and solutions found for coping with problems encountered.

The water quality assessment process is in its substance 'learning by doing'. The lessons learned during a huge monitoring exercise, as JDS2 certainly was, will contribute to a better and optimised planning of future monitoring activities in the Danube River Basin, as well as to development of properly tailored procedures for assessment of the status of water bodies. The issues described in this chapter and the suggestions made came into view during JDS2 activities and are to be taken into account by experts on monitoring and assessment in the Danube River Basin and beyond, where applicable.

This chapter does not discuss in detail the conclusions of the assessment of ecological and chemical status as these were thoroughly dealt with in previous chapters.

## 25.2 Hydromorphology

The first survey of this kind covering the whole reach of the Danube was a good opportunity to test the available methodologies. The sampling sites selected for JDS2 were not always found to be representative for characterisation of the hydromorphological features of the Danube as they had often been selected to detect discharges from agglomerations (focus of the JDS2 was given to water quality aspects). Many stations were located upstream of dams in the impounded areas, within the cities and transition stretches (e.g. from cities to more natural stretches, or just downstream of dams) which were not typical for the prevailing character of long Danube reaches. The survey only allowed the analysis of the main navigable channel; there were no opportunities to examine side channels.

The hydromorphological characteristic of sampling sites with regard to microhabitats should be more detailed in future to support the biological assessment. The continuous longitudinal survey seems to be more relevant than the site survey due to its higher representativeness. Such surveys can provide a good baseline information for the future.

The quality of the navigation map decreases and the data is less up-to-date downstream of Belgrade. Unfortunately the new digital navigation maps do not show all river regulation works, shallows, bars/islands and indicative values for channel depth and flow velocity.

To ensure high quality of data and reliability of results, it is crucial that the assessment of hydromorphological features of the Danube River is done by a single expert during the whole survey.

The results of the survey enabled formulation of the following recommendations for action:

–  If stretches do not reach 'good' *ecological status*, countries should put more effort into restoring additional stretches and preserving those that still have 'good' hydromorphological conditions. An update of preliminary risk assessments and HMWB designations should be considered.

–  Consideration should be given to using environmentally sustainable alternatives for further bank revetments and reinforcement along the Lower Danube.

–  Continuation and improvement of restoration measures along the Upper Danube (by further reconnecting floodplains and providing more space for channel development) should be undertaken (considerably reducing bank reinforcement to the absolute necessary minimum).

–  Large scale restoration of floodplains should be carried out along the Lower Danube (beginning with the reconnection of islands with ring dikes).

–  Environmental impact assessment studies and long-term monitoring of hydromorphological features for relevant planning control (checking of exemptions) should be undertaken.

## 25.3    Biology

In **macrozoobenthos** sampling a thorough comparison between the Air-lift / MHS / Multicorer methods and Kick & Sweep / dredging was difficult because the two approaches are not only different in terms of technique but they also sample different spatial zones of the river. More taxa were collected with Air-lift / MHS / Multicorer compared to the Kick & Sweep / dredging method (362 and 202 respectively). In general, Air-lift / MHS / Multicorer seems to be more effective regarding a standardised documentation of benthic invertebrates as it is a quantitative method and covers the largest area of the river ecosystem. On the other hand, Kick & Sweep / dredging seems to be more effective in documenting Mollusca, Odonata, Mysidacea and Heteroptera taxa. It underlines that, regarding diversity, a combination of both Air-lift / MHS and Kick & Sweep / dredging is useful. For a decision on methods for future WFD-compliant monitoring programmes, the objectives for the use of the biological quality element (BQE) macrozoobenthos have to be considered.

The biological quality element **phytobenthos** indicated significant differences between the right and left side of the river channel, the effect of one-sided discharge of organic and nutrient pollution. Other biological quality elements often exhibited a similar, but less distinct, pattern. However, for the future WFD-compliant assessment of ecological status, these local differences should be integrated into an assessment result for the whole water body.

The length used in the JDS2 for **macrophyte** analysis was 3 km on each river side. This seemed to be the absolute minimum to reflect the occurrence and abundance of macrophyte species. In the JDS2, as well as in the JDS1, an emphasis was given to survey sites in power plant reservoirs. It would be highly interesting to also sample the reaches of the Danube where neither water depth nor flow conditions are influenced by reservoir-typical conditions. For assessing the impacts of the tributaries and municipal wastewater discharges, downstream sampling sites should be located closer to those discharges and site planning should take into account the discharge magnitude and also the mixing conditions. National surveys supporting the future JDS could cover longer reaches for macrophyte sampling.

For the ecological assessment of **phytoplankton**, one single sampling date is not sufficient due to the high natural variability of this quality element. Hence, a future JDS programme should be complemented by additional national sampling programmes to provide reliable results for the assessment of *ecological status*.

Comparable **fish** sampling in a large river is rather complicated. Even during ideal conditions, with perfect planning and much experience, there will always be a high degree of variation in the sampling efficiency. The JDS2 results demonstrated the possibilities for fish sampling in large rivers as needed for the evaluation of *ecological status*. It was shown that with a standardised sampling programme, based on electrofishing in the shallow (littoral) areas, it is possible to collect detailed information on the fish community in a cost efficient way. The results indicate that even a large river can be

sufficiently sampled by a boat using electrofishing. Moreover, night sampling proved to be very effective in the case of a large river. Therefore, these results should be considered as a motivation for updating the CEN-Standard for electrofishing.

The JDS2 demonstrated the use of **biological quality elements relevant for *ecological status* according to the WFD** and identified further needs for harmonisation of sampling and assessment methods, and reference conditions, and for the selection of representative sampling sites.

In the **microbiological analysis**, the use of the Colilert system for *E. coli* detection proved to be the most appropriate and robust parameter to predict faecal contamination. The comparison of on-board microbiological data with those from the three national reference laboratories revealed a good correspondence (with the only exception being the enterococci microtiter plate technique due to its high detection limits). For the next JDS, sampling in the middle and at both river banks is recommended to improve significantly the detection probability of faecal contamination from smaller sources. Moreover, a more sensitive protocol for Enterococci detection has to be considered. The integration of more reference laboratories from other countries should be enforced. For a better comparison the use of the same methodology in the next JDS should be a prerequisite. Moreover, on-board measurements of *E. coli* and Enterococci using standardised membrane filtration methods should be done in parallel. Quantitative microbial source tracking to quantify human faecal pollution has shown great potential and should be extended to other relevant faecal source specific markers (e.g. animal sources).

The longitudinal study for basic microbial parameters (bacterial numbers, biomass, morphotypes and bacterial secondary production) provided an overview of biological data missing for the whole Danube to date. The data obtained was promising, however, a comparative analysis with data from the biological quality elements (according to the WFD) is still required to make any conclusions on the application of these parameters for the assessment of *ecological status*.

## 25.4    Chemistry

The application of results on chemical parameters supportive for the assessment of *ecological status* is discussed in the previous chapter. However, it is essential to stress again the need to develop appropriate type-specific classification systems/guiding values for those supportive physico-chemical parameters in Danube countries. Comparison of the results using Austrian and Czech classification schemes for dissolved oxygen shows remarkable differences. Only five sampling sites on the Danube and four at the mouths of tributaries did not comply with the Austrian standard for the 'good' category. On the other hand, using the Czech standard, 68 sites on the Danube and half of the tributaries did not comply with the 'good' category criterion.  Even though in the case of nutrients the results of the two classification schemes were similar, a harmonised classification is one of the key challenges for the near future.

Trace analyses of priority substances revealed two key problems in the basin: reaching sufficient quantification limits and comparability of results from different laboratories. For most of the priority substances analysed during JDS2, the limits of quantification (LOQ) were below or at the level of the environmental quality standards (EQS). For some compounds however the LOQ exceeded the EQS, disabling an assessment according to WFD rules. Comparing the results for nonylphenol in SPM collected at 23 selected sampling sites, more than 60% of the overlapping samples showed significant differences of more than 35%. Despite the fact that the differences could be explained by the various analytical methods applied using different solvents and extraction techniques, strengthening of proficiency testing for WFD priority substances is a must.

# 26   Conclusions

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## 26.1   Introduction

Shared by 19 countries, the Danube River Basin is the most international river basin in the world. In 1996, the Trans National Monitoring Network (TNMN) was launched, constituting the main data source about water quality for the Danube and its major tributaries. It provides a structured and well-balanced overall view of the basin's pollution status, as well as the long-term development of water quality and pollution loads in terms of relevant determinands for the basin's major rivers. Recent monitoring upgrades will also help the TNMN meet the requirements of the EU Water Framework Directive (WFD), especially by broadening its scope to consider biological monitoring and WFD priority substances.

To attain a full overview of the water quality needed for EU WFD implementation, the ICPDR organises monitoring surveys on the entire stretch of the river. The results of the first `Joint Danube Survey (JDS1)´ in 2001 were a key information source. During the second `Joint Danube Survey (JDS2)´ in 2007, 96 sites were successfully sampled along the 2600 km stretch of the Danube River, as well as 28 sites on its major tributaries. The survey gathered comprehensive information about riverine hydromorphology, biology and chemistry; strengthened basin-wide cooperation among the scientific community and increased public awareness towards protection of the Danube. Following the survey's completion, the collected data was analysed in laboratories and scientific institutes across Europe.

## 26.2   Hydrological conditions during JDS2

During JDS2 no significant hydrological situation occurred in the Upper Danube that would reach long-term values of low or high water levels. Two weeks before the JDS2 launch, the discharge of the Danube at Regensburg nearly reached the one year flood return period.

The middle course of the Danube was characterised by discharges slightly below the mean water whereas the Lower Danube was subject to a continuous increase of discharge from an annual low water situation (about 3000 m³/s at Zimnicea, rkm 550) towards levels above average (over 6000 m³/s): The survey faced an increasing water flow downstream of the Iron Gate and then rode that wave downstream to the delta.

## 26.3   Hydromorphology

Hydromorphological alterations have been identified as a basin-wide significant water management issue in the Danube River Basin. JDS2 included the first ever systematic survey of hydromorphological parameters in the entire navigable longitudinal Danube stretch using a single method.

The continuous longitudinal hydromorphological assessment showed that the situation in the Lower Danube is better than in the upper part. About 40% of the investigated Danube was satisfactory, meaning that there are still many healthy ecological areas – a status that is generally more positive

than earlier perceived. Any deterioration of this *status quo* in the future should be prevented and specific programmes of measures should take place to enhance the situation for migratory species and to support floodplain restoration.

The hydromorphological analysis identified areas with significant alterations requiring attention. Suggested remedial measures include:

– Reconnection of floodplains and side-arms;

– Removal of bank reinforcements where possible (e.g. east of Vienna);

– Enhanced discussions with the navigation sector about channel maintenance and with the hydropower sector to improve the naturalness of impounded Danube sections.

Overall, the relatively positive status found for Danube hydromorphology indicates a good basis for protecting that status into the future as well as for additional restoration activities.

## 26.4    Biology

### 26.4.1 Macrozoobenthos
The macrozoobenthic community documented during JDS2 comprises 411 taxa. Regarding diversity, the most heterogeneous groups are Diptera and Oligochaeta. The fauna is dominated by Crustacea (Amphipoda and Isopoda) in terms of abundance while Mollusca are the predominant group regarding biomass. However, aquatic insects, especially EPT-taxa, play only a minor role in the Danube River.

Delineation of the three major reaches of the Danube (upper – middle – lower) could be confirmed by multivariate analysis of the macrozoobenthos data. Additionally, the Danube typology could be revised at a smaller scale resulting in five identified section types of the Danube River.

The saprobic indices, characterising organic pollution, varied in the Danube between 1.83 and 3.15. Most of the sites (58) can be classified as "*indication of good ecological status*" according to the WFD and for 9 other sites a "*high ecological status*" is indicated. For 8 sites the SI shows an "*indication of moderate ecological status*"; for 3 sites "*poor ecological status*". The comparison of different national classification systems showed a clear need for harmonisation.

The tributaries (near their confluence with the Danube) had saprobic indices between 2.1 and 3.26 (Austrian SI). In the rivers Sio, Jantra and Russenski Lom, the SI-values were higher than 3.0. The Arges River was found excessively polluted and did not host any macroinvertebrate specimens.

The bottom fauna of the upper and middle reach of the Danube is dominated by Ponto-Caspian Neozoa (mostly Crustacea and Mollusca). Their relative abundance observed during the JDS2 ranged between 60% and 80% and they represented up to 40% of the total number of taxa. Neozoa are not locally abundant but cover the whole Danube stretch. As Neozoa dominate the fauna, their classification is a crucial point in assessing ecological status. Most of them indicate ß-mesosaprobic water quality due to their national classification, which results in an overall *good ecological status* due to their dominance. Omitting Neozoa from the analyses leads to zero-values of the saprobic index in some cases.

### 26.4.2  Phytobenthos
Phytobenthos as a part of the aquatic flora, contrary to the aquatic fauna, reacts directly to the nutrient content (mostly phosphorus) and is considered as a reliable indicator of eutrophication processes on a long-term basis. The *indication of ecological status* based on phytobenthos analysis suggested an increase of nutrients in the longitudinal profile of the Danube. This was confirmed by a longitudinal increase of phytobenthos biomass, as well as by varying species diversity. The results of the JDS2 showed a high species diversity of phytobenthos with 443 identified taxa. Concerning the individual

Danube River types, the biomass had the highest concentrations in Type 7 (Iron Gate reservoir). When comparing the results of phytoplankton and phytobenthos biomass, the Middle Danube shows the highest variability; the Upper and Lower Danube show lower phytoplankton levels, resulting in better growing opportunities for phytobenthos. In spite of a large similarity in diatom samples, there were significant differences between the River Danube and some tributaries.

### 26.4.3 Macrophytes

In the regulated non-impounded stretches of the Danube, the macrophytes often meet the conditions required for *good ecological status*; however the situation is unsatisfactory in the impounded stretches upstream of hydro-electric power plants and a negative influence from some tributaries in the Lower Danube was observed.

69 macrophyte species were identified during the JDS2. The unexpected spread of duckweeds in the main river channel was probably triggered by the warm winter period. The fern, *Salvinia natans,* has been found in an oxbow system near Vienna over the last 3 years and could be an indication of climate change induced migration of thermophilic species up the Danube River. Another migrating species that was found around Novi Sad is the helophyte, *Chamaesyce glyptosperma*. The submerged invasive species, *Elodea nuttallii,* migrated from Western Europe down the Danube into the delta area and is replacing *Elodea canadensis*.

### 26.4.4 Phytoplankton

Phytoplankton analysis found most of the Danube in acceptable conditions; the unsatisfactory sites occurred only in the middle reach. From the distribution of phytoplankton chlorophyll-a and biomass along the river corridor, three sections can be defined: an upstream section from upstream of Iller to Baja with chlorophyll-a values below 10 µg/l and biomass concentration below 2 mg/l (km 2600–1481); a middle section where values exceed this threshold from downstream of Baja to Grocka (km 1481–1132) and a downstream section with generally low values again. Maximum values of both parameters have decreased by about fivefold compared to results from the JDS1.

River phytoplankton was largely characterised by centric diatoms while tributaries were very rich in species diversity.

Comparison with the JDS1 data indicated a quality improvement; but the high trophic potential in the Middle Danube (based on phosphorus concentrations) necessitates intensification of efforts towards the use of P-free detergents. The most polluted river indicated by the phytoplankton analysis was the Arges.

### 26.4.5 Fish

During the JDS2, the first ever fish survey on the entire Danube was carried out bringing a homogeneous dataset for the whole reach, but also contributing to methodological harmonisation between EU and non-EU countries. The assessment of the fish population showed that only about one third of the investigated sites on the Danube and the tributaries presented a indication of *good status*. The hydromorphological alterations can be considered as the main pressure on fish populations in the Upper Danube section, while water quality is a key pressure in the middle and lower sections. Furthermore, the general lack of migratory species in the Danube indicates a loss of river connectivity, which emphasises the need for actions already proposed by the ICPDR concerning the reopening of migration routes for the Danube sturgeon and other migratory species. In general, a very high species diversity was found in the Danube (over 64000 fish of 71 species were sampled) indicating that the Danube could be ranked as 'top' river in Europe in terms of number of fish species. Invasive fish species however are a rising threat that needs to be evaluated.

### 26.4.6 Zooplankton

In the main branch of the Danube River and its tributaries, 126 zooplankton taxons were found, out of these 87 Rotatoria, 30 Cladocera and 9 Copepoda were registered. The high zooplankton numbers were observed in the slow-flowing middle reach.

There was no increase in abundance or number of species observed in the reservoir sections. Only the effect of the Morava was registered in the Danube zooplankton composition; other tributaries did not influence the community of the main river. The density of zooplankton was lower than in 2001.

In general, the biological indicators have different requirements concerning sampling time in the year and site selection and a joint survey will always be a compromise for all the scientific needs.

Overall, the biological indicators investigated during the JDS2 revealed a good potential to achieve healthy *ecological* conditions. However, some specific problems need to be addressed such as hydro-electric dam reservoir areas, specific tributaries and rising invasive species populations. The above suggestions on hydromorphological measures should assist in correcting some of the biological problems.

### 26.4.7 Microbiology

Microbial analysis found about one third of the sites polluted. The highest microbial contamination levels for the Danube River were found in the stretch between Budapest and Belgrade, while the tributaries, Arges and Russenski Lom, and side-arms, Rackeve-Soroksar and Moson Danube, can be considered as hot spots. This emphasises the need for ensuring the sufficient treatment of wastewaters.

A comparison of the data with the preceding JDS1 (2001) indicated a slight decreasing trend in faecal pollution of the Danube River. However, due to methodological reasons, no definite assessment is possible.

The application of the human faecal specific quantitative microbial source tracking BacH marker for all tributary and side arm samples clearly demonstrated the relevance of human faecal contamination. BacH indicated that the dominant fraction of *E. coli* was derived from human sewage or excreta.

The longitudinal study of the Danube River and its tributaries revealed conspicuous differences between bacterial parameter values from the Danube River water samples and the merging tributaries. Bacterial numbers, biomass and secondary production values from tributaries were always higher than in the Danube River. Only in the case of tributaries with higher discharge volumes (e.g. Inn and Drava) did bacterial parameter values remain below those of the Danube River.

In general, the Danube River seems to be rather unaffected by the tributaries input. As measured parameter values were usually lower than the calculated expected values after the inflow of tributary waters, it may be assumed that beside the impact of microzooplankton, both the filter feeding macrozoobenthos and cell retention by river bank sediments are responsible for the observed decrease of bacterial biomass. The results suggest that the Danube River may function as a purification system for bacterial loads imported from tributaries. The evolution of the bacterial parameters along the longitudinal transects reveals clear trends for an increase of bacterial numbers and biomass and a marked decrease in bacterial volume. These observations are in agreement with the River Continuum Concept.

## 26.5    Chemistry

Water temperature distribution during the JDS2 ranged within the pattern typical for the timing of the survey (August – September) both in the Danube and in the mouths of selected tributaries. Maximum values were recorded in the middle reach of the Danube River.

The dissolved oxygen concentration pattern demonstrated near to 100% saturation along the Danube River, with slightly higher values in the upper and middle Danube reaches.  Although these higher values corresponded to the primary productivity characterised by chlorophyll-a concentration, there

was no such algal blooming during the JDS2 as occurred in the JDS1. Tributaries at their confluence to the Danube displayed slightly higher dissolved oxygen levels than in the recipient Danube. Significant depletion of dissolved oxygen was recorded at the mouth of the Arges, due to the discharge of untreated municipal wastewater.

The longitudinal profile of pH values along the Danube River was similar to the results for dissolved oxygen. The good correlation occurred during both the JDS1 and JDS2, demonstrating the balanced effect of primary production and decomposition of organic matter, or in other words, healthy conditions in this particular aquatic ecosystem.

A relatively constant profile for N-ammonium concentration was observed along the Danube River; the maximum concentration peak was located in the Iron Gate reservoir backwaters. A highly elevated concentration was measured in the mouth of the Arges tributary. A significantly decreasing nitrate profile down the Danube was observed. A very low content of organic nitrogen was found in the JDS2 water samples.

A strong decrease in orthophosphate concentrations was observed in the Upper Danube followed by a slight increasing profile in the lower reach, mainly caused by discharges of municipal wastewater with P-containing detergents. Except for two very elevated concentrations (0.635 and 1.000 mg/l in the mouths of the Russenski Lom and Arges respectively), most of the tributaries had concentration levels similar to the Danube River. Total phosphorous concentrations in water increased slightly down the Danube, while dissolved silicates showed a generally decreasing trend down the Danube.

Compared to the JDS1 results, N-ammonium, nitrites and TP profiles were relatively similar. Concentrations of nitrates and dissolved silica recorded during JDS2 were higher than those measured during JDS1. On the other hand, a decrease in orthophosphate concentrations was observed during the JDS2 when compared to the JDS1, with few exceptions located in the middle reach of the Danube.

Among the priority substances, di-(2-ethylhexyl)phthalate (DEHP) was found at relatively high concentrations in nearly all JDS2 water samples and in 44% of the water samples the proposed environmental quality standard (EQS) was exceeded. At several sites an indication of non-compliance with WFD was found for tributyltin. The highest concentrations of organotins in suspended particulate matter (SPM) were found in the Danube downstream of Pancevo. The Arges and Russenski Lom rivers showed the highest concentrations of alkylphenols in water with 4-iso-nonylphenol exceeding EQS at three sites. Highest concentration of 4-iso-nonylphenol in SPM was found downstream of Budapest. Most of the analysed  volatile organic compounds (VOCs) were not detected in the JDS2 samples; in the case of a few detected substances no EQS was exceeded. These results are in harmony with the findings of the JDS1. Among organochlorinated compounds in water, only 1,2,4-trichlorobenzene slightly exceeded EQS at one site. The results for organochlorine compounds in sediments and SPM do not indicate that these substances are relevant pollutants in the Danube catchment area, which is a clear improvement of the past situation as described in the Danube Roof Report 2004.

The analytical results obtained for polar compounds in the Danube (pharmaceuticals, pesticides, perfluorinated acids (PFOS/PFOA) and phenolic endocrine disrupting compounds) are similar to those in other large European rivers such as the Rhine, Elbe or Po. The most relevant polar compounds identified in the Danube River in terms of frequency of detection, persistency and concentrations were anticorrosives benzotriazoles, pesticide 2,4-D, and antiepileptics pharmaceutical carbamazepine.

Results of the complementary GC-MS screening of "unknown" emerging pollutants suggested that the basin-wide presence of phthalate plasticisers, organophosphate flame retardants and siloxanes belonging to the group of fuel additives and personal care products should be investigated in more detail.

Most of the polyaromatic hydrocarbons (PAH) in water samples were far below the WFD AA-EQS values and values in sediments were about one order of magnitude lower than those typically found in the Elbe River. The exception were summary concentrations of benzo (g,h,i)perylene and indeno

(1,2,3-cd)pyrene in water (being close to the EQS of 2 ng/l at most sites and at 6 sites the EQS was slightly exceeded). In addition, the sum of the benzo(b)fluoranthene and benzo(k)fluoranthene, and the sum of benzo(g,h,i)perylene and indeno(1,2,3-cd)pyrene, exceeded the EQS in the upper section of the Tisa tributary.

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and dioxin-like PCBs were more than one order of magnitude lower in all compartments when compared to the Elbe River and only one site slightly exceeded the "safe sediment value" for PCDD/Fs. EC-6 PCBs did not exceed the related German quality standards in sediment.

Polybrominated diphenylethers (PBDEs) concentrations in SPM were an order of magnitude lower than in Dutch rivers for c-deca BDE, and c-penta BDE was a around a factor of 5 below the WFD EQS value in all water samples.

The concentration profiles down the Danube suggests that PAHs and PCDD/Fs arise from diffuse sources, whereas PBDEs and PCBs display distinct zones of contamination. This fits into the picture of PAHs and PCDD/Fs as combustion by-products being dispersed mainly into the atmosphere, whereas "intentionally produced industrial chemicals" such as PCBs and PBDEs stem from emissions through industrial and urban effluents.

The comparison of left and right bank sediment data suggests a diffuse emission from both sides of the catchment for PAH.  PCDD/Fs and PCBs show some distinct signals from the left side. The results of PBDEs show a clear impact from the tributaries Drava, Sava and Velika Morava, all being right tributaries of the Danube River.

Pollution profiles in the sediments are scarcely reflected by the data in the water column, where (except for PBDEs) the spatial gradients are lower and maxima often appear at different sites. This underlines the historic character of the findings in the sediments.

Concentrations of metals in water were found above the quality targets at only three sites. The concentration of mercury slightly exceeded the EQS at two sites downstream of Budapest, and the concentration of nickel exceeded the EQS in the Timok confluence with the Danube. The evaluation, however, suffered from a lack of natural background values for metals, which might increase the EQS levels for certain areas and substances. The concentration ranges of the heavy metals, as well as arsenic in water, SPM and bottom sediments during the JDS2, were rather similar to those observed in the JDS1 samples. For several substances the maximum levels observed during the JDS2 were lower than those in the JDS1.

In general, the average concentrations of priority substances detected during the JDS2 tend to be lower than those measured during the JDS1, especially for organic substances. This indicates that measures taken to reduce their emissions are starting to be successful. However, several priority substances as well as newly emerging substances are becoming of concern in the Danube basin and require measures to be taken to minimise their emissions.

The results of the ecotoxicological analysis of the Danube sediments showed no significant toxic effects.

## 26.6    Isotope analysis

The regional distribution of ^{137}Cs contamination mainly originated from the Chernobyl accident in May 1986. The JDS2 results demonstrate a clear general decrease by a factor of 10 in the ^{137}Cs activity concentration of Danube sediments between 1988 and 2007. Since 2004, a generally constant ^{137}Cs level has been detected along the Danube except for the middle section where a slight increase was detected. This effect could be explained by the downstream transport of remobilised sediment and by locally increased ^{137}Cs input by soil erosion. The observed radioecological behaviour is a good illustration of an impact of climatic change causing seasonal and regional changes in contaminated soil

erosion in the Danube catchment. There is a clear relation of ^{137}Cs concentrations in sediment to the hydrological conditions in the Danube Basin.

Due to the decreased artificial radioactivity levels in the Danube River, there are no associated health risks. Naturally occurring radionuclides, such as **[226]Ra** and **[228]Ra**, in the Danube and tributary sediments were found in normal geochemical activity concentration levels. It can be concluded that the Danube River was found in a good radioecological status in 2007, however, locally elevated concentrations in the tributaries (e.g. Inn and Velika Morava), caused either by contaminated soil erosion (Chernobyl accident) or by emissions from industrial sites (mining activities increasing natural radioactivity), should be mentioned.

A variety of isotope data (i.e. $\delta^2$H, $\delta^{18}$O, ^{3}H, and [222]Rn) were collected during the JDS2 to improve hydrological and geochemical characterisation of the Danube Basin. The JDS2 findings support previous results emphasizing the dominant role of tributaries and in-channel mixing over direct groundwater inflows from aquifers along the Danube. The Inn has the largest impact on $\delta^2$H and $\delta^{18}$O values in the Danube as a result of its large discharge and its more negative isotope composition. $\delta^2$H and $\delta^{18}$O data do not indicate a strong evaporation process in the Danube, however there are exceptions in some tributaries such as the Sio. The ^{3}H results show that the Váh and Morava tributaries are influenced by nuclear power plant discharges. The values detected during the JDS2 are however well below any health limits.

The [222]Rn data suggest that groundwater inputs to the river are largest in the Upper Danube and only small along the Middle and Lower Danube.

## 26.7    Conclusions

The JDS2 is an important integral part of the ICPDR Monitoring Strategy. It will provide valuable support to Danube countries in their national status assessments and, in addition, provided a unique dataset for river basin management planning at the basin-wide level due to the comparability of results. Joint longitudinal surveys of the Danube and its major tributaries can be considered an important tool for the preparation of the Danube River Basin Management Plan.

The results of the Joint Danube Surveys 1 and 2 create the most comprehensive and homogeneous database on the status of the aquatic ecosystem of the Danube and its major tributaries.

The survey confirmed the earlier TNMN conclusions of a generally improving trend along the main Danube River. However, it also reinforced evidence of specific problems, especially at a number of tributaries and downstream of large cities.

It identified the need for actions to address:

– Hydromorphology (e.g. reconnecting side-arms);

– Continuing the building and expansion of sewage treatment plants;

– Specific industrial pollution problems (e.g. hot spots);

– More intensive investigations and measures on some tributaries.

A common understanding about methods and assessments was generated among Danube scientists and governments. New methods used during the survey proved valuable (e.g. air-lift sampler, biological elements, fish and hydromorphology). The JDS2 will be made available for future research efforts and dialogue with different stakeholders and users (e.g. concerning problem locations and parameters).